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COMPOSIÇÃO APROXIMADA DE QUATRO ALGAS COMUNS À COSTA PORTUGUESA

COMPOSICIÓN APROXIMADA DE CUATRO ALGAS COMUNES EN LA COSTA PORTUGUESA

PROXIMATE COMPOSITION OF FOUR SEAWEEDS COMMONLY FOUND ON THE PORTUGUESE SHORE

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ABSTRACT

Four macroalgae species, three red algae *(Palmaria palmata, Gracilaria gracilis* and *Porphyra umbilicalis)* and one green algae (*Ulva rigida*) were analysed to calculate and compare their proximate composition. These algae were grown in aquaculture in the same geography, and were also harvested in the same season. *Ulva rigida* differentiates the most from the other with higher contents of carotenoids and lower content in ashes. *Palmaria palmata* shows the lower contents in total sugars, lipids, and protein, but higher in ashes. *Porphyra umbilicalis* and *Gracilaria gracilis* have similar contents showing high contents of both lipids and protein.

Keywords: Seaweeds, Algae, Portuguese shore, Chemical composition of seaweeds.

INTRODUCTION

Algae can be classified into microalgae (microscopic and unicellular) and macroalgae

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also known as seaweeds (macroscopic and multicellular). This last and larger group is divided into three main types according to their pigmentation: *Phaeophyceae* (brown algae), *Rhodophyceae* (red algae), and *Chlorophyceae* (green algae) (Rodrigues et al., 2015). The chemical contents of the algae vary widely with the species, geography, and season (Afonso et al., 2021).

Seaweeds have a biomass composed of valuable biomolecules, which have grown in scientific interest over the years for different industrial sectors due to their abundance and characteristics (Matos et al., 2021). The search for healthy foods of natural origin and produced sustainably, has increased the demand for algae for direct human consumption, or for the production of food compounds, around the world (Hosseinkhani et al., 2022). According to Fleurence (1999), the algae proteins are more suitable for human consumption when compared with those of vegetable origin due to their composition, and in addition algae also contain essential amino acids such as the aspartic and the glutamic acids.

Approximately 291 species of algae are utilised by humans, most of which by the food sector (Ścieszka & Klewicka, 2019) and hydrocolloids industry such as carrageenans, alginates and agar (Liao et al., 2021). Another fraction is used in the production of pharmaceuticals, cosmetics, animal feed, fertilisers, and paper (White & Wilson, 2015). Rodrigues et al. (2015) refers the important content in beneficial functional ingredients, deserving detailed attention. About 32 million tons of macroalgae (wet weight) are currently processed worldwide, with Southeast Asia standing out as the largest harvesting area (Jönsson et al., 2020).

Due to its high potential, seaweeds represent an important raw material to produce different bioproducts that are categorized into fuel and non-fuel. Non-fuel products are composed by carbohydrates, lipids, minerals, pigments and other bioproducts (Chandra et al., 2019).

Seaweeds are used in the diet of the human being in many communities around the world, mainly in Southeast Asia, and is becoming an important source of food in western societies due to their nutritional and pharmaceutical qualities (Rodrigues et al., 2015). The aim of this study was to explore the different chemical composition of four seaweeds commonly found in the Portuguese shore: three red algae (*Palmaria palmata*, *Gracilaria gracilis* and *Porphyra umbilicalis*) and one green alga (*Ulva rigida*). Furthermore, determine which of the species studied would have the greatest nutritional potential for human nutrition.

METHODOLOGY

For this study four replications of the four different seaweeds were completed. Therefore, a total of sixteen repetitions for each of the laboratory procedures was performed, to determine each of the seaweed contents. The chemicals used in all the processes were analytical or High-Performance Liquid Chromatography (HPLC) grade. The results were recorded in percentage of dry matter (% DM) for protein, carbohydrates, total sugars, lipids, and ashes, and in ppm for carotenoids.

Algae samples

The food-grade seaweed used in this study were supplied by AlgaPlus (Ilhavo, Portugal), under standardized conditions, sanitized, dried, crushed and packaged in hermetically closed vials. These were stored at room temperature as per manufacturer labelled advice. The seaweeds were grown in marine aquaculture ponds. *Gracilaria gracilis* and *Ulva rigida* were harvested in the summers of 2018 and 2020, respectively, while *Palmaria palmata* and *Porphyra umbilicalis* were collected in the summer of 2019.

Moisture and ash quantification

The moisture and ash content in the algae were determined according to the National Renewable Energy Laboratory (NREL) protocol. For the quantification of moisture, the convection oven method was adopted. The samples were oven dried (Gallenkamp) at 103 ºC until a constant weight was obtained. The weight difference before and after drying was expressed as percentage of moisture. Please keep in mind that this is moisture of the commercial product and not from fresh algae. Then, the oven-dried samples were placed in a muffle (Heraeus Instruments) furnace for 24 h at 575 ºC to determine the ash content. Finally, the crucibles were cooled down to room temperature in a desiccator, and weighted. The difference between the initial (after drying) and final weight gives the content of ashes in the algae.

Lipids quantification

The lipids were extracted following the procedure of Batista et al. (2013). A Soxhlet

extraction using petroleum ether as an extraction agent, after sample hydrolysis, was used for total lipids quantification. Acid hydrolysis has been used to break the lipids bonds with other compounds, increasing the extraction yield (Cuellar-Bermudez et al, 2015). Samples weighing 5 g were heated until boiling, with 50 ml of 4N hydrochloric acid in an Erlenmeyer flask, covered with a watch glass for 1 hour. The mixture was filtered, with filters incorporated into cellulose cartridges and then placed in extraction ampoules of the Soxhlet device. The extraction was carried out with petroleum ether for 5 hours. The recovered lipids were concentrated in a rotary evaporator, followed by the evaporation of the remaining solvent in an oven at 103 ºC. After solvent evaporation, the tube was weighed again to obtain, by difference, the total lipids content.

Protein quantification

To find the protein content, the Kjeldahl method, described by the AOAC (920.11G) (AOAC International, 1995) was used. Samples of the algae of 0.6 g were weighed and subjected to acid digestion (Buchi) of the protein, at a temperature of 420 ºC, with two catalyst pellets: concentrated sulfuric acid (H_2SO_4) and 30% hydrogen peroxide (H_2O_2). After the digestion, a distillation and neutralization (Tecator) were conducted, with the addition of excess base, sodium hydroxide (NaOH), collecting the distillate in a boric acid (H_3BO_3) solution containing methylene blue and methyl red indicators. Finally, the substrate herein obtained substrate was graduated with a standard solution of chloridric acid (HCl), allowing the calculation of the amount of nitrogen in the sample. The protein content was calculated by converting the total nitrogen content, multiplying by an overall conversion factor (6.25 × N) (FAO, 2012).

Carbohydrate quantification

To determine the carbohydrate content, the DNS colorimetric method (3,5-dinitrosalicylic acid) was used (James, 1995). For the preparation of the samples 0.5 g of algae were weighed and hydrolysed with sulfuric acid (H_2SO_4) 1.5 M. Standard glucose solutions were also prepared (0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.25 and 1.5 mg glucose per ml). For the measurement of the absorbance, the different samples and the standard glucose solutions were read in 96-well microplates at 540 nm, for which a microplate reader for UV-Vis absorbance

(Thermo Fisher) was used. The results were obtained and then recorded as carbohydrate content.

Total sugars quantification

The total sugars were determined using the gravimetric method, according to the Munson and Walker technique, with reference to NP 1419 (IPQ, 1984).

For the preparation of the samples, 0.5 g were weighed, and a digestion was carried out with Carrez I and II solutions, to determine the reducing sugars directly or, after inversion, the total sugars, as a function of the copper oxide I (cuprous oxide) obtained by copper reduction II. For measurement, the absorbance of different samples and glucose standard solutions, as explained previously, were read in 96-well microplates at 540 nm, for which a microplate reader for UV-Vis absorbance (Thermo Fisher) was used. The results were obtained and recorded as total sugars content.

Carotenoids quantification

The extraction of carotenoids was performed according to Rodriguez-Amaya (2001). Initially, 5 g of algae samples were weighed, 40 ml of acetone was added and shake in the dark for 15 minutes. Then, the mixture obtained was vacuum filtered within a Buchner funnel and into a 250 ml kitassato. The residue was washed with 3 ml of petroleum ether (repeated 3 times). The kitassato extract was then transferred into a separatory funnel. For the total removal of acetone and transfer of carotenoids to the second solvent, washes were performed with distilled water three times (100 mL). The quantification of carotenoids extracted from the samples, previously separated, and measured volumetrically, was performed in 96-well microplates at 450nm, using a microplate reader for UV-Vis absorbance (Thermo Fisher). The results were obtained and recorded as carotenoids content.

Data analysis

The different algae were initially tested for significant differences in their chemical composition (ashes, protein, carotenoids, carbohydrates, total sugars, and lipids) with a MANOVA via Wilk's lambda test. Univariate ANOVA tests were performed for the referred contents of the algae chemical composition to test significant differences between algae. The

prerequisites for the parametric tests were tested via Kolmogorov-Smirnov tests for the normal distribution of the residuals and via Levine's test for the homogeneity of variances. As the prerequisites were not met, the non-parametric Kruskal-Wallis H test was used, followed by the Bonferroni adjustment as post-hoc. All the significant levels were set to $P < 0.05$. All the analysis were performed using Statistica® 14.0.0.15 by TIBCO software Inc, Palo Alto, CA, USA.

To further explain the significant differences found, the data was then explored using multivariate methods, namely principal components and classification analysis based on the correlations between the variables. A cluster analysis was also performed based on the cases, with the Ward aggregation method together with the Manhattan method to calculate the linkage distances.

RESULTS AND DISCUSSION

All the different chemical contents but the carbohydrates show significant differences between the different algae. Tables 1 summarises these differences.

In Table 2 the scores of the different variables in the principal components factors, can be found. Figure 1 shows the biplot of principal components one and two applied to the chemical constituents (variables) of the algae being studied. Total sugars, lipids and proteins are the main variables contributing to factor one, while carbohydrates, carotenoids and ashes are the main variables contributing to the factor one. Factors one and two together, explain 81.11% of the variability of the data, as can be visualised in Figure 1 and 2.

Table 01: Results of the Kruskal-Wallis test applied to the different chemical contents of the algae studied.

Composition	Kruskal-Wallis P-value	Median contents for the different algae			
		Palmaria palmata	*Ulva rigida*	*Porphyra umbilicalis*	*Gracilaria gracilis*
Ash (%)	< 0.01	39.40^{b}	24.72^{a}	29.78^{b}	26.78^{ab}
Protein (%)	< 0.01	9.59^{a}	14.59^{ab}	29.73^{b}	27.93^{ab}
Lipids (%)	< 0.01	1.06^{a}	1.59^{ab}	2.04^{ab}	2.44^{b}
Carbohydrates (%)	> 0.05	25.94	27.02	26.16	26.33
Total Sugars (%)	< 0.05	14.16^{a}	19.45^{ab}	17.72^{ab}	21.44^{b}
Carotenoids (ppm)	< 0.01	8.19^{ab}	14.19^{a}	12.21^{ab}	5.34^{b}

Different letters in superscript are indicative of significant differences ($P < 0.05$)

Source: Own (2022)

Table 02: Scores of the difzxferent variables and their contribution for the different factors. Principal components median analysis performed on the correlations between variables.

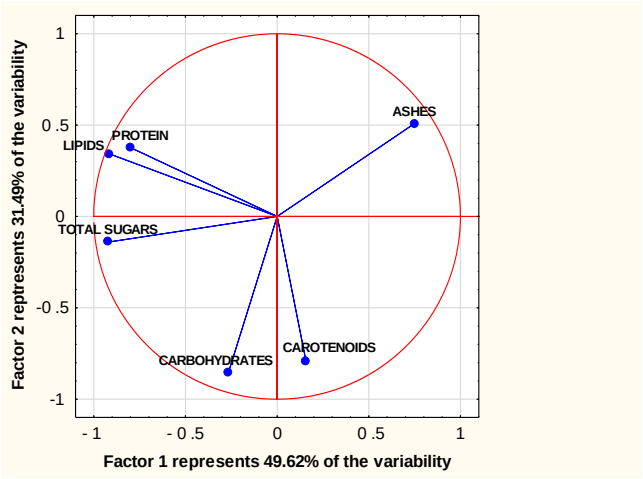
	Scores					
Variables	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Ash	0.189	0.137	0.047	0.326	0.301	0.001
Protein	0.213	0.075	0.294	0.060	0.068	0.290
Carotenoids	0.008	0.332	0.509	0.060	0.081	0.010
Carbohydrates	0.024	0.384	0.031	0.535	0.024	0.002
Total sugars	0.285	0.010	0.088	0.010	0.525	0.083
Lipids	0.281	0.062	0.031	0.010	0.002	0.615

Source: Own (2022)

In Figure 3 shows the projection of the different algae replicates (cases) on the axis formed by factors one and two from the principal components analysis. It becomes clear that *Palmaria palmata* is isolated to the right hand side of the graph, once, as found by the Kruskal-Wallis analysis, it has lower contents of lipids, protein, and total sugars. In the other hand the factor discriminating *Ulva rigida* is the second factor, mainly commanded by the lower content in ashes. *Ulva rigida* is also discriminated from the other algae by its lower content in carotenoids.

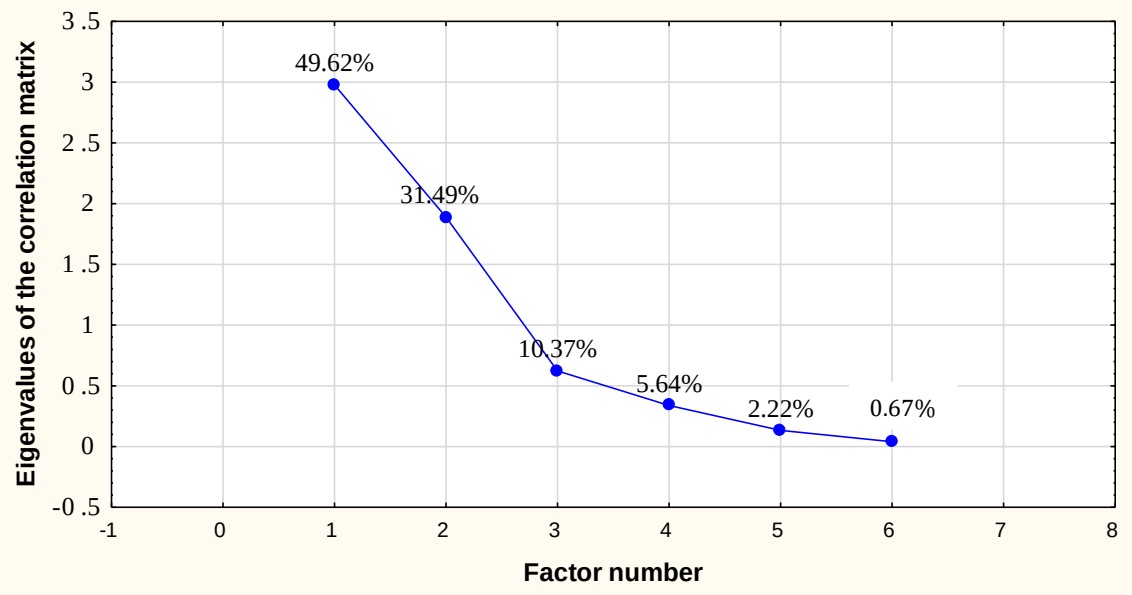
Figure 4 represents the tree formed after the cluster analysis putting in evidence the differences between the algae being studied, as they are clustered together creating homogeneous groups differentiating them.

Figure 01: Biplot of variables on the axis formed by factors one and two of the principal components analysis. Based on the correlations between variables.



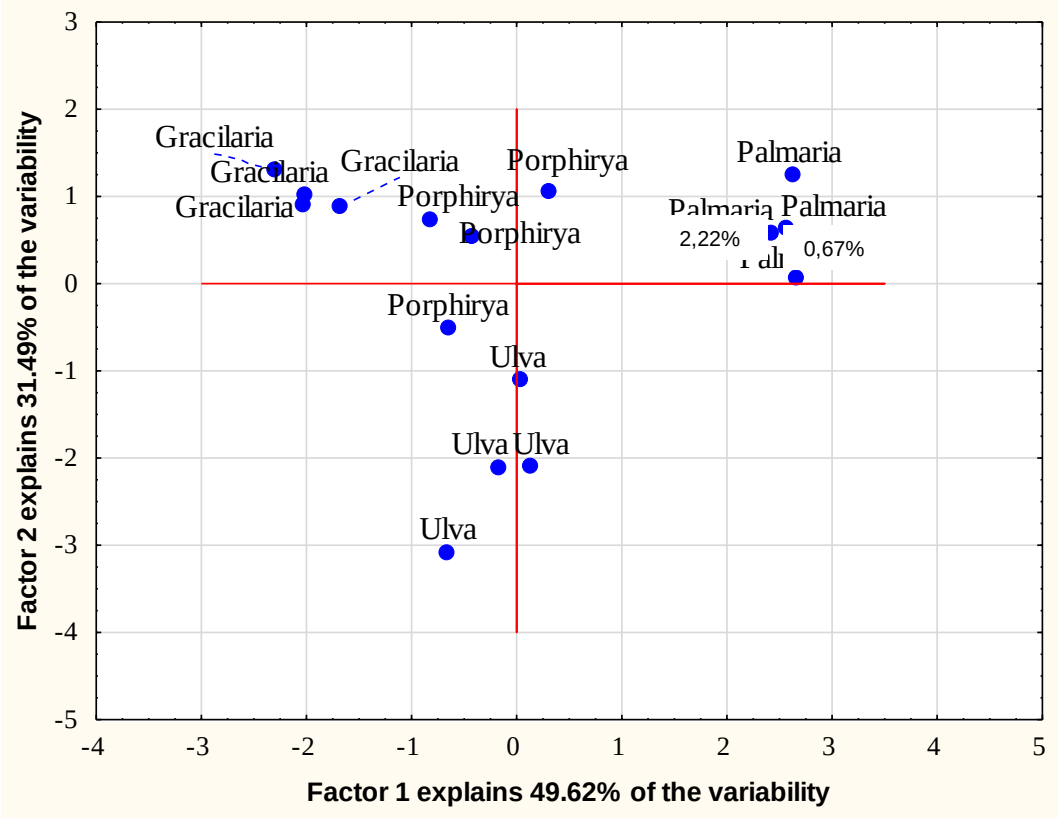
Source: Own (2022)

Figure 2: Scree plot displaying the variation captured by each of the factors in the principal components analysis.



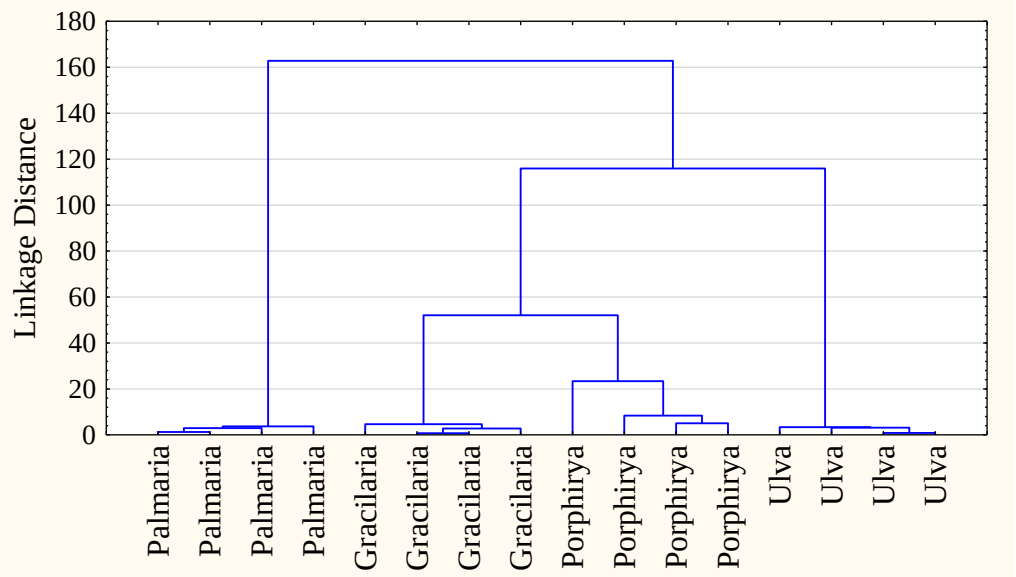
Source: Own (2022)

Figure 03: Projection of the algae cases in the factor plan



Source: Own (2022)

Figure 04: Cluster tree executed on the algae cases using the Ward's method and the Manhattan linkage distance.



Source: Own (2022)

Algae are organisms capable of synthetise a wide range of carotenoids through a diversity of carotenogenesis pathways, which can be used as taxonomic markers (Liaaen-Jensen, 1998). Carotenoids such as α-carotene, and zeaxanthin, are found in the macrophytic red algae, while in green, violaxanthin, and 9′-cis neoxanthin can be found. Lutein and β-carotene are found in both red and green algae (Takaichi, 2011). While red algae are mainly marine, the green algae occur mainly in fresh water. Authors such as Nunes et al. (2017) have reported contents for carotenoids in macroalgae ranging between the limit of detection and 2978 ppm. These authors reported 204.1 ppm for *Ulva rigida*, which are well above the values found in this study.

The content in ashes is reported to vary in macroalgae between 5.8 and 46.2% of the DM (Gamero-Vega et al., 2020). Cofrades et al. (2010) report values of 10% for *Porphyra umbilicalis*, well below the values of this study, while Rodrigues et al. (2015) reported 20.5% for *Gracilaria gracilis* slightly below those found in this study. For *Ulva rigida* authors have found values varying between 25.7 and 37.8%, tallying the results of our study (24.72%). Echave et al. (2021) found contents in ashes (24%) for *Palmaria palmata* lower than those

found in this study (39.4%). In this study the ashes' content of *Ulva rigida* shown the lower values (24.7%).

Red algae are known by their higher protein content, especially with the genus *Porphyra* (Gamero-Vega, 2020). Accordingly to Fleurence (1999), the high content in protein of the red algae (up to 47%), is as high as animal products such as eggs, milk, or meat. Other authors report different protein contents regarding the studied algae: For *Gracilaria gracilis* values vary from 22.5% (Rodrigues et al., 2015) to 40-45% (Francavilla, 2013); Cofrades et al. (2010) reported protein contents for *Porphyra umbilicalis* around 40%; Echave et al. (2021) 21 % for *Palmaria palmata*; and Nunes et al. (2017) 7.16% for *Ulva* sp. These values agree with those obtained in this study, being the exception, the low values found in *Palmaria palmata* tallying those of Machado et al. (2020).

Nunes et al. (2017) identifies a positive correlation between the contents in protein and lipids of algae, which agrees with the results obtained in this study. The lipidic content reported by other authors for the algae used in this study are similar. Rodrigues et al. reported 2.2% for *Gracilaria gracilis*, and Paiva et al. (2017) 1.02 for *Ulva rigida*. In relation to *Porphyra umbilicalis*, the values are in agreement with those reported in other literature (e.g. Paiva et al., 2014). The lower content in lipids in this study is shown by *Palmaria palmata*, tallying the results of other authors (e.g. Paiva et al., 2014).

In carbohydrates no differences were found between the species studied. Their values agree with those reported by authors such as Murugaiyan (2020) founding contents of 32% for *Ulva* sp., and 28% for *Gracilaria* sp. Some authors (e.g. Taboada et al., 2010) report higher values of carbohydrates for *Ulva rigida* (42%).

For total sugars, *Ulva rigida* shows values like those reported in the literature, such as by Hardouin et al. (2016) reporting 24 % for *Ulva* sp. Some authors (e.g. Rosemary et al., 2019) relate the carbohydrates content of algae with their metabolism, deducting a correlation with their growth. *Palmaria palmata* shows the lower content in sugars within the species of this study, and its content in carbohydrates shown also to be lower than values (71%) reported by authors such as Parjikolaei et al. (2016). *Gracilaria gracilis* however shown a carbohydrate content (26%) similar to those found by Francavilla (2013), but lower than those reported by Rodrigues (2015) (24 and 46.6%, respectively). Overall, the chemical content of the macroalgae present a wide variation between and across the different species that depend on many factors

such as harvesting season, geography, and growing conditions (Afonso et al., 2021).

CONCLUSIONS

This study confirms the wide variability of the chemical content of macroalgae within the same species and across different species. Although the studied species having been grown in the same geography, growing conditions, and having also been harvested in the same season, variations exist among species.

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