

Article

Effect of Asparaginase on Neo-Formed Contaminants and Quality Attributes in Wholemeal Biscuits

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Featured Application

This study provides information on the effectiveness of asparaginase treatment in reducing acrylamide levels in wholemeal wheat shortbread biscuits, indicating the possibility of secondary effects on sensory quality and therefore suggesting further investigation into this aspect.

Abstract

The effects of asparaginase treatment and conditions of its use (enzyme dose and dough resting time) on the formation of heat-induced contaminants, namely, acrylamide (ACR) and 5-hydroxymethylfurfural (HMF), were investigated in wholemeal wheat biscuits. In addition, potential impacts on sensory-related quality attributes were assessed through instrumental analysis of colour, texture, and volatile organic compounds (VOCs). Three levels of asparaginase addition (100, 300, and 500 ASNU kg^{−1} flour) were tested and compared with an untreated control, with and without the application of a 15 min dough resting step. Results confirmed the effectiveness of asparaginase in reducing ACR levels in the final biscuits, showing a dose-dependent response with reductions of up to 68% at 500 ASNU kg^{−1} flour. A dough resting step tend to further lower ACR content. In contrast, asparaginase treatment did not exert a consistent effect on HMF formation, whereas dough resting tended to decrease it. Moreover, asparaginase addition at the highest dose reduced the level of several Maillard reaction-derived VOCs by approximately 30%. Overall, asparaginase treatment markedly reduced ACR formation while causing changes in some odour-active compounds in biscuits. Further sensory evaluation will be necessary to determine whether these changes are perceptible and may influence consumer acceptance.

Keywords: acrylamide; 5-hydroxymethylfurfural; cookies; baked confectionary; enzymes; Maillard reaction; aroma; volatile organic compounds; colour; texture



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1. Introduction

Thermal processing of cereal-based foods induces a complex network of chemical reactions that strongly influence both product quality and safety. Among these, the Maillard

reaction (MR) is a major contributor to the development of desirable sensory characteristics such as colour, aroma, and texture, while at the same time being responsible for the formation of a range of undesired compounds [1,2]. In particular, the generation of process contaminants, such as acrylamide (ACR) and 5-hydroxymethylfurfural (HMF), has raised considerable concern, leading to increasing regulatory attention and to the need for effective mitigation strategies in bakery products [3,4]. Acrylamide formation in cereal-based baked foods is primarily associated with the reaction between free asparagine and reducing sugars during high-temperature baking [5]. In wheat-based products, free asparagine is generally recognised as the limiting factor for acrylamide formation, rather than the availability of reducing sugars [6]. Its concentration in cereal flours can vary widely depending on genotype, agronomic practices, and degree of refinement, with wholemeal flours typically exhibiting higher levels due to the presence of bran and germ fractions [6,7]. As a consequence, wholemeal bakery products are often characterised by a higher acrylamide-forming potential compared to their refined counterparts, making mitigation strategies particularly relevant for this category [6,8].

The enzymatic conversion of asparagine by asparaginase represents one of the most effective and technologically feasible approaches to reduce ACR formation in bakery products. Asparaginase hydrolyses asparagine into aspartic acid and ammonia, removing the key ACR precursor without directly targeting other amino acids mainly responsible for colour and flavour development [9,10]. Most asparaginases are known to be quite specific for asparagine, even though low activity towards other amino acids has been reported [10]. Commercial preparations of asparaginase, commonly obtained from *Aspergillus oryzae* or *Aspergillus niger*, are applied at the industrial level and have been assessed as safe by international regulatory authorities [10]. Numerous studies have demonstrated that the use of asparaginase, often in combination with a controlled dough resting time, can substantially reduce acrylamide levels in biscuits and other baked cereal products [4,6,11,12]. Despite this extensive body of literature, most studies have focused on formulations based on refined wheat flour, whereas the application of asparaginase in wholemeal bakery products has received far less attention. The more complex composition of wholemeal doughs may affect both the efficiency of enzymatic treatments and the overall progress of the MR [6,8]. Moreover, while the impact of asparaginase on acrylamide formation is well documented, much less information is available on its influence on other MR-related contaminants, such as HMF [4], particularly in wholemeal systems.

Beyond food safety, the potential effects of asparaginase treatment on sensory quality attributes deserve careful consideration. Changes in the amino acid profile of the dough may influence the formation of volatile organic compounds (VOCs), which arise from multiple pathways, including the MR, caramelisation, and lipid oxidation, and play a central role in defining the aroma of baked products [2,13]. Key odorants identified in cereal-based baked goods belong to several chemical classes, including aldehydes, alcohols, pyrazines, ketones, furans, acids, and sulphur-containing compounds, whose formation is strongly dependent on formulation and processing conditions [2,13]. Previous studies have generally reported limited effects of asparaginase treatment on VOC formation in biscuits from refined wheat flour [14]. On the contrary, there are no available data on the effects on aroma-related VOCs in wholemeal biscuits. Colour development, largely associated with the advanced stages of the MR, is another critical quality parameter in baked products and has often been reported to correlate with acrylamide formation [15]. However, the extent to which enzymatic mitigation strategies may influence browning reactions in wholemeal biscuits, which are intrinsically darker than refined flour products, is still not fully understood [16,17].

Therefore, this study aimed to evaluate the effect of asparaginase treatment and processing conditions on the formation of heat-induced contaminants in wholemeal wheat biscuits. Three levels of asparaginase addition (100, 300, and 500 ASNU kg⁻¹ flour) were investigated, in combination with the application of a dough resting time. In parallel, the impact of these treatments on sensory-related quality attributes was assessed through instrumental analysis of colour, texture, and VOC profile. To the best of the authors' knowledge, this study provides one among the first studies of the effects of asparaginase use on both safety and sensory quality-related parameters in wholemeal wheat biscuits. By addressing both aspects of biscuits quality, this study aims to provide new insights into the feasibility of asparaginase-based mitigation strategies in wholemeal bakery products and to contribute to the development of safer wholegrain biscuits without compromising sensory quality.

2. Materials and Methods

2.1. Experimental Biscuits

Shortbread biscuits were produced using the following formulation: wholemeal wheat flour (350 g), butter (105 g), sugar (88 g), baking powder (8 g), and milk (64 g), with or without the addition of asparaginase. The wholemeal flour complied with Italian legislative requirements and had the following composition: moisture 12.4%, ash 1.38%, protein 12.1%, fibre 7.8%, and an initial asparagine content of 250.5 µg/g. The baking powder consisted of a commercial formulation containing disodium diphosphate (E450i), sodium bicarbonate (E500ii), corn starch, and stabilising sodium, potassium, and calcium salts of fatty acids (E470a). Asparaginase was incorporated at levels of 100, 300, or 500 ASNU kg⁻¹ flour, while a control treatment (C) was prepared without enzyme addition. ASNU (Asparaginase Units) is defined as the amount of enzyme capable of producing 1 µmol of ammonia per minute under the specified assay conditions. The commercial asparaginase preparation used in this study was Acrylaway[®] L (Novozymes/Novonesis, Bagsvaerd, Gladsaxe, Denmark). For conventional biscuit formulations, the optimum asparaginase dosage for acrylamide mitigation generally ranges from 500 to 1000 ASNU kg⁻¹ flour, although the effective level may vary according to product formulation and processing conditions. In this study, the selected enzyme dosages were established based on the manufacturer's recommendations, previously published investigations [11,12], and preliminary experiments conducted in our laboratory. In addition, for each level of asparaginase supplementation, two conditions were evaluated to assess enzyme activity: (i) immediate baking following dough preparation, with no resting period after mixing; and (ii) a dough resting period of 15 min at ambient temperature (20 ± 1 °C) before baking. The experimental treatments are summarised in Table 1.

Table 1. Variables of wholemeal biscuit preparation investigated in the experimental design: levels of asparaginase addition (ASNU kg⁻¹ flour) and the application of a dough resting time (min).

Sample Code	Asparaginase Addition Level (ASNU kg ⁻¹ Flour)	Resting Time (min)
Ca	0	0
Cb	0	15
100a	100	0
100b	100	15
300a	300	0
300b	300	15
500a	500	0
500b	500	15

Dough preparation was carried out using a planetary mixer equipped with a 3 L bowl and a C-shaped dough hook (KitchenAid, Whirlpool, Benton Harbor, MI, USA). Butter was first placed in the mixing bowl and mixed for 1 min to obtain a soft and homogeneous consistency. Sugar was then added, and mixing was continued for another minute. Subsequently, flour, baking powder, and milk were incorporated, and the mixture was kneaded for a further 6 min. When included in the formulation, asparaginase was dispersed in the milk immediately before being added to the mixing bowl. After mixing, the dough was removed from the bowl and divided into two equal portions (A and B), which were subjected to different pre-baking treatments. Portion A was immediately sheeted to a thickness of 5 mm using a rolling pin equipped with thickness guides. Biscuits were then cut using a circular stainless-steel cutter with a diameter of 5 cm and baked in a steam-convection oven (Angelo Po, Carpi, Italy) at 200 °C for 18 min, with air circulation disabled. Portion B was shaped into a loaf, wrapped in cling film, and allowed to rest for 15 min at ambient temperature (20 ± 1 °C). At the end of the resting period, the dough was sheeted, cut, and baked under the same conditions described for Portion A. One baking trial was conducted for each experimental condition. The following physical characteristics were determined for all biscuits: length (5.00 ± 0.31 cm), width (4.63 ± 0.25 cm), thickness (0.84 ± 0.08 cm), weight (10.5 ± 0.6 g), and moisture content ($5.0 \pm 0.5\%$).

2.2. Chemical Determinations

2.2.1. Acrylamide

Acrylamide was quantified using an in-house validated LC–MS/MS method developed in our laboratory. Method validation was performed in accordance with European and U.S. guidelines for bioanalytical LC–MS/MS methods, while full methodological details and validation data will be reported separately. Briefly, homogenised biscuit samples were accurately weighed and spiked with acrylamide-d3 as internal standard prior to aqueous solid–liquid extraction. Chromatographic separation was achieved on a Restek Allure Acrylamide column (50×2.1 mm) using gradient elution with formic acid in water (A) and acetonitrile (B). Detection was performed in MRM mode monitoring the transitions m/z 72 $\rightarrow$ 55 and 72 $\rightarrow$ 27 for acrylamide, and m/z 75 $\rightarrow$ 58 for acrylamide-d3. Quantification was based on solvent-matched calibration using isotopic internal standardisation. The method showed a limit of detection (LOD) of $3 \mu\text{g kg}^{-1}$ and a limit of quantification (LOQ) of $10 \mu\text{g kg}^{-1}$. Precision was satisfactory across the validated concentration range, with relative standard deviation (%RSD) values below 10% at all concentration levels evaluated. Validation results demonstrated satisfactory accuracy, precision, recovery, and control of matrix effects within the concentration range relevant to this study. Two analytical replicates were carried out for each of the eight biscuits samples.

2.2.2. 5-Hydroxymethylfurfural (HMF)

HMF was determined as reported by Prata et al. [18], with minor modifications. Briefly, 2 g of biscuits were transferred to a screw-capped glass tube and mixed with 2 mL of acetonitrile. The mixture was vortexed for 1 min, then 2 mL of hexane was added, and the sample was vortexed again for 1 min. After centrifugation at 5000 rpm for 15 min, the hexane phase was discarded, and the acetonitrile phase was recovered. The remaining pellet was re-extracted as described above. The supernatants were pooled and transferred to a 20 mL volumetric flask, and deionised water was added to volume. An aliquot of the aqueous extract was centrifuged at 10,000 rpm using a mini centrifuge (LLG uniCFUGE 5, LLG Labware, Teltow, Germany), then filtered through a $0.2 \mu\text{m}$ RC filter and subjected to HPLC analysis. HPLC analysis was performed using a Varian ProStar system (Varian Inc., Walnut Creek, CA, USA) equipped with a photodiode array (PDA) detector. Separation

was achieved on a Nucleosil 100-5 C18 column (5 μm , 250 $\times$ 4 mm) maintained at 32 °C. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B). The gradient programme was as follows: 95% A and 5% B at the start; after 10 min, solvent B was increased to 100%, held for 5 min, and then returned to 5% B. Chromatograms were recorded at 280 nm, and HMF was quantified using external standard calibration. Two analytical replicates were carried out.

2.2.3. Volatile Organic Compounds (VOCs)

Biscuit samples were cryogenically frozen using liquid nitrogen and subsequently ground with a laboratory grinder (IKA, Staufen, Germany) to obtain a homogeneous powder. The resulting powders were stored at -70 °C until analysis, which was performed within three weeks of grinding. Volatile organic compounds (VOCs) were analysed using a semi-quantitative headspace solid-phase microextraction coupled with the gas chromatography–mass spectrometry (HS-SPME/GC-MS) method previously developed in our laboratory [19], with minor modifications. For each analysis, 0.5 g of biscuit powder was weighed into a 15 mL glass vial. The vial was immersed in a thermostatically controlled water bath maintained at 60 °C. VOC extraction was then carried out by exposing a 50/30 μm DVB/CAR/PDMS fibre (Supelco, Sigma-Aldrich, Milan, Italy) to the sample headspace for 45 min. Following extraction, the fibre was immediately transferred to the split/splitless injection port of the gas chromatograph for thermal desorption, and the chromatographic run was initiated. Analyses were performed using an Agilent 6890 GC coupled to a 5973N mass spectrometer equipped with a quadrupole mass analyser (Agilent Technologies, Palo Alto, CA, USA). Desorption and injection were conducted in splitless mode at 240 °C. Chromatographic separation was achieved on a DB-WAX capillary column (60 m $\times$ 0.25 mm i.d., 0.5 μm film thickness; J&W, Agilent Technologies, Palo Alto, CA, USA). The inlet temperature was maintained at 240 °C. The oven temperature programme consisted of an initial hold at 40 °C for 10 min, followed by a ramp to 210 °C at 4 °C min^{-1} and a subsequent increase to 220 °C at 30 °C min^{-1} , with a final hold of 5 min. The total run time was 57.83 min. Helium was used as the carrier gas at a constant flow rate of 2 mL min^{-1} , corresponding to a linear velocity of 36 cm s^{-1} . Mass spectrometric detection was performed using electron ionisation (EI) at 70 eV. The transfer line, ion source, and quadrupole temperatures were maintained at 220, 230, and 150 °C, respectively. Data acquisition was carried out in both full-scan and single-ion monitoring (SIM) modes for compound identification and semi-quantitative determination, respectively. Volatile compounds were identified by comparing the linear retention indices (LRI) and mass spectra of chromatographic peaks with data reported in the literature and in the NIST/EPA/NIH Mass Spectral Library (version 2.4, 2020). The complete list of the 41 VOCs identified, together with their retention indices and the selected m/z ions used for SIM detection, is reported in Supplementary Table S1. Semi-quantitative determination of each VOC was based on the peak area obtained from SIM chromatograms. All analyses were performed in triplicate on the powder obtained from each biscuit sample.

2.3. Instrumental Measurement of Quality Attributes of Biscuits

2.3.1. Colour

Biscuit colour was measured using a CR-400 chromameter (Konica Minolta, Milan, Italy) operating in the CIE 1976 $L^* a^* b^*$ colour space under D65 illuminant conditions. For each biscuit, colour measurements were recorded twice on the upper surface and twice on the lower surface. Ten biscuits were analysed for each treatment, resulting in a total of 40 measurements per biscuit type ($n = 40$). The Browning Index (BI) was subsequently

calculated from the measured L^* , a^* , and b^* values according to the equations reported by [20]:

$$BI = 100 \times [(X - 0.31)/0.17], \text{ where } X = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*)$$

2.3.2. Texture

Biscuit texture was evaluated by measuring the breaking force using a three-point bending test. Ten biscuits were analysed using a TA.XTplus Texture Analyser (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 5 kg load cell. Samples were placed on two supports set 13 mm apart, and measurements were performed using an HDP/3PB probe. The instrument was operated at a pre-test speed of 3 mm s⁻¹, a test speed of 3 mm s⁻¹, and a post-test speed of 10 mm s⁻¹. Hardness, stiffness, and fracturability were derived from the force–distance curves generated during the test. Hardness (N) was defined as the maximum force (breaking force) recorded. The gradient (N mm⁻¹), corresponding to the initial slope of the force–distance curve, was used as an indicator of sample stiffness and reflects the elastic behaviour of the biscuit prior to fracture. Fracturability, expressed as distance (mm), was defined as the deformation at which the maximum force occurred and is commonly associated with biscuit brittleness. Eleven biscuits were analysed for each kind of treatment ($n = 11$).

2.4. Statistical Analysis

The results obtained for ACR, HMF, VOC, and colour and texture parameters were subjected to one-way ANOVA and Tukey's HSD post-hoc test for multiple comparisons ($p < 0.05$). Additionally, Principal Component Analysis (PCA) was performed on the VOC dataset. Prior to PCA analyses, the dataset was pre-processed by averaging replicate measurements and applying autoscaling. Linear correlations among VOCs levels, colour, texture parameters, and ACR, HMF contents were assessed by calculating the corresponding Pearson correlation coefficients (r) and related p values. All statistical analyses were carried out using XLStat software (version 2025.02.0; Addinsoft, New York, NY, USA).

3. Results

3.1. Acrylamide (ACR) Content

Figure 1 shows the effects of asparaginase addition and of dough resting time on ACR levels. Experimental data confirm the effectiveness of asparaginase use also in the case of wholemeal wheat biscuits, highlighting a reduction of ACR content from $172 \pm 8 \mu\text{g kg}^{-1}$ (without resting) and $137 \pm 2 \mu\text{g kg}^{-1}$ (with resting) in the control biscuits to a range of values between $103 \pm 1 \mu\text{g kg}^{-1}$ and $45 \pm 1 \mu\text{g kg}^{-1}$ in the treated biscuits. On average, the reduction in ACR level reached 39%, 46%, and 68% in biscuits treated, respectively, with 100, 300, and 500 ASNU doses (Figure S1), suggesting a progressive reduction effect associated with increasing enzyme doses. When the two preparation methods (with and without dough resting time) were considered separately, each increase in asparaginase dose resulted in a significant reduction in acrylamide (ACR) levels, except in a single case (from 100a to 300a, Figure 1). When applying the highest dose of asparaginase, the reduction in ACR with respect to the untreated control amounted to 69% in biscuits obtained without the application of dough resting time, and to 67% with resting time. Furthermore, the application of a 15 min resting time tended to further decrease ACR content (Figure 1); the average reduction amounted to 23%, $108 \mu\text{g kg}^{-1}$ to $83 \mu\text{g kg}^{-1}$ (Figure S1). The combined effect of the highest dose of asparaginase and the application of the resting step resulted in a 74% reduction in ACR compared with the content in biscuits produced without the application of the enzyme and the resting step.

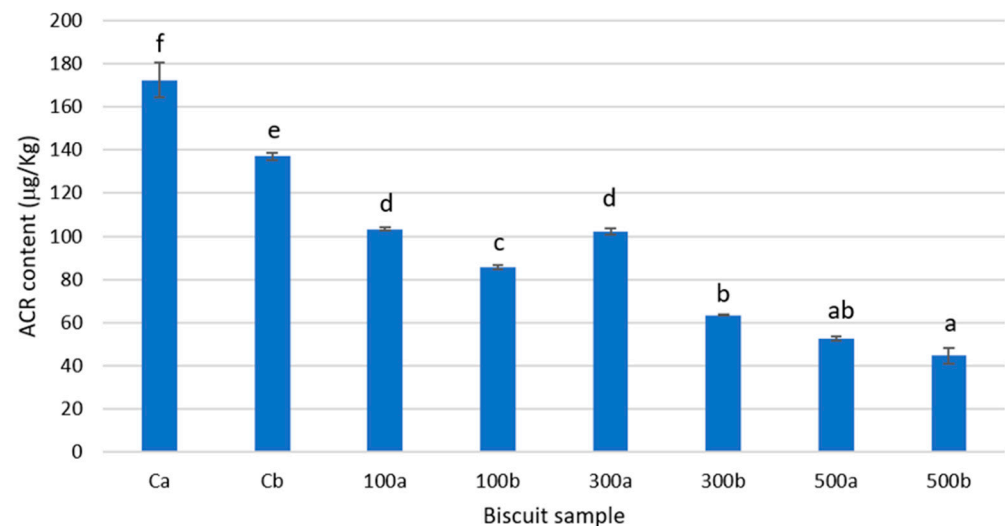


Figure 1. Acrylamide level ($\mu\text{g kg}^{-1}$) in the experimental biscuit samples, treated with asparaginase (at 100, 300, 500 ASNU kg^{-1}) and untreated Control, without (a) and with (b) resting of dough during preparation. Mean values, standard deviations ($n = 2$). Difference letters indicate statistically significant differences (Tukey's test at $p < 0.05$).

3.2. 5-Hydroxymethylfurfural (HMF) Content

The influence of asparaginase treatment and resting time on HMF formation is shown in Figure 2. It can be observed that there is no effect consistently associated with the asparaginase dose (Figures 2 and S2). Conversely, a more consistent trend was observed regarding dough resting time: at all asparaginase addition levels, HMF showed significantly lower levels in biscuits obtained with dough resting time, except for the 500 ASNU kg^{-1} addition level (Figure 2). By considering all biscuit samples, on average, a 33% reduction (from 1.72 mg kg^{-1} to 1.15 mg kg^{-1}) was observed in biscuits prepared using a resting time of 15 min (Figure S2).

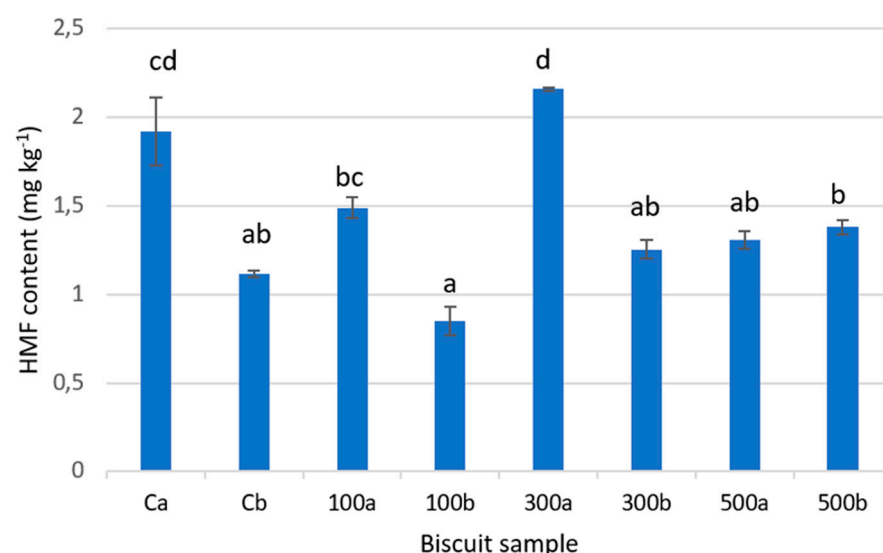


Figure 2. HMF level (mg kg^{-1}) in the experimental biscuits, treated with asparaginase (at 100, 300, 500 ASNU kg^{-1}) and untreated Control, without (a) and with (b) resting of dough during preparation. Mean values, standard deviations ($n = 2$). Difference letters indicate statistically significant differences (Tukey's test at $p < 0.05$).

3.3. Volatile Organic Compounds (VOCs)

The VOC profile was analysed by determining the levels of 41 identified compounds. Figure 3 shows the bi-plot obtained from the PCA of the corresponding dataset, indicating that the first two Principal Components explained 70% of the total variance (PC1 44% and PC2 26%, respectively). PC1 discriminated most samples according to asparaginase treatment, as asparaginase treated biscuits, except for one (300 A), were located along the negative axis of PC1. Notably, most VOCs generated through the MR were instead associated with the positive axis of PC1, as suggested by the high loading values of these compounds for the PC1 (Table S2). MR-related VOCs, such as 2-methylpyrazine, 2,5, 2,6, and 2,3-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2-acetylfuran, 2-furanmethanol, and 2-furanone, were characterised by loading values higher than 0.9, whereas others, such as 2- and 3-methylbutanal, furfural, furaneol, and *p*-vinylguaiacol, still showed high loadings, although lower than that value, indicating high levels of these compounds in untreated biscuits (Table S2). Also, some methyl ketones (2-heptanone and 2-octanone), which are products of lipid oxidation, are associated with the positive axis of PC1. For all these compounds, a progressive decrease was observed with increasing asparaginase dose, and, on average, biscuits treated at 500 ASNU showed a reduction of approximately 30% compared to untreated samples (Table S3). For furfural, a potential marker of MR progression, this reduction amounted to 28%. For the same compounds, a reduction effect associated with the application of dough resting time was also observed, although it was generally less pronounced. In contrast, other compounds derived from lipid oxidation (E-2-nonenal, E-2-octenal, 2,4-decadienal, and 2-pentylfuran) were collectively associated with the positive axis of PC2 (loadings in Table S2) and did not appear to be clearly related to the effects of asparaginase treatment. Finally, the analysis of bootstrap ellipses from PCA suggests a separation between the overall VOC profiles of biscuits with higher levels of addition and those not treated with asparaginase (500a vs. Ca and, separately, 500b vs. Cb) (Figure S3).

3.4. Colour and Texture

Figure 4 shows the results regarding the chromatic coordinate measurements and the Browning Index (BI). No clear and marked trends consistently related to the asparaginase dose were observed. The *a** and *b** coordinates, and consequently the BI, tended to exhibit higher values only in samples treated with 300 ASNU, but this did not indicate a consistent effect of asparaginase addition on the colour characteristics of the biscuits. Moreover, only in biscuits treated with asparaginase, the BI tended to show higher values in samples subjected to dough resting (Figure 4).

Figure 5 presents the results of the texture measurements. Among the three parameters evaluated, only hardness showed an effect associated with the studied variables, specifically, an increase of hardness related to the addition of asparaginase at the highest level with respect to untreated biscuits (500b vs. Cb).

3.5. Relationships Between ACR and HMF and Sensory-Related Parameters

The analysis of Pearson correlation coefficients between neo-formed contaminants and sensory-related analytical parameters (Table 2) shows an association between the formation of ACR and that of several VOCs derived from the MR (2-methylpyrazine, 2,5, 2,6, and 2,3-dimethylpyrazine, acetic acid, 3-ethyl-2,5-dimethylpyrazine, furfural, 2-ethyl-3,5-dimethylpyrazine, 2-acetylfuran, benzaldehyde, 2-furanmethanol, 2-furanone) and some volatile products of lipid oxidation (2-heptanone and 2-octanone). By contrast, no correlations were observed between ACR and colour or texture parameters, nor between HMF and any of the other parameters analysed, except for a single volatile compound.

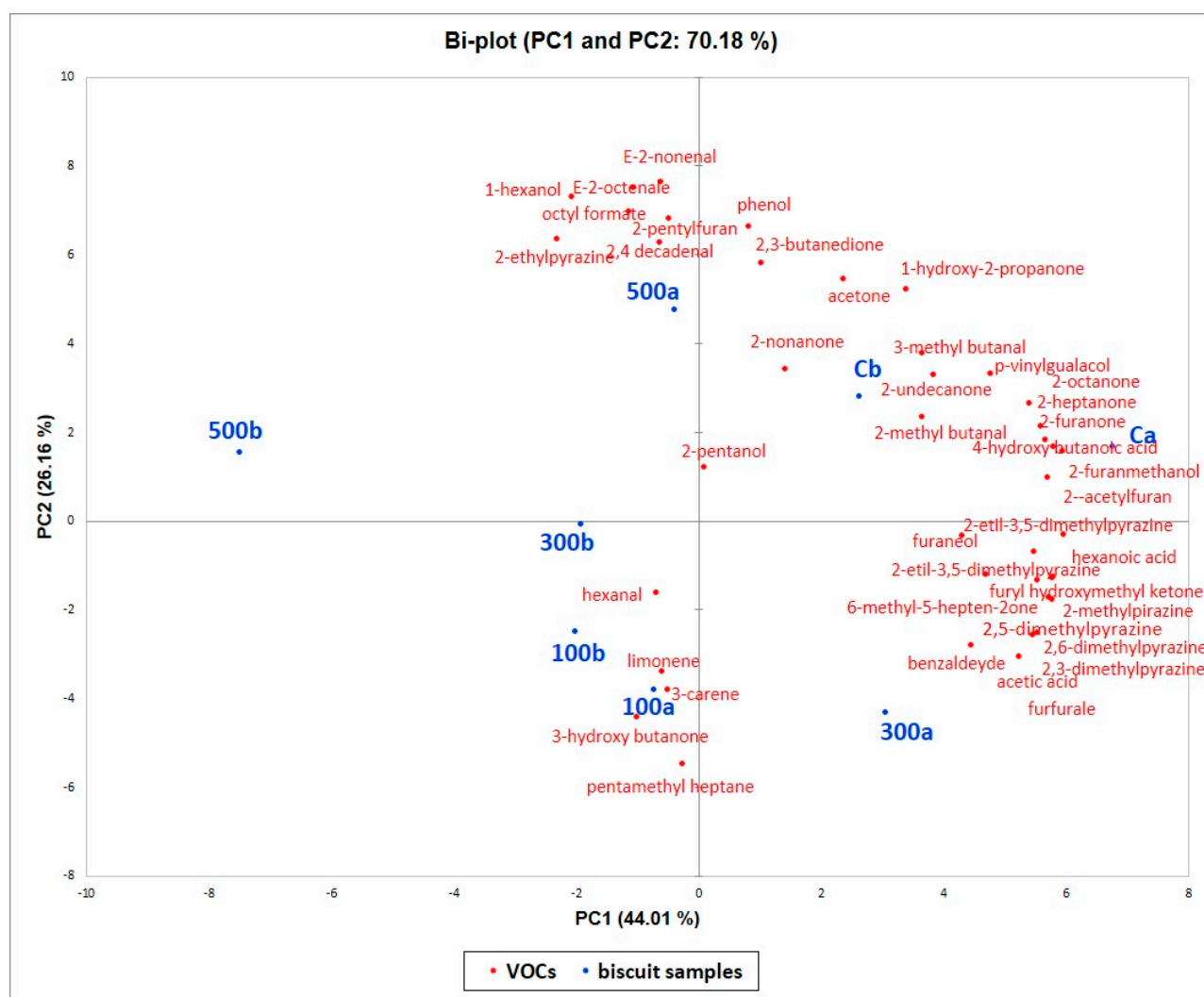


Figure 3. PCA bi-plot of VOCs of biscuit samples treated with asparaginase (at 100, 300, 500 ASNU kg^{-1}) and untreated Control, without (a) and with (b) resting of dough during preparation.

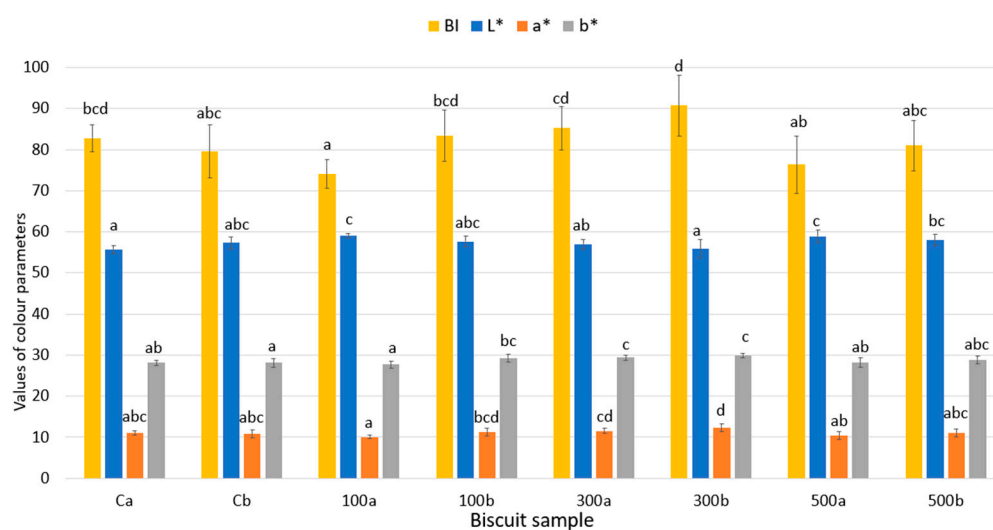


Figure 4. Browning Index (BI) and chromatic coordinates L^* , a^* , and b^* of all experimental biscuits, treated with asparaginase (at 100, 300, 500 ASNU kg^{-1}) and untreated Control, without (a) and with (b) resting of dough during preparation. Mean values and standard deviations ($n = 40$). For each colour parameter, different letters indicate statistically significant differences (Tukey's test at $p < 0.05$).

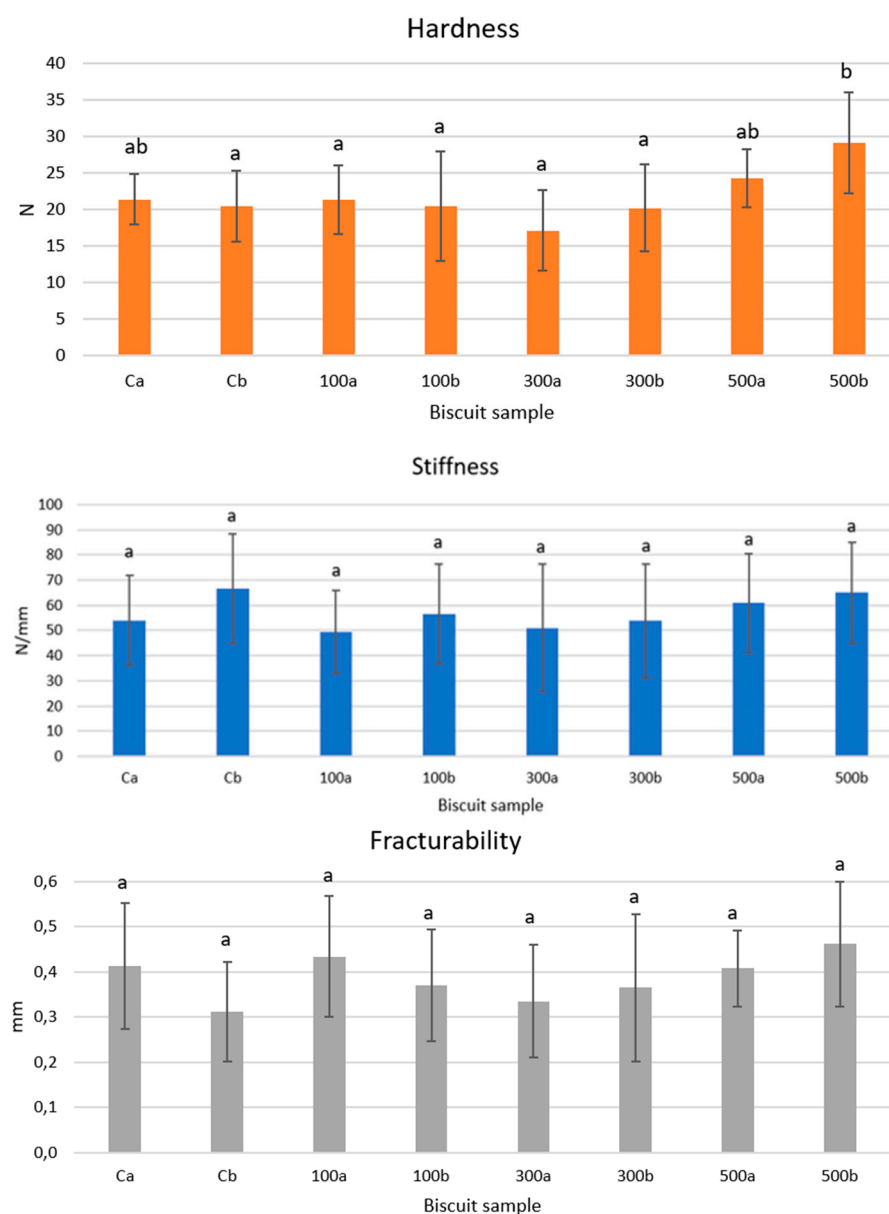


Figure 5. Instrumental texture characteristics, hardness, stiffness, and fracturability measured on all experimental biscuits, treated with asparaginase (at 100, 300, 500 ASNU kg^{−1}) and untreated Control, without (a) and with (b) resting of dough during preparation. Mean values and standard deviations (n = 11). Different letters indicate statistically significant differences (Tukey's test at $p < 0.05$).

Table 2. Pearson correlation coefficients (r), along with p values, between neo-formed contaminants (acrylamide, ACR, and 5-hydroxymethylfurfural, HMF) content and sensory-related parameters (VOC level, texture, and colour parameters). Only parameters with significant correlation ($p < 0.05$) were reported.

Experimental Variable	ACR		HMF	
	r	p	r	p
VOCs				
2-heptanone	0.901¹	0.002	0.269	0.519
2-methylpyrazine	0.862	0.006	0.591	0.123
2-octanone	0.857	0.007	0.324	0.434
2,5-dimethylpyrazine	0.837	0.009	0.459	0.252
2,6-dimethylpyrazine	0.739	0.036	0.581	0.131
6-methyl-5-hepten-2-one	0.837	0.010	0.745	0.034

Table 2. Cont.

Experimental Variable	ACR		HMF	
	r	p	r	p
2,3-dimethylpyrazine	0.810	0.015	0.450	0.263
acetic acid	0.876	0.004	0.532	0.175
3-ethyl-2,5-dimethylpyrazine	0.717	0.045	0.357	0.385
furfural	0.727	0.041	0.693	0.057
2-ethyl-3,5-dimethylpyrazine	0.770	0.025	0.495	0.212
2-acetylfuran	0.853	0.007	0.672	0.068
benzaldehyde	0.717	0.046	0.480	0.229
4-hydroxybutanoic acid	0.869	0.005	0.402	0.323
2-furanmethanol	0.900	0.002	0.369	0.368
2-furanone	0.726	0.041	0.571	0.139
hexanoic acid	0.940	0.001	0.277	0.506

Note: ¹ Values in bold are different from 0 with a significance level $\alpha = 0.05$.

4. Discussion

4.1. Acrylamide (ACR)

From the perspective of acrylamide formation potential, the differences between the use of wholemeal wheat flour and refined flour mainly lie in the higher asparagine content of wholemeal flour [6]. Regarding the effectiveness of the enzyme asparaginase in reducing asparagine content, the differences associated with the use of wholemeal flour could, in principle, primarily be related to a reduction in water activity due to the higher fibre content, which, by increasing the amount of bound water, decreases the proportion of free water. This reduction in free water availability may limit enzyme diffusion within the dough matrix and consequently decrease the extent of asparagine hydrolysis. However, lower free water content during baking may also reduce the interaction between free asparagine and reducing sugars, potentially slowing MR development, as reported by Anese et al. [12]. Second, the presence of bran, by making the dough structure less homogeneous, could hinder the accessibility of the asparagine substrate to the enzyme asparaginase. Furthermore, the slight reduction in pH and the slight increase in lipid content [12] in the dough obtained from wholemeal flour could further contribute to decreasing asparaginase activity. Despite these potential negative influences associated with wholemeal wheat flour, the results of this study show that the effectiveness of asparaginase in reducing ACR levels in shortbread biscuits is substantially preserved. The reduction levels observed at the highest dose were similar to those obtained in previous studies on biscuits made from refined flour under comparable conditions: the same dose of asparaginase and a dough resting time of 20 min at 20 °C resulted in a 71% reduction in short-dough biscuits [12], whereas a slightly higher dose (525 ASNU kg⁻¹) combined with a resting time of 15 min achieved a 65% reduction in ACR levels in semisweet and ginger cookies [11]. Even greater reductions in ACR, up to 96%, have been achieved in rotary-cut biscuits from refined wheat flour, although with the addition of significantly higher levels of asparaginase, up to 3000 ASNU kg⁻¹ [21]. From an application perspective, considering that the cost of the enzyme represents one of the main factors limiting the widespread industrial use of asparaginase [10], it is important to evaluate on a case-by-case basis the optimal cost–benefit ratio associated with the use of the most appropriate dose of asparaginase for the specific product under consideration. Moreover, results showed that, as observed on biscuits from refined flour, the application of a 15 min dough resting period further reduced ACR content, likely by allowing a more extensive enzymatic hydrolysis of free asparagine prior to baking [12]. Modelling proposed by Anese et al. [12] on the effect of asparaginase in reducing ACR formation in refined flour biscuits established that dough resting time could have a significant effect on reducing the

final ACR content in biscuits, although to a lesser extent than asparaginase dosage and temperature during the resting period. The results of this study appear to confirm this minor yet significant effect of resting time also in the case of wholemeal biscuits.

4.2. 5-Hydroxymethylfurfural (HMF)

The HMF levels observed in this study fall within the lower range of values reported in the literature for biscuits [4]. This may be related to the formulation used, in which sucrose was the primary sugar source, as the type of sugar is known to significantly influence HMF formation during baking [4,22]. Furthermore, it is well established that the use of sodium bicarbonate, included in the formulation here used as a leavening agent, contributes to lowering the levels of HMF formed during biscuit baking, particularly when baking temperatures do not exceed 200 °C, as in this study [23]. The results do not show effects of the addition of asparaginase on HMF level that are consistently associated with the added dose. Minor variations, although at overall very low HMF levels, may be attributed to minor heterogeneities in dough composition, enhanced by the relatively heterogeneous nature of wholemeal flour [16]. The lack of consistent effects is in agreement with previous observations indicating that HMF formation in biscuits is primarily driven by the caramelisation of reducing sugars [17,22]. Therefore, it appears to be decoupled from ACR formation when, as in this study, the concentration of asparagine is altered through asparaginase treatment. Similarly, no effect of asparaginase addition on HMF formation was observed during the toasting of bread crisps [8]. A more consistent influence on HMF formation was observed in relation to dough resting, which tended to reduce HMF levels in the biscuits. This suggests that dough resting, regardless of asparaginase addition, may promote a decrease in the pool of reducing sugars present in the dough and available for caramelisation reactions during the subsequent thermal treatment. Overall, these results reinforce the concept that acrylamide and HMF formation in cereal-based products are governed by distinct chemical pathways. While acrylamide mitigation is effectively achieved through targeted precursor depletion, HMF appears to be more responsive to process-related parameters affecting sugar chemistry.

4.3. VOCs

In a previous study on shortbread biscuits made from refined flour, it was observed that the application of asparaginase doses (up to 1000 ASNU kg⁻¹), even higher than those employed in this study (up to 500 ASNU kg⁻¹), although leading to a marked depletion of asparagine in the dough, produced only limited and inconsistent modifications in the VOC profile. This suggested a minor role of asparagine, as a MR reactant, in the formation of VOCs in shortbread biscuits [14]. In this study, however, a tendency towards a reduction in the levels of several MR-related VOCs was observed in shortbread biscuits produced from wholemeal wheat flour following the addition of asparaginase. At the highest asparaginase dose, this reduction reached approximately 30% of the levels detected in untreated biscuits. One limitation of this study lies in the lack of data regarding the changes in the amino acid composition of the dough resulting from the addition of asparaginase. Consequently, the collected data do not allow us to establish whether the observed reductions in MR-related VOC formation were due to the, albeit likely, decrease in asparagine content or to potential secondary effects of asparaginase on other amino acids, such as glutamine [10]. However, the fact that the addition of asparaginase produced more pronounced effects on VOCs compared to analogous biscuits made from refined flour could be attributable, at least in part, to the higher concentration of reducing sugars in wholemeal flour dough [8]. A higher content of reducing sugars could make more evident differences in MR products resulting from modifications in amino acid

composition. Nevertheless, a potentially significant role of asparagine in the formation of certain MR-related VOCs was suggested by several experimental observations. In model systems, asparagine has previously been shown to participate in the MR leading to the formation of 2-methylpyrazine and 2,5-dimethylpyrazine [24]; this association appears to be consistent with this study, in which a reduction in the levels of these VOCs was observed, corresponding to the dose of asparaginase added. Similarly, studies in model systems have reported a contribution of asparagine to the formation of furfural, as well as HMF and 5-methylfurfural [25]. Therefore, the dose-dependent reduction observed in furfural levels in biscuits following the addition of asparaginase may be consistent with a corresponding reduction in asparagine levels in the dough. The lack of reduction in HMF may instead be explained by the fact that, in the biscuit matrix, HMF appears to be formed primarily through caramelisation reactions, as mentioned above [17,22]. Significantly, this study also highlighted a linear correlation between the reduction in ACR and the reduction in several MR-related VOCs, although the latter was quantitatively less pronounced. In biscuits treated with 500 ASNU/kg of asparaginase, an approximately 68% reduction in ACR paralleled an average reduction of about 30% in these VOCs. As noted above, the lack of data on the amino acid composition of the dough does not allow us to propose mechanistic explanations for the different extent of the effects observed on ACR and the MR-related VOCs discussed above. However, it is well established that most asparaginases exhibit high specificity toward asparagine, whereas some enzymes also show limited activity toward glutamine [10]. This suggests that minor changes in the levels of amino acids other than asparagine in the dough cannot be ruled out. Furthermore, since asparagine is a direct precursor of ACR, it is plausible that the effect of asparaginase treatment on ACR levels is more pronounced than its effect on other MR products. In fact, the formation of these other MR products, such as several aroma-related VOCs, may be influenced by several amino acids and, presumably, only secondarily by the asparagine content of the dough. On the other hand, to date, limited information is available on the role of individual amino acids in the formation of specific VOCs under defined biscuits formulations and processing conditions [2,13].

It remains to be established whether the modifications in the content of several MR-related VOCs observed in this study may translate into perceivable changes in the sensory characteristics of the biscuits. Most of the MR-related VOCs showing an average reduction of approximately 30% have been recognised as key odorants in bakery products [13]. The semi-quantitative method used for VOC determination in this study does not provide information on the actual concentrations of these compounds in the food matrix and, therefore, does not allow us to establish whether their concentrations exceed their respective odour thresholds. Consequently, it is not straightforward to infer the potential contribution of a given compound to the olfactory characteristics of the product under investigation. Nevertheless, the literature indicates that several of the VOCs identified in this study have previously been reported as key odorants in biscuits, although not necessarily in biscuit types comparable to those investigated in this study [13]. Specifically, 2- and 3-methylbutanal are key odorants in bakery products and are characterised by malty notes; 2,3-, 2,5-, and 2,6-dimethylpyrazine are associated with roasty and nutty notes; the two ethyl-dimethylpyrazines are key odorants known for their relatively low odour thresholds and cocoa-, nutty-, and roasty-like notes; the group of furans has been associated with bready, almond, pungent, and sweet aromas in biscuits, while 2-furanmethanol and furaneol, in particular, have been associated with caramel-like notes [13]. Further investigations, including sensory analysis of the products, will be necessary to establish whether the observed effects on the chemical composition of the volatile fraction may result in differences in the sensory profile and sensory quality of the biscuits.

As a secondary effect, a reduction in MR-related VOCs associated with dough resting time was observed, confirming a finding already reported in the study on biscuits made from refined flour [14]. In this case, the additional time allowed by the resting step for enzymatic processes promoted by asparaginase addition, as well as for other processes, may have further depleted the pool of MR reactants, ultimately reducing VOC formation during baking. Unlike the previous study on shortbread biscuits made from refined flour [14], this study did not reveal an accumulation effect of lipid oxidation-derived VOCs associated with dough resting time. On the contrary, a reduction associated with dough resting was observed for some of these compounds.

4.4. Colour and Texture

While it is well established that acrylamide formation is generally correlated with biscuit browning [15], it has also been observed that the addition of asparaginase may decouple the reactions leading to acrylamide formation from those responsible for browning, since it does not affect colour development while markedly reducing acrylamide levels [12,23]. These observations were reported for biscuits produced from refined wheat flour. In agreement with these findings, this study did not show consistent effects of asparaginase application on colour characteristics also in wholemeal biscuits, which are generally characterised by a darker colour compared to those produced from refined flour. Minor changes observed at only one addition level may be explained by small inhomogeneities in a dough obtained from wholemeal flour, rather than by an effect of asparaginase addition. Moreover, a slight tendency towards increased browning associated with the application of dough resting time appears to parallel a similar effect previously observed, to a greater extent, in biscuits produced from refined flour [23]. In contrast, very limited information is available regarding the effects of asparaginase treatment on biscuit texture [16]. Overall, similarly to what has been observed in biscuits produced from other cereal flours treated with asparaginase [16], these results confirm the absence of marked effects consistently associated with asparaginase addition. The increase in hardness observed only at the highest asparaginase level did not appear sufficient to establish a clear effect of the enzyme on the biscuit texture characteristics.

5. Conclusions

The presence of acrylamide in food is regulated by Commission Regulation (EU) 2017/2158 [3], which establishes the application of an ALARA risk-reduction approach for this contaminant. The aim is to achieve “as low as reasonably achievable” levels below the benchmark levels set out in the Regulation, through the implementation of all relevant mitigation measures. In addition, regulatory amendments are currently under discussion within the food sector, aiming to lower the benchmark levels for certain food categories, convert some of them into legally binding maximum limits, and expand the range of food categories subject to monitoring and control. Consequently, there is a growing need for more comprehensive and detailed information regarding the applicability of currently available mitigation strategies, among which the use of asparaginase represents one of the most promising approaches for the bakery products category. This study provides evidence on the effectiveness of asparaginase application in reducing acrylamide levels in wholemeal biscuits, despite the limitations of the experimental design employed. In particular, the study did not include process replicates for firmly establishing the effects of the variables under investigation (asparaginase dosage and dough resting time), nor did it examine the changes in the amino acid and sugar composition of the dough induced by these variables. These limitations made it difficult to attribute with certainty some of the observed changes in contaminants and VOCs to the variables under study. Moreover, the

limitations of the approach employed in the semi-quantitative analysis of VOCs, together with the absence of sensory evaluations, do not allow the establishment of the existence of significant effects on the sensory characteristics of the biscuits. However, the experimental data collected suggest the plausibility of such effects and highlight the need for further investigations to clarify these aspects.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16136750/s1>, Table S1: List of determined Volatile Organic Compounds. Table S2: Loadings values for the first two PCs obtained by the PCA of the VOCs of the biscuits dataset. Table S3: Effects of asparaginase addition dose and dough resting time on VOCs levels. Figure S1: Mean acrylamide levels (mean values and standard deviations) as a function of the added asparaginase dose and the application of dough resting time. Figure S2: Mean HMF levels (mean values and standard deviations) as a function of the added asparaginase dose and the application of dough resting time. Figure S3: Bootstrap ellipses obtained from PCA analysis of the VOC dataset determined in the biscuits.

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References

1. van Boekel, M.; Fogliano, V.; Pellegrini, N.; Stanton, C.; Scholz, G.; Lalljie, S.; Somoza, V.; Knorr, D.; Jasti, P.R.; Eisenbrand, G. A Review on the Beneficial Aspects of Food Processing. *Mol. Nutr. Food Res.* **2010**, *54*, 1215–1247. [[CrossRef](#)] [[PubMed](#)]
2. Parker, J.K. Thermal Generation or Aroma. In *Flavour Development, Analysis and Perception in Food and Beverages*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 151–185.
3. Regulation-2017/2158-EN-EUR-Lex. Available online: <https://eur-lex.europa.eu/eli/reg/2017/2158/oj> (accessed on 8 November 2024).
4. Capuano, E.; Fogliano, V. Acrylamide and 5-Hydroxymethylfurfural (HMF): A Review on Metabolism, Toxicity, Occurrence in Food and Mitigation Strategies. *LWT-Food Sci. Technol.* **2011**, *44*, 793–810. [[CrossRef](#)]
5. EFSA. Scientific Opinion on acrylamide in food. *EFSA J.* **2015**, *13*, 4104. [[CrossRef](#)]
6. Schouten, M.A.; Tappi, S.; Rocculi, P.; Romani, S. Mitigation Strategies to Reduce Acrylamide in Cookies: Effect of Formulation. *Food Rev. Int.* **2023**, *39*, 4793–4834. [[CrossRef](#)]

7. Cankaya, S.; Günal, R.; Somuncu, E.İ.; Navruz-Varlı, S. Acrylamide Mitigation in Cereal-Based Foods: A Comprehensive Review of Enzymatic Treatments, Additives and Novel Technologies. *Eur. Food Res. Technol.* **2025**, *251*, 2077–2096. [\[CrossRef\]](#)
8. Capuano, E.; Ferrigno, A.; Acampa, I.; Serpen, A.; Açar, Ö.Ç.; Gökmen, V.; Fogliano, V. Effect of Flour Type on Maillard Reaction and Acrylamide Formation during Toasting of Bread Crisp Model Systems and Mitigation Strategies. *Food Res. Int.* **2009**, *42*, 1295–1302. [\[CrossRef\]](#)
9. Siopa, J.; Ribeiro, M.; Cosme, F.; Nunes, F.M. Survey of Acrylamide in Marketed Biscuits and Industrial Mitigation via Dough Composition and Asparaginase. *J. Food Compos. Anal.* **2025**, *148*, 108375. [\[CrossRef\]](#)
10. Xu, F.; Oruna-Concha, M.-J.; Elmore, J.S. The Use of Asparaginase to Reduce Acrylamide Levels in Cooked Food. *Food Chem.* **2016**, *210*, 163–171. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Hendriksen, H.V.; Kornbrust, B.A.; Østergaard, P.R.; Stringer, M.A. Evaluating the Potential for Enzymatic Acrylamide Mitigation in a Range of Food Products Using an Asparaginase from *Aspergillus Oryzae*. *J. Agric. Food Chem.* **2009**, *57*, 4168–4176. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Anese, M.; Quarta, B.; Frias, J. Modelling the Effect of Asparaginase in Reducing Acrylamide Formation in Biscuits. *Food Chem.* **2011**, *126*, 435–440. [\[CrossRef\]](#)
13. Garvey, E.C.; O'Sullivan, M.G.; Kerry, J.P.; Kilcawley, K.N. Factors Influencing the Sensory Perception of Reformulated Baked Confectionary Products. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 1160–1188. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Masciola, F.; Baiamonte, I.; Marconi, E.; Melloni, S.; Nardo, N.; Narducci, V.; Sanchez Del Pulgar, J.; Turfani, V.; Raffo, A. Effect of Asparaginase Treatment on Biscuit Volatile Compounds. *Appl. Sci.* **2025**, *15*, 3779. [\[CrossRef\]](#)
15. Gökmen, V.; Açar, Ö.Ç.; Arribas-Lorenzo, G.; Morales, F.J. Investigating the Correlation between Acrylamide Content and Browning Ratio of Model Cookies. *J. Food Eng.* **2008**, *87*, 380–385. [\[CrossRef\]](#)
16. Musa, S.; Becker, L.; Oellig, C.; Scherf, K.A. Influence of Asparaginase on Acrylamide Content, Color, and Texture in Oat, Corn, and Rice Cookies. *J. Agric. Food Chem.* **2024**, *72*, 22875–22882. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Mesias, M.; Morales, F.J. Acrylamide- and Hydroxymethylfurfural-Forming Capacity of Alternative Flours in Heated Dough Systems. *Foods* **2025**, *14*, 1597. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Prata, R.; Petrarca, M.H.; Filho, J.T.; Godoy, H.T. Simultaneous Determination of Furfural, 5-Hydroxymethylfurfural and 4-Hydroxy-2,5-Dimethyl-3(2H)-Furanone in Baby Foods Available in The Brazilian Market. *J. Food Compos. Anal.* **2021**, *99*, 103874. [\[CrossRef\]](#)
19. Raffo, A.; Carcea, M.; Castagna, C.; Magrì, A. Improvement of a Headspace Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry Method for the Analysis of Wheat Bread Volatile Compounds. *J. Chromatogr. A* **2015**, *1406*, 266–278. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Pathare, P.B.; Opara, U.L.; Al-Said, F.A.-J. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. *Food Bioprocess Technol.* **2013**, *6*, 36–60. [\[CrossRef\]](#)
21. Gazi, S.; Göncüoğlu Taş, N.; Görgülü, A.; Gökmen, V. Effectiveness of Asparaginase on Reducing Acrylamide Formation in Bakery Products According to Their Dough Type and Properties. *Food Chem.* **2023**, *402*, 134224. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Nguyen, H.T.; Van Der Fels-Klerx, H.J.; Peters, R.J.B.; Van Boekel, M.A.J.S. Acrylamide and 5-Hydroxymethylfurfural Formation during Baking of Biscuits: Part I: Effects of Sugar Type. *Food Chem.* **2016**, *192*, 575–585. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Kukurová, K.; Ciesarová, Z.; Mogol, B.A.; Açar, Ö.Ç.; Gökmen, V. Raising Agents Strongly Influence Acrylamide and HMF Formation in Cookies and Conditions for Asparaginase Activity in Dough. *Eur. Food Res. Technol.* **2013**, *237*, 1–8. [\[CrossRef\]](#)
24. Cho, H.; Park, M.K.; Kim, Y.-S. Study on Volatile Compounds Formed from the Thermal Interaction of Hydrolyzed Vegetable Proteins with Reducing Sugars. *Food Sci. Biotechnol.* **2023**, *32*, 283–298. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Luo, C.; Yin, Q.; Zeng, L.; Zhang, Q.; Wang, B.; Yu, G.; Shen, S.; Xie, W. Insight into Co-Pyrolysis Behavior of Asparagine/Fructose Mixtures Using on-Line Pyrolysis-GC/MS. *J. Anal. Appl. Pyrolysis* **2025**, *186*, 106969. [\[CrossRef\]](#)

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