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Physicochemical and Microbiological Properties of Hazelnuts from Three Varieties Cultivated in Portugal

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Abstract

Hazelnut is an important crop worldwide, and the characteristics of the fruits are quite variable according to a number of factors, including variety and cultivation conditions, which in turn can vary according to harvest year. This study aimed to investigate some physical and chemical characteristics of three hazelnut varieties grown in Portugal (Grada de Viseu, Tonda di Giffoni and Butler) along two different harvesting years (2021 and 2022). Also, the microbial quality was investigated for its relevance to the conservation of the fruits. The physical properties evaluated were biometric characteristics and colour, the chemical components analysed were moisture, lipids, protein, ash and fibre, and the microbial properties investigated were the microorganisms, moulds and yeasts. The results showed that, generically, statistically significant differences were found between the three varieties under study on several properties investigated. The kernel was confirmed as the lighter part of all hazelnuts, and when comparing between varieties, Tonda di Giffoni presented the lighter fruits in both harvesting years. With respect to weight, the Tonda di Giffoni variety was the lightest in both harvest years. Moisture content was observed to be higher than the recommended limits for two of the samples (Grada de Viseu in 2021: 6.01 ± 0.26 g/100 g and Butler in 2022: 6.02 ± 0.37 g/100 g), although the difference was marginal given that the recommended limit is 6%. Not surprisingly, lipids were the major chemical component, ranging from 66.46 ± 1.67 to 70.14 ± 1.75 g/100 g in 2021 and from 64.38 ± 1.67 to 77.77 g/100 g in 2022. It was further observed that the three varieties presented a satisfactory microbiological quality. Finally, applying factor analysis with principal components and Varimax rotation, a solution that explains 92.8% of the variance was obtained. This study provided information that is relevant for the characterisation and evaluation of variability according to the year of hazelnuts of three varieties cultivated in Portugal.

Keywords: *Corylus avellana* L.; physicochemical properties; microbiological quality; harvesting year

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1. Introduction

The hazelnut (*Corylus avellana* L.) is the fruit of the hazel tree, and it is considered a good source of different macro- and micronutrients associated with different health benefits [1]. Globally, in 2023, hazelnut production was approximately 1,090,583 tonnes, with Turkey leading the world in production. In the same year, Portugal's hazelnut production was equal to 240 tonnes, with a harvested area of 410 ha [2]. Regarding their chemical properties, hazelnuts contain 50–73% lipids, 10–22% carbohydrates, 10–24% protein, and an ash content varying from 2.4 to 2.8%. Furthermore, this dry fruit is also rich in different phytochemicals and fatty acids, mainly monounsaturated fatty acids [3]. In addition, hazelnut oil is a good source of vitamin E, mainly in the α-tocopherol form. Potassium and β-sitosterol are the major minerals and phytosterols, respectively, that are present in hazelnuts [4]. All around the world, there are different hazelnut varieties, and their characteristics are affected by numerous factors such as the geographical location, agricultural techniques, year of harvest, and climatic conditions, among others [5]. For example, in the study performed by Ferrão et al. [6], where the properties of the Grada de Viseu variety were compared through different harvesting years and production locations, it was found that a higher average annual temperature and a lower average annual precipitation resulted in smaller fruits.

Moreover, hazelnut's physical properties are also of utmost importance since they are important parameters for the industry and influence consumer food choices. In the case of the food industry, physical properties of the agricultural products influence different processes and operations, such as classification, drying, packaging, transportation and storage [7]. Hazelnut colour and biometric characteristics are two of the most important physical characteristics of this dried fruit. The knowledge of the biometric characteristics allows for the differentiation of hazelnut varieties and also the designing of processing equipment, as well as the ability to decide on hazelnut utilisation (fresh consumption or to industry) [4,8,9]. According to Romero-Aroca et al. [10], hazelnut kernel size is the main quality characteristic for the consumption market. Colour also affects hazelnut quality and therefore consumer acceptance for this product, with a preference for hazelnut kernels with a clear and uniform colour [11,12].

Due to hazelnuts' lipid content, during storage, besides lipid rancidity, moulds, such as, for example, *Aspergillus*, *Penicillium*, *Cladosporium*, and *Phomopsis* spp., can also grow [13]. In order to guarantee safety and quality, it is of utmost importance to control these aspects. Moreover, mould contamination is a serious food safety problem because it can produce mycotoxins or even allergens that are a risk for human health [14]. Human acute and chronic exposure to mycotoxins is associated with organ damage, immunity disorders and carcinogenesis [15].

To ensure that hazelnut quality is preserved throughout shelf-life, it is crucial to know their chemical and physical characteristics. However, in the literature, there are different studies about the hazelnuts cultivated in Turkey [16–18] and Italy [19–22]. Moreover, studies on the physicochemical properties and microbiological quality of the most cultivated hazelnuts in Portugal are still scarce. In a survey performed by Ferrão et al. [23], it was found that the main hazelnut varieties cultivated by the Portuguese producers were Grada de Viseu, Segorbe, Fertile de Coutard, Butler and Negreta. This study aims to perform a multi-parameter quality assessment of Portugal's principal hazelnut cultivars over two consecutive harvest seasons in order to disentangle the relative contributions of cultivar and year to quality traits.

2. Materials and Methods

The hazelnut samples used in this work were from three varieties, Grada de Viseu, Tonda di Giffoni and Butler, harvested in two different agricultural years (2021 and 2022) and cultivated in the same orchard situated in Viseu, in the centre of Portugal. The nuts were harvested at their maturity state and then sun-dried until the moisture content was less than 6%. After that, the whole fruit was stored refrigerated at 5 °C with a relative humidity of 61.3% until the experiment began in order to guarantee the safety and quality of this dried fruit. The hazelnuts were supplied from each producer on a bulk lot, meaning that they collected the hazelnuts from the whole field. Lately, a random sample has been taken from the whole set of trees from the same orchard. From each lot, a set of random hazelnuts was taken and ground, and from that mixture the weight necessary for each of the analyses in the study was then extracted.

2.1. Biometric Analysis

Biometric analysis was performed using a calliper rule (Digital Calliper, Model CD-15APX, from Mitutoyo Corporation, Kanagawa, Japan) with a precision scale, and it was performed on 50 hazelnuts from each variety. Different biometric parameters were accessed for the whole hazelnut and also the kernel, more specifically length (distance between centres), width (wider equatorial zone) and thickness (narrow equatorial zone perpendicular to the latter). With these values, it was possible to calculate two more biometric parameters:

$$\text{Shape index} = \frac{(\text{width} + \text{thickness})}{2 \times \text{length}} \tag{1}$$

$$Compression\ ratio = \frac{\text{width}}{\text{thickness}} \tag{2}$$

Furthermore, the kernel percentage was also calculated as follows [24]:

$$\text{Kernel percentage} = \frac{\text{Kernel weight}}{\text{Nut weight}} \times 100 \tag{3}$$

2.2. Colour Analysis

Colour was measured using a colourimeter Konica Minolta CR-400, which directly read the Cartesian coordinates L*, a* and b*. The L* coordinate ranges from 0 (black) to 100 (white) and represents the lightness. The a* and b* coordinates vary between −60 and +60 and represent chromaticity coordinates. Regarding the a* coordinate, it varies from red (+a) to green (+a), while the b* coordinate goes from yellow (+b) to blue (−b) [25–27]. Thirty nuts were used from each sample, and two measurements were performed on the hazelnuts' brown shell, two on the hilum, two on the skin and two on the kernel.

2.3. Chemical Analysis

To perform the chemical analysis, we used only the kernels of each hazelnut sample. These were milled and homogenised before the experimental procedures were performed. Standard methods of the Association of Official Analytical Chemists were followed to assess the moisture, ash, fibre, protein (%N × 5.30) and lipid contents [28]. Water activity was measured at a constant temperature, 25 °C, using a Hygroscope (from Rotronic, Bassersdorf, Switzerland). All reagents were of analytical grade (Nitric acid 65% from ChemLab Analytical, Zedelgem, Belgium, Petroleum ether from Fisher Chemical Waltham, Massachusetts, USA, sodium hydroxide 99% purity from Absolve Industries Private Limited, Nagpur, Maharashtra, India, sulfuric acid 95–97% p.a. from ChemLab Analytical, acetone ≥ 99.8% purity from Fisher Chemical, copper (II) sulphate 5-hydrate from Panreac Química SA, Castellar del Vallès, Barcelona, Spain, boric acid ≥ 99.8% purity from Fisher Chemical and hydrochloric acid 37% from Riedel de Häen, Seelze, Germany).

2.4. Microbiological Analysis

To perform the microbiological analysis, 10 g of each sample were introduced into a sterile stomacher bag, to which 90 mL of peptone water was added and then homogenised in a Stomacher 400 (Seward, Worthing, West Sussex, UK) for 2 min. Then, the homogenates (dilution 10^{-1}) were subjected to successive decimal dilutions with peptone water, up to the dilution of 10^{-4}. For the determination of the microorganisms at 30 °C the procedure described in the ISO 4833-2:2013 [29] was followed. For that, the surface scatter sowing method was used and 1 mL of each dilution was pipetted onto the surface of Plate Count Agar (PCA, PanReac Applichem ITW Reagents, Castellar del Vallès, Barcelona, Spain). The determination of moulds and yeasts was performed by the plate count technique, using the culture medium Dichloran Glycerol Agar (DG-18, Biokar diagnostics, Allonne, France), and again, 1 mL of each dilution was pipetted onto the surface of DG-18. Afterwards, the PCA and DG-18 agar plates were incubated at 30 °C for 48 h and at 25 °C for 5 days. Two plates were inoculated per dilution. After the incubation period, the colony count was performed according to ISO 7218:2007, and the results were expressed as log CFU/g [30].

This procedure was performed in triplicate for each hazelnut variety, and only the kernel with the skin was used.

2.5. Statistical Analysis

The samples under study were compared for all properties measured using the statistical test of one-way analysis of variance (ANOVA), complemented with the Tukey post hoc test. The level of significance considered for statistical analysis was 5% ($p < 0.05$), and the software used for the statistical analysis was SPSS version 28.

To determine if there was a grouping structure between the items, exploratory factor analysis (FA) was applied using the method of Principal Component Analysis (PCA). After verification of the adequacy of the data, FA was applied with extraction using PCA and using Varimax rotation with Kaiser Normalisation. The Eigenvalues greater than one were used to determine the number of components [31]. Factor loadings with an absolute value lower than 0.4 were excluded [32,33]. Regarding the reference values for alpha, it is dependent on the authors, but in general, values over 0.7 are desirable, with values over 0.8 being considered very good. Nevertheless, according to some authors, values over 0.5 could be acceptable [34–36].

3. Results

3.1. Biometric Parameters

As can be observed in Table 1, Grada de Viseu presented the heaviest nuts for both agricultural years (3.90 ± 0.92 g for 2021 and 3.69 ± 0.74 g for 2022), while Tonda di Giffoni had the lightest ones, again for both agricultural years (2.94 ± 0.39 g for 2021 and 3.21 ± 0.56 g for 2022). The results of the ANOVA test showed that the differences encountered between the varieties were statistically significant, with Tonda di Giffoni being significantly different from the other hazelnut varieties in both years. However, no statistically significant differences were found in the same variety according to the year of harvest, and this tendency was also observed for the kernel weight. In 2021, kernel weight varied from 1.26 ± 0.22 g (Tonda di Giffoni) to 1.70 ± 0.20 g (Butler), and in 2022 it varied between 1.46 ± 0.23 g (Tonda di Giffoni), 1.60 ± 0.27 g (Grada de Viseu) and 1.60 ± 0.37 g (Butler).

Regarding the kernel percentage (Table 1), the variety with the highest value was Butler (44.81 ± 6.08%) in the agricultural year of 2021, whereas Tonda di Giffoni presented the highest kernel percentage in 2022 (46.88 ± 11.76%), but in this case, there were no significant differences between the varieties under study (2021: $p = 0.629$, 2022: $p = 0.719$).

The results in Table 2 show that the hazelnuts of the Butler variety had a longer length in the two agricultural years (17.44 ± 0.89 cm in 2021 and 17.26 ± 1.47 cm in 2022) and a wider width in 2022 (17.84 ± 28.43 cm). In 2021, the sample of Grada de Viseu-2021 presented the largest width (14.87 ± 1.55 cm) and thickness (13.11 ± 1.83 cm), while in 2022, the largest value was for Tonda di Giffoni-2022 (13.41 ± 1.43 cm). Regarding the shape ratio, the hazelnuts of the Tonda di Giffoni variety harvested in 2021 and 2022 are the ones with the highest value (1.02 ± 0.10 and 0.98 ± 0.10, respectively). The same tendency was observed for the compression ratio, and again, the Tonda di Giffoni variety presented the highest value for both agricultural years (1.02 ± 0.10 in 2021 and 0.98 ± 0.10 in 2022). Furthermore, some statistically significant differences between the varieties were found in 2021 and 2022.

Table 1. Weight and kernel percentage (mean ± standard deviation).

Sample	Nut Weight [1] (g)	Kernel Weight [1] (g)	Kernel Percentage [1] (%)
Grada de Viseu-2021	3.90 ± 0.92 ^{bA}	1.62 ± 0.28 ^{bA}	44.11 ± 14.44 ^{aA}
Tonda di Giffoni-2021	2.94 ± 0.39 ^{aA}	1.26 ± 0.22 ^{aA}	42.98 ± 5.09 ^{aA}
Butler-2021	3.81 ± 0.35 ^{bA}	1.70 ± 0.20 ^{bA}	44.81 ± 6.08 ^{aA}
p-value	<0.0005	<0.0005	0.629
Grada de Viseu-2022	3.69 ± 0.74 ^{bB}	1.60 ± 0.27 ^{abA}	45.48 ± 15.88 ^{aA}
Tonda de Giffoni-2022	3.21 ± 0.56 ^{aA}	1.46 ± 0.23 ^{aA}	46.88 ± 11.76 ^{aB}
Butler-2022	3.68 ± 0.71 ^{bB}	1.60 ± 0.37 ^{abB}	44.70 ± 12.80 ^{aB}
p-value	<0.0005	<0.0005	0.719

[1] To compare among varieties for one year, mean values in the same column with different lower letters are statistically different ($p < 0.05$). To compare the same variety through different years, mean values in the same column with different upper letters are statistically different ($p < 0.05$).

Table 2. Biometric characterisation of the samples under study (mean ± standard deviation).

Sample	Length [1] (cm)	Width [1] (cm)	Thickness [1] (cm)	Shape Ratio [1]	Compression Ratio [1]
Grada de Viseu-2021	15.88 ± 1.12 ^{cA}	14.87 ± 1.55 ^{bA}	13.11 ± 1.83 ^{aA}	0.88 ± 0.11 ^{aA}	0.88 ± 0.11 ^{abA}
Tonda di Giffoni-2021	13.23 ± 1.02 ^{aA}	13.87 ± 1.45 ^{aA}	12.95 ± 1.42 ^{aA}	1.02 ± 0.10 ^{cA}	1.02 ± 0.10 ^{bA}
Butler-2021	17.44 ± 0.89 ^{dA}	14.29 ± 1.22 ^{abA}	12.53 ± 1.11 ^{aA}	0.77 ± 0.06 ^{aA}	0.77 ± 0.06 ^{aA}
p-value	<0.0005	<0.0002	0.137	<0.0005	0.002
Grada de Viseu-2022	15.77 ± 1.27 ^{bA}	14.75 ± 1.20 ^{aA}	13.25 ± 1.29 ^{bA}	0.89 ± 0.08 ^{aA}	0.89 ± 0.08 ^{aB}
Tonda di Giffoni-2022	14.24 ± 1.15 ^{aA}	14.50 ± 1.35 ^{aA}	13.41 ± 1.43 ^{bA}	0.98 ± 0.10 ^{aA}	0.98 ± 0.10 ^{aA}
Butler-2022	17.26 ± 1.47 ^{cB}	17.84 ± 28.43 ^{aA}	11.25 ± 1.75 ^{aB}	0.84 ± 0.79 ^{aA}	0.84 ± 0.79 ^{aA}
p-value	<0.0005	0.530	<0.0005	0.277	0.109

[1] To compare among varieties for one year, mean values in the same column with different lower letters are statistically different ($p < 0.05$). To compare the same variety through different years, mean values in the same column with different upper letters are statistically different ($p < 0.05$).

When the results were analysed according to the year of harvest, some statistically significant differences were also found. More specifically, in 2021, there were no statistically significant differences, except in the case of thickness ($p = 0.137$). On the other hand, in the harvest year of 2022, there were statistically significant differences in the cases of length and thickness. Furthermore, the length of Butler hazelnuts harvested in 2021 was statistically different from the nuts of that variety harvested in 2022 ($p = 0.002$), with the hazelnuts of 2021 being longer than those of 2022. In the case of thickness, the differences encountered were also for the Butler variety ($p = 0.03$), with the hazelnuts harvested in 2022 being thinner than those harvested in 2021.

3.2. Colour

Table 3 shows the colour coordinates for the shell, the hilum, the skin and the kernel of the hazelnuts. Regarding lightness, in both years, the nuts of the Grada de Viseu variety were found to be lighter when compared to the other varieties. In fact, Grada de Viseu was statistically different from the other hazelnut varieties for both agricultural years.

Moreover, Grada de Viseu also revealed the lowest values for the coordinates a* and b*. The results of the ANOVA test showed that the differences encountered between the samples were statistically significant ($p < 0.0005$) for the three coordinates of the shell. As for the year of harvest, it was found that for all of the colour coordinates of the shell, the values of the Grada de Viseu variety increased from 2021 to 2022, while the values of the other two varieties decreased. In the case of the a* coordinate, in 2021, the three samples were statistically different from each other, with the same trend observed for coordinate b* in 2022.

Table 3. Colour coordinates in the different parts of the hazelnuts (mean ± standard deviation).

Hazelnut Part	Sample	L*	a*	b*
Shell	Grada de Viseu-2021	36.45 ± 2.41^{aA}	11.63 ± 2.99^{aA}	10.40 ± 1.60^{aA}
	Tonda di Giffoni-2021	49.67 ± 2.92^{bA}	15.93 ± 2.07^{bA}	26.83 ± 3.52^{bA}
	Butler-2021	49.39 ± 2.52^{bA}	17.97 ± 2.28^{cA}	27.55 ± 2.93^{bA}
	p-value	<0.0005	<0.0005	<0.0005
	Grada de Viseu-2022	37.84 ± 1.92^{aA}	14.55 ± 2.03^{aB}	11.34 ± 1.88^{aA}
	Tonda di Giffoni-2022	43.09 ± 3.26^{bA}	15.40 ± 2.31^{abA}	14.80 ± 2.44^{bB}
	Butler-2022	43.23 ± 3.61^{bB}	16.64 ± 4.42^{bA}	16.34 ± 1.97^{cB}
	p-value	<0.0005	<0.0005	<0.0005
Hilum	Grada de Viseu-2021	38.11 ± 3.82^{aA}	9.00 ± 1.01^{bA}	12.69 ± 1.43^{aA}
	Tonda di Giffoni -2021	55.31 ± 5.84^{eA}	8.38 ± 1.64^{aA}	21.42 ± 2.69^{bA}
	Butler-2021	47.27 ± 2.54^{bA}	12.25 ± 1.41^{cA}	22.89 ± 1.99^{cA}
	p-value	<0.0005	<0.0005	<0.0005
	Grada de Viseu-2022	46.50 ± 3.26^{bA}	10.75 ± 0.79^{bA}	14.67 ± 1.02^{cB}
	Tonda di Giffoni-2022	51.54 ± 4.47^{cB}	9.92 ± 1.25^{aA}	14.22 ± 0.99^{abB}
	Butler-2022	43.76 ± 4.19^{aA}	11.58 ± 1.49^{cA}	14.13 ± 1.41^{aB}
	p-value	<0.0005	<0.0005	0.025
Skin	Grada de Viseu-2021	36.11 ± 3.30^{aA}	13.92 ± 1.91^{aA}	13.37 ± 1.49^{aA}
	Tonda-2021	50.61 ± 5.98^{bA}	15.35 ± 1.97^{bA}	25.69 ± 2.68^{bA}
	Butler-2021	51.14 ± 2.68^{bA}	18.78 ± 1.67^{cA}	28.61 ± 1.62^{eA}
	p-value	<0.0005	<0.0005	<0.0005
	Grada de Viseu-2022	35.43 ± 3.69^{aA}	14.72 ± 1.91^{bA}	14.69 ± 1.02^{aB}
	Tonda di Giffoni-2022	42.10 ± 4.77^{bA}	13.86 ± 1.38^{aB}	14.21 ± 1.39^{aB}
	Butler-2022	42.60 ± 7.55^{bB}	15.44 ± 1.38^{cB}	15.55 ± 2.13^{cA}
	p-value	<0.0005	<0.0005	<0.0005
Kernel	Grada de Viseu-2021	69.64 ± 4.64^{aA}	1.78 ± 0.45^{bA}	18.43 ± 1.66^{aA}
	Tonda di Giffoni-2021	78.86 ± 3.14^{bA}	1.59 ± 0.32^{aA}	24.88 ± 2.83^{bA}
	Butler-2021	76.70 ± 8.85^{bA}	1.62 ± 0.33^{aA}	25.00 ± 2.50^{bA}
	p-value	<0.0005	<0.0005	<0.0005
	Grada de Viseu-2022	72.64 ± 4.18^{bA}	4.50 ± 0.58^{bA}	17.11 ± 2.53^{aB}
	Tonda di Giffoni-2022	69.81 ± 4.87^{aB}	4.74 ± 0.67^{bB}	16.24 ± 1.36^{aB}
	Butler-2022	77.73 ± 4.57^{cA}	2.42 ± 0.88^{aB}	26.99 ± 2.51^{bA}
	p-value	<0.0005	0.013	<0.0005

To compare among varieties for one year, mean values in the same column with different lower letters are statistically different ($p < 0.05$). To compare the same variety through different years, mean values in the same column with different upper letters are statistically different ($p < 0.05$).

As can be observed in Table 3, in general, the hilum was revealed to had higher values for coordinate L*, when compared to the rest of the shell, but lower values for coordinates a* and b*, meaning that this hazelnut part is lighter and less yellow and red than the rest of the shell. Furthermore, Tonda di Giffoni was the variety with the highest value of L* in both years (55.31 ± 5.84 in 2021 and 54.54 ± 4.47 in 2022), with this trend also observed for coordinate a* (12.25 ± 1.41 in 2021 and 11.58 ± 1.49 in 2022). Regarding the b* coordinate, except for the Grada de Viseu variety, the hazelnuts harvested in 2021 were more yellow than those harvested in 2022. Again, statistically significant differences were found for the three colour coordinates of the hilum. Moreover, statistically significant differences were also found for the values of L* in the hazelnuts of the Tonda di Giffoni variety in the years of 2021 and 2022. In the case of the b* coordinate, the differences encountered were for the three varieties according to the year of harvest.

According to the results shown in Table 3 for the colour coordinates in the skin, it was found that the Butler variety presented the highest values of L*, a* and b* in both agricultural years, meaning that the film of that variety was lighter, and had a more intense red and yellow tone when compared to the other varieties. Again, statistically significant differences were found between the samples for the three coordinates. When the results are analysed according to the same variety in different harvest years, there were also found to be some statistically significant differences.

The results for colour coordinates of hazelnuts' kernels presented in Table 3 show that the kernels were lighter, with a red colour practically absent and a yellow colour still present when compared to the other parts of the hazelnuts (Tables 3–5). For the fruits harvested in 2021, the Grada de Viseu variety was the one with the lowest value of L* (69.64 ± 4.64), meaning that it had a lighter kernel than the other varieties, whereas in 2022, the variety with the lowest value of L* was Tonda di Giffoni (69.81 ± 4.87). Furthermore, for the L* coordinate, in 2021, Grada de Viseu was statistically different from the other varieties, while in 2022, the three varieties were statistically different from each other.

Table 4. Chemical properties of the samples under study (determined on the kernel, mean ± standard deviation).

Sample	Moisture [1] (g/100 g)	Water Activity [1]	Fat [1] (g/100 g)	Ash [1] (g/100 g)	Fibre [1] (g/100 g)	Protein [1] (g/100 g)
Grada de Viseu-2021	6.01 ± 0.26 ^{bA}	0.56 ± 0.01 ^{aA}	67.82 ± 1.68 ^{aA}	2.73 ± 0.08 ^{bA}	6.35 ± 0.25 ^{bA}	17.98 ± 0.34 ^{cA}
Tonda di Giffoni-2021	4.78 ± 0.40 ^{aA}	0.54 ± 0.01 ^{aA}	66.46 ± 5.33 ^{aA}	2.28 ± 0.04 ^{aA}	5.70 ± 0.27 ^{abA}	12.50 ± 0.36 ^{aA}
Butler-2021	5.79 ± 0.13 ^{bA}	0.55 ± 0.01 ^{aA}	70.14 ± 1.75 ^{aA}	2.34 ± 0.01 ^{aA}	5.15 ± 0.28 ^{aA}	15.31 ± 0.15 ^{bA}
p-value	<0.0005	0.090	0.452	<0.0005	<0.0005	<0.0005
Grada de Viseu-2022	5.65 ± 0.27 ^{bA}	0.56 ± 0.01 ^{aA}	64.38 ± 1.67 ^{aA}	1.69 ± 0.16 ^{aA}	6.12 ± 0.03 ^{bcB}	22.84 ± 0.18 ^{dA}
Tonda di Giffoni-2022	4.86 ± 0.33 ^{aA}	0.54 ± 0.01 ^{aA}	78.18 ± 1.71 ^{bA}	2.22 ± 0.20 ^{bB}	5.92 ± 0.28 ^{bA}	16.11 ± 0.55 ^{bA}
Butler-2022	6.02 ± 0.37 ^{bA}	0.55 ± 0.01 ^{aA}	77.77 ± 0.59 ^{bA}	2.31 ± 0.18 ^{bA}	5.37 ± 0.42 ^{abA}	18.03 ± 0.24 ^{cA}
p-value	0.013	0.090	<0.0005	0.012	0.045	<0.0005

[1] To compare among varieties for one year, mean values in the same column with different lower letters are statistically different ($p < 0.05$). To compare the same variety through different years, mean values in the same column with different upper letters are statistically different ($p < 0.05$).

When the results are analysed according to the same variety harvested in different years, it was observed that for the L* coordinate, there were only significant differences between the hazelnuts of the Tonda di Giffoni variety harvested in 2021 and those harvested in 2022. In the case of the values of coordinate a*, significant differences were found between the Tonda di Giffoni and Butler varieties harvested in 2021 and those harvested in 2022. For the b* coordinate, the differences encountered were for the Grada de Viseu and Tonda di Giffoni varieties harvested in 2021 and 2022. It is important to highlight that the values of the a* coordinate are higher in 2022 when compared to the ones in 2021, meaning that the kernels of the hazelnuts harvested in 2022 had a more red tone than the ones harvested in 2021.

Table 5. Mean count (log CFU/g ± standard deviation) of total microorganisms at 30 °C and moulds and yeasts at 25 °C of the samples under study.

Sample	Microorganisms at 30 °C [1]	Moulds and Yeast [1]
Grada de Viseu-2021	2.86 ± 0.03 ^{cA}	2.42 ± 0.07 ^{bA}
Tonda di Giffoni-2021	2.62 ± 0.03 ^{bA}	2.37 ± 0.06 ^{bA}
Butler-2021	2.28 ± 0.07 ^{aA}	1.67 ± 0.13 ^{aA}
p-value	<0.0005	<0.0005
Grada de Viseu-2022	2.84 ± 0.03 ^{bA}	2.40 ± 0.07 ^{aA}
Tonda di Giffoni-2022	2.61 ± 0.03 ^{aA}	2.46 ± 0.04 ^{aA}
Butler-2022	2.90 ± 0.01 ^{cB}	2.47 ± 0.03 ^{aB}
p-value	<0.0005	0.059

[1] To compare among varieties for one year, mean values in the same column with different lower letters are statistically different ($p < 0.05$). To compare the same variety through different years, mean values in the same column with different upper letters are statistically different ($p < 0.05$).

3.3. Chemical Properties

As can be seen in Table 4, in 2021, the variety with the highest value of moisture was Grada de Viseu (6.01 ± 0.26 g/100 g), whereas in 2022, it was Butler (6.02 ± 0.37 g/100 g). The ANOVA test showed statistically significant differences between the varieties, both in 2021 and 2022; the nuts of the Tonda di Giffoni variety were significantly different from the fruits of the other varieties.

As expected, lipids were the major chemical component for the three varieties. For the hazelnuts harvested in 2021, the Butler variety was the one that presented a higher lipid content (70.14 ± 1.75 g/100 g). Again, significant differences were found between the varieties, but only in 2022, with the Grada de Viseu variety significantly different from the other two varieties. Furthermore, there were no significant differences in the varieties according to the year of harvest.

For the nuts collected in 2021, the Grada de Viseu variety presented the highest values of ash (2.73 ± 0.08 g/100 g), fibre (6.35 ± 0.25 g/100 g) and protein (17.98 ± 0.34 g/100 g). As for the hazelnuts harvested in 2022, Butler was the variety with the highest content of ash (2.31 ± 0.18 g/100 g), while Grada de Viseu was again the variety with the highest values of fibre (6.12 ± 0.03 g/100 g) and protein (22.84 ± 0.18 g/100 g). For these three chemical properties, statistically significant differences were found among the varieties under study. When the results were analysed according to the year of harvest, some significant differences were also found between the nuts harvested in 2021 and 2022.

3.4. Microbiology

The results presented in Table 5 showed that in 2021, the Grada de Viseu variety had the highest values for the microorganisms at 30 °C (2.86 ± 0.03 log CFU/g) and also for the moulds and yeast at 25 °C (2.42 ± 0.07 log CFU/g), while in 2022, the variety with the highest values for both analyses was Butler (2.90 ± 0.01 log CFU/g for microorganisms at 30 °C and 2.47 ± 0.03 for moulds and yeasts at 25 °C). These results may be explained by the fact that in 2021, Grada de Viseu was the variety with the highest moisture content, while in 2022, it was Butler that presented the highest moisture content, as can be seen in Table 5. The ANOVA test showed that the differences encountered between the varieties were statistically significant ($p < 0.05$) with the exception of the values of moulds and yeasts for the hazelnuts harvested in 2022 ($p = 0.059$). When the results were analysed according to the same variety harvested in different years, it was observed that there were only significant differences for the Butler variety in both analyses.

3.5. Factor Analysis

The correlation matrix has a large number of values greater than 0.5, which implies that there are correlations between the variables, which is a basic condition for using AF. The solution obtained by FA with PCA and Varimax rotation showed that the percentages of variance explained (VE) by nine factors were, F1—24.35%, F2—19.67%, F3—13.12%, F3—6.92%, F5—6.65%, F6—6.36%, F7—5.93%, F9—4.06%, resulting in a total variance explained of 92.82%, which is very relevant (Table 6).

Table 6. Solution obtained through factor analysis.

Factor	%VE [1]	Items	Loadings
F1	24.35	a* value of the hilum	0.515
		Nut width	0.731
		Nut thickness	0.865
		Nut weight	0.842
		Kernel width	0.860
		Kernel thickness	0.908
		Kernel weight	0.898
		Kernel volume	0.917
		Hilum length	0.763
		Hilum width	0.831
F2	19.67	L* value of the shell	0.577
		b* of the shell	0.774
		b* of the hilum	0.682
		L* of the skin	0.839
		a* of the skin	0.842
		b* of the skin	0.933
		Microorganisms at 30 °C	0.901
		Moulds and yeasts	0.869
		Protein	0.571
		Fibre	0.655
F3	13.12	Nut length	0.823
		Nut shape ratio	0.811
		Kernel length	0.853
		Kernel shape ratio	0.828
		Moisture	0.809
F4	6.92	L* of the kernel	0.845
		Fat	0.836
F5	6.65	L* of the hilum	0.768
		Shell thickness	0.810
		Nut volume	0.688
F6	6.36	a* of the kernel	0.719
		b* of the kernel	0.527
		Ash	0.760
F7	5.93	Nut compression ratio	0.893
		Water activity	0.517
F8	5.77	a* of the shell	0.615
		Kernel percentage	0.856
F9	4.06	Kernel compression ratio	0.853

[1] VE = Variance explained.

As can be observed in Table 6, in general, the item loadings for all factors were high (varying from 0.515 to 0.917 for F1;varying from 0.571 to 0.933 for F2; varying from 0.809 to

0.853 for F3; varying from 0.836 to 0.845 for F4; varying from 0.688 to 0.810 for F5; varying from 0.527 to 0.760 for F6; varying from 0.517 to 0.893 for F7; varying from 0.615 to 0.856 for F8 and with a loading equal to 0.853 for F9). High loadings are indicative of the high contribution of the items to the definition of the factors. Items with the highest loadings are item "b* of the skin" (loading of 0.933 into factor F2), followed by the item "Kernel volume" (loading of 0.917 into factor F1), meaning that these items are most strongly associated with the respective factors.

4. Discussion

Fruit size is an important factor for consumers' food choices, and for predicting the dimensions of the machines in the industry. For example, varieties with a medium calibre are more suitable for table consumption [37]. The results of the statistical analysis revealed significant differences between the dimensions of the hazelnuts, with Tonda di Giffoni being different from the other two varieties under study, regardless of the harvesting year. On the other hand, for each of the varieties, the biometric characteristics did not vary according to year. In the study of Lopes et al. [27], it was found that nut weight for the Butler, Grada de Viseu and Tonda di Giffoni varieties was equal to 3.68 ± 0.41 g, 3.67 ± 0.45 g and 3.16 ± 0.48 g, respectively. In the same study, kernel weight was equal to 1.65 ± 0.26 g (Butler), 1.47 ± 0.23 g (Grada de Viseu) and 1.39 ± 0.26 g (Tonda de Giffoni). These values were comparable to the ones found in the present study.

The information on hazelnuts' biometric characteristics is of the utmost importance for all the players in the hazelnut sector, since they can evaluate the fruit quality and therefore take more profits from the market [8]. Kernel percentage provides information about hazelnuts' edible part and, therefore, is an important parameter. Regarding the kernel percentage (Table 1), the variety with the highest value was Butler in the harvest year of 2021, whereas Tonda di Giffoni presented the highest kernel percentage in 2022. However, these results were not statistically different. In the work performed by Ferrão et al. [9], seven hazelnut varieties cultivated in Portugal were examined, and it was found that kernel percentage ranged from 44.14 ± 6.24%. to 67.68 ± 10.33%. In this work, the kernel percentage varied from 42.98 ± 5.09% to 46.88 ± 11.76%, which is a narrower range. The study performed by Pacchiarelli et al. [38] found similar results for the Tonda di Giffoni variety regarding the nut and kernel weight, particularly in the harvesting year of 2022. As referred to by Valentini et al. [39], if there is a lack of water during nut growth, the nuts will be smaller, whereas if this happens during kernel growth, it will result in poorly filled nuts and wrinkled kernels.

With respect to the dimensions of the evaluated fruits, the hazelnuts of the Butler variety tend to be bigger in both years. On the other hand, hazelnuts of the Tonda di Giffoni variety presented the highest shape ratio, meaning that the nuts of that variety were more rounded in the equatorial zone when compared to the nuts of the other varieties. A similar trend was observed for the compression ratio. According to Lopes et al. [27], values of the compression ratio close to one are associated with hazelnuts that are more rounded in the equatorial zone. In the industry, hazelnuts with a round shape are preferred because they are easier to work with than long or large-shaped hazelnuts [13].

One of the primary quality attributes evaluated by consumers when purchasing food products is the visual appearance [40]. Each type of food typically falls within a specific acceptable colour range, which can be influenced by various factors, including consumer variability, age, ethnic background, and the physical context in which the evaluation occurs [41]. Consumers generally prefer foods exhibiting a higher chroma and more vivid colouration [42]. Moreover, colour is influenced by the chemical, biochemical, microbial and physical changes which occur during growth, maturation, postharvest handling and processing [41]. Since colour affects consumers' food choice and preferences, understanding consumer preferences related to food colour is essential for effective marketing within the food industry. In the present study, colour was measured in different parts of the fruits (shell, hilum, skin and kernel). For the shell, L* varied between 36.45 ± 2.41 (Grada de Viseu-2021) and 49.67 ± 2.92 (Tonda di Giffoni-2021), a* ranged from 11.63 ± 2.99 (Grada de Viseu-2021) to 17.97 ± 2.28 (Butler-2021) and b* ranged from 10.40 ± 1.60 (Grada de Viseu-2021) to 27.55 ± 2.93 (Butler-2021). In the study by Ferrão et al. [6], where only the Grada de Viseu variety was studied from different harvest years and also from different geographical locations of production, it was observed that, for the shell, the L* coordinate ranged from 42.49 ± 1.80 to 44.63 ± 1.51, a* ranged from 16.65 ± 2.59 to 20.33 ± 2.04 and b* varied between 20.66 ± 2.65 and 27.50 ± 3.78, which are higher values than the ones obtained in this study.

The results for colour coordinates of hazelnuts' kernels presented in Table 3 show that the kernels were lighter, with L* ranging between 69.64 ± 4.64 (Grada de Viseu-2021) and 78.86 ± 3.14 (Tonda di Giffoni-2021), with a red colour practically absent (less than 2% in 2021 and less than 5% in 2021) and a yellow colour that is still present when compared to the rest parts of hazelnuts (Tables 3–5). Similar results were found in previous studies [6,27].

The differences encountered in hazelnuts' colour are aligned with the results of previous scientific research, where it was also described that hazelnuts' quality and physico-chemical characteristics are dependent not only on the genotype but also on the climatic conditions, among other factors [43]. According to previous works, temperature plays a vital role in the growth and development of hazelnuts, influencing various physiological processes through either average temperatures or extreme temperatures. Additionally, hazelnut trees have chilling requirements that can impact critical stages such as floral differentiation, blooming, and fruit nut setting [44–46].

Hazelnuts' chemical composition serves as a key quality parameter, influencing processing conditions such as roasting temperature and duration, as well as playing a significant role in human nutrition [47,48]. Variations in cultivation practices can also impact the chemical profiles of food products [49]. Changes in fatty acid composition may affect the susceptibility of foods to lipid oxidation. Consequently, lipid oxidation can lead to alterations in aroma, colour, flavour, and nutritional content, potentially diminishing the health benefits associated with consumption [50]. In particular, the value of moisture content assumes a pivotal role in the composition of hazelnuts and is intimately related to the water acidity. These are key factors that ensure the microbial safety of the fruits and influence shelf-life. It is recommended by the European Union that the moisture content of hazelnut kernels should not exceed 6.0% [51] because higher values can compromise hazelnut quality and consequently diminish their shelf-life [9]. According to Guiné [52], all microbial activity ceases with values of water activity lower than 0.62. In this study, none of the samples exceeded that limit, and there were no statistically significant differences between the varieties.

Hazelnuts are particularly rich in lipids, which were found to be the major chemical component for the three varieties studied. Similar results were found in previous studies [53]. Nuts are an excellent source of fatty acids, more specifically unsaturated fatty acids, the consumption of which is associated with the reduction of cardiovascular risk and blood cholesterol [54]. According to Amaral and Oliveira [55], twenty different fatty acids are present in hazelnuts, with oleic acid being the most predominant. In a study developed by Amaral et al. [56], involving nineteen hazelnut varieties cultivated in Portugal, it was found that oleic acid varied between 76.7% and 82.8%, followed by linoleic (9.2%), palmitic (5.6%), stearic (2.7%) and vaccenic (1.4%) acids, with the content of other fatty acids being lower than 1%. A higher content of oleic acid is associated with higher oxidative stability and, consequently, a longer shelf-life of a product. On the other hand, a higher linoleic acid content corresponds to a lower oxidative stability [4]. According to previous studies, the hazelnuts with the best shelf-life quality are those from Italy, Portugal and Poland [53,57,58]. The hazelnut is a good source of mono- and polyunsaturated fatty acids (MUFA and PUFA, respectively) and has low amounts of saturated fatty acids (SFA) [59,60]. In fact, when compared to other nuts, such as almonds, cashews, chestnuts, macadamias and walnuts, the hazelnut presents higher amounts of MUFAs and lower contents of SFAs [61]. According to the literature, the hazelnuts with the highest concentrations of MUFAs are those cultivated in Italy (81.92–83.91%), Portugal (81.60–83.05%), and Poland (81.74–82.15%), while the ones with the lowest concentrations are from Turkey (77.81–80.90%) and Iran (79.3%) [16,53,57,58,62]. The differences encountered among hazelnut varieties are due to climate conditions, geographical origin, state of growth, storage conditions and storage time [4]. For example, according to Ghirardello et al. [20], during storage, the ratio between unsaturated and saturated fatty acids decreases, which can be explained by the decrease in linoleic acid content, possibly because of its peroxidation and subsequent loss.

The results obtained in this study for hazelnuts' chemical composition are in line with previous scientific research, where it was described that the chemical composition of hazelnuts is dependent on different factors, including the cultivar and the harvest year [62]. Ozdemir and Akinci studied some properties of four Turkish hazelnut varieties and found that the protein content varied from 18.25 $\pm$ 0.25 to 22.06 $\pm$ 0.69 g/100 g, fat varied between 57.39 $\pm$ 0.68 g/100 g and 62.90 $\pm$ 1.05 g/100 g, fibre ranged from 2.91 $\pm$ 0.07 to 3.69 $\pm$ 0.14 g/100 g and ash varied between 2.22 $\pm$ 0.02 and 2.29 $\pm$ 0.01 g/100 g.

According to the limits established for the count of microorganisms at 30 °C and mould and yeast by the National Health Institute Doutor Ricardo Jorge ($<10^6$ CFU/g for microorganisms at 30 °C, $<10^5$ CFU/g for yeast and $<5 \times 10^2$ CFU/g for moulds), all the varieties studied, in both harvest years, presented satisfactory microbiological quality [63].

Nuts, including hazelnuts, are often susceptible to contamination by different fungi, which can produce mycotoxins, with *Aspergillus* species being an example of that. The contamination by aflatoxins during storage, particularly when nuts are inadequately dried after harvest, affects the health, quality and shelf stability of both in-shell and shelled hazelnuts [13,64,65]. Aflatoxins are associated with different toxic effects in animals and humans, such as, for example, mutagenic, teratogenic and immunotoxic outcomes [13,66].

5. Conclusions and Limitations

This work provided interesting results that allowed us to compare not only the differences between three of the most important varieties cultivated in Portugal but also the differences that exist in the same variety according to the year of harvest. Regarding the biometric properties, Grada de Viseu was the variety with the heaviest shelled nuts in both harvesting years. On the other hand, in 2021, Butler presented the heavier kernels, and in 2022, it was Grada de Viseu and Butler. It is important to highlight that Tonda di Giffoni was the variety with the highest luminosity for shelled hazelnuts and kernels in both harvesting years. The Tonda di Giffoni variety was more rounded in the equatorial zone. The results also showed that hazelnut kernels were lighter and had a less intense red tone when compared to the other parts of this dried fruit. As for the chemical properties, for all the varieties, independently of the harvesting year, lipids were the major chemical component, followed by protein and fibre. The samples of Grada de Viseu-2021 and Butler-2022 had values of moisture that were above the recommended limit, and therefore, these samples are more susceptible to fungal activity. It is important to highlight that none of the varieties had values of water activity higher than the recommended limit. Moreover, according to the established limits, all the varieties showed a satisfactory microbiological quality for both harvesting years. It was also possible to see that, in general, there were statistically significant differences between the varieties under study. This study showed that there were differences between the hazelnut varieties under study, which is very important for all the players in the hazelnut sector, since it allowed the characterisation of three of the most important hazelnut varieties cultivated in Portugal, in terms of some physicochemical properties and also microbiological quality. This information is crucial for industries, so that they can make sure the properties stay the same over the years, in order to guarantee that these dried fruits can be sold in international markets, which are very competitive. On the other hand, microbiological quality is essential to make sure the fruits stay safe and in good condition for sale and subsequently for consumption.

Despite the conclusions obtained, this study has some limitations, namely the fact that it focuses on only two consecutive harvest years. Furthermore, in future research, it is also important to include other analyses, such as sensory analysis, flavour compounds (including volatile compounds), and mycotoxin analysis. We understand that these could be very useful in complementing the data reported here about the physicochemical characteristics of the hazelnuts.

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