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Influence of Fertilization and Rootstocks in the Biomass Energy Characterization of *Prunus dulcis* (Miller)

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Abstract: The importance of replacing fossil fuels with new energy routes such as the use of biomass leads to the improvement of sources such as agricultural and forest systems through adequate management techniques.The selection of the vegetal material and the management practices can influence the properties and quality of the obtained biofuel. The properties of the biomass obtained from pruning almond trees (*Prunus dulcis* (Mill)) have been analyzed in this study. Two varieties were tested, Marcona and Vayro, with three rootstocks, GF305, GF677 and GN Garnem, under different fertilization systems. The quality of the biofuel was evaluated with respect to the chemical composition and gross calorific value. We observed that the variables that mostly influenced the gross calorific value of the biomass were the variety, the rootstock and, primarily, the variety-rootstock interaction. Marcona presented better biomass properties than Vayro. Trees grafted on GF305 obtained a higher gross calorific value than the ones grafted on GF677 and GN Garnem. The percentage of nitrogen highly depended on the fertilization treatment applied, with saccharides and aminoacid fertilization accumulating a higher level of nitrogen than the humic and fluvic fertilization.

Keywords: biomass; variety and rootstock selection; almond tree; agricultural practices

1. Introduction

Energy efficiency is a basic pillar when it comes to achieving a certain degree of social and environmental sustainability, guaranteeing an adequate level of energy security [1]. In this sense, the European Union has set achieving greater efficiency in the development of renewal energies as a priority objective [2,3], committing to increase the exploitation of renewable energy by 20% [4].

Under this premise, the research of fuels as an alternative to crude oil and coal has led scientists to analyze materials that come from agriculture and forest environments [5–7]. Among the biofuels obtained from these systems, lignocellulosic materials such as wood chips and pellets take on an important role [8–10], as they can be used in combustion boilers to produce heat in both industrial—especially in oil mills—and domestic applications [11–13]. Improved cogeneration systems have also been developed to simultaneously produce heat and electricity [14,15]. Another very relevant application is the use of lignocellulosic materials in pyrolysis processes [16–18]. The primary biofuels used in small-scale combustion systems commonly have a woody origin, and as the demand for these biofuels grows, the pressure on forest exploitation will increase, with possible negative environmental consequences, which is why it is important to find new sources from which to obtain biofuel [4].

Ignorance regarding the energetic properties of the lignocellulosic materials from agricultural environments has led to numerous investigations determining the calorific value, ash, and elemental composition of the wood of different crops, such as the orange tree [19–21], the olive tree [8,20,21], the almond tree [19,20,22], the apple tree [23], the vine [8,19,20], and even herbaceous plant remains from greenhouses [24,25]. The heating or calorific value is one of the most important aspects related to the use of biomass, as it expresses the energy content of the biomass fuel and is a key parameter that has been widely used for the development of calorific power prediction models based on elemental, proximal and structural composition [26–30], although unfortunately the accuracy of the correlations based on those analyses are generally not very high [31].

In fact, just as the quality of the fruit varies according to the cultivation techniques used, the energy properties of the wood can be affected by the growing conditions and agricultural practices, such as the variety or rootstock selection or the fertilization.

Considering the lack of accurate biomass standardization, especially in relation to physicochemical, process and environmental parameters, the study and choice of raw materials for achieving better process efficiencies is not devoid of difficulties [32]. If the agricultural practices for obtaining materials influence their composition, obviously they will also do so in their energetic properties. Therefore, a proper characterization is required for the adequate use of the wastes obtained for biofuel uses.

The aim of this work was to demonstrate this hypothesis on the wood of almond tree (*Prunus dulcis* (Mill.)), which is one of the most important crops in both the Mediterranean region and also the Californian coast, which generates a huge amount of available biomass for energy uses [22], and has potential calorific powers superior to other common sources of biomass such as the almond shell, the olive pomace from oil mills, or the pressed grape waste from the wine industry [32]. Two varieties were tested, Marcona and Vayro, with two rootstocks and different fertilizations. The novelty offered by this study consists of the introduction of new parameters, such as rootstock selection and fertilization, in the study and classification of biomass products.

If the hypothesis is true, it would be necessary to consider those practices that improve the obtained products for food production, on the one hand, and on the other, the energetic material coming from the pruning waste. The use of both resources could significantly contribute to regional and national bio-economies in the future [9,14].

2. Materials and Methods

2.1. Field Study

The research area was located on the east coast of Spain, in the province of Valencia (latitude 39°28′50″ N, longitude 0°21′59″ W). The region is characterized by an average annual temperature of 18.3 °C. Maximum temperatures (30.2 °C) are observed in the month of August, and minimum temperatures (7.1 °C) in January. The average rainfall is around 475 mm, with a relative humidity of 65%; the driest period corresponds with the month of August, and the rainiest with October [33]. For study purposes, a total of 108 individuals of Prunus dulcis (Mill.).

(Mill.) were selected. The procedure of each trial consisted in the selection of a minimum of 36 individuals for the different combinations of factors that could influence the generated waste: variety, pattern and fertilization type. Table 1 shows the factors studied and their different levels.

Table 1. Factors and levels analyzed for the characterization of almond trees biomass.

Factor	Number of Levels	Levels
Variety	2	Marcona Vayro
Rootstock	3	GF 305 GF 677 GN Garnem
Fertilization	3	Biostimulant 1 Control Biostimulant 2

2.2. Vegetal Material and Treatments

For this research, we analyzed two almond tree varieties (both of them were characterized following the UPOV norm TG/56/4 Corr): Marcona and Vayro. Marcona is an autochthonous variety from the east of Spain that is known worldwide for the high quality of its fruits. It is a highly ramified, medium-high vigor tree with low cold requirements [34]. Vayro is a late-flowering variety obtained through crosses within the IRTA (Instituto de Investigación y Tecnología Agroalimentarias) genetic hybridization program. Trees of this variety show very strong vigor and medium branch density [35].

The behavior of 3 rootstocks widely used in almond cultivation was tested. GF 305 rootstock is a medium-vigor frank peach pattern that adapts adequately to irrigated conditions [36] and is tolerant to drought. GF 677 comes from the interbreeding of peach trees (*Prunus persica* L. Batsch) and almond trees (*Prunus dulcis* Miller) obtained in France by the INRA (Institut National de la Recherche Agronomique) [37]. It is a vigorous rootstock with good agronomic behavior in both dry and irrigated conditions. Finally, GN Garnem, obtained by the Agricultural Research Service of the Government of Aragón (CITA-DGA) as the result of crossing between *Prunus dulcis* (Mill.) (cv. Garrigues) and *Prunus persica* L. Batsch (cv. Nemared). It stands out for its strong vertical growth, inducing greater vigor than GF 677 [38].

Regarding fertilization, the samples were subjected to 3 types of treatment. Biostimulant 1, which is mainly composed of saccharides and amino acids; Biostimulant 2, rich in humic and fulvic extract, and a control group of test plants that were only irrigated with water.

2.3. Laboratory Analysis

2.3.1. Proximate Analysis

The evaluation of the drying process was carried out according to the norm ISO 18134-2:2017 [39]. The process took place in oven drying conditions under controlled temperature (105 $\pm$ 2) °C. In order to avoid the loss of volatile compounds, the drying process did not exceed 24 h. Ash content and volatile compounds were determined according to the norms ISO 18122:2015 [40] and ISO 18123:2015 [41], respectively.

2.3.2. Determination of Gross Calorific Value and Elemental Composition Analysis

Gross calorific values (CGV) of samples were analyzed using a LECO AC500 Automatic Calorimeter, based on norm ISO 18125:2017 [42]. Carbon (C), Hydrogen (H) and Nitrogen (N) determinations were carried out according to norm ISO 16948:2015 [43].

3. Results and Discussion

An overview of the chemical composition of almond tree biomass was conducted in order to describe the biomass potential of this species and how the selection of the vegetal material (variety and rootstock) and management practices can influence the properties of the obtained biofuel.

Table 2 presents the values of proximate analysis, elemental composition and gross calorific value of the studied samples. All variables, except the percentage of hydrogen, present standard kurtosis and standard skewness between −2 and +2, ANOVA analysis can be performed, as they all follow normal distributions. The average gross calorific value obtained in our analysis shows similar results to those obtained in almond hulls and shells by Nhuchhen [44] or by Fernández-González [19] or Yin [26] for biomass. The results are also close to those found for other *Prunus* species, such as *Prunus Avium* L. [45] or *Prunus armeniaca* L. [46]. As can be observed, the concentrations of C and H were 48.11% and 5.77%, respectively, which are the same as those observed by Jenkins [47] for almond residues. Percentages of C, H, N are in the range of published data for other biomass groups (C = 42–71%; H = 3–11%; N = 0.1–12%) [48]. Low concentration of N, as is the case, is a positive characteristic, as high N produces a negative impact on the environment due to nitrogen dioxide emissions [19]. The greatest disadvantage of biomass when it is used as fuel is its high moisture content, which is inversely correlated with its calorific value [49]. Almond trees presented an average moisture content (8.40%) much lower than the average for woody species (20%) [50], which therefore makes it convenient for energetic uses.

Table 2. Characterization of examined biomass.

	Average	Standard Deviation	Standard Skewness	Standard Kurtosis	Minimum	Maximum
Carbon (%)	48.41	2.22	−1.99	2.01	41.50	53.50
Hidrogen (%)	5.77	0.30	−4.81	4.96	4.83	6.60
Nitrogen (%)	0.97	0.27	1.62	−0.59	0.44	1.67
C/H	8.40	0.38	−1.53	0.70	7.29	9.26
GCV (joule/g)	18503.44	555.99	−6.73	7.99	17067.37	19628.39
Moisture content (%)	8.36	1.83	1.87	0.98	5.78	12.84
Ash (%)	3.41	0.80	2.05	−1.39	2.15	5.01
Volatile compounds (%)	82.89	5.48	−1.88	0.58	69.59	92.74

The dependence of the studied variables is shown in Table 3. It has been proved in other studies that moisture content and percentages of C and H have a high influence on gross calorific value [45,51]. However, in our study, we observed that the variables that mostly influence the gross calorific value are not the composition of C, H, N, but variety, rootstock and, above all, variety-rootstock interaction. We found that the variable that influenced the percentage of N the most was the treatment applied. This result is consistent with other research [52,53] that concludes that fertilizers and pesticide doses are highly important for some elements, such as the nitrogen content.

Given the obtained results, a variance analysis was carried out in order to compare the influence of the different factors analyzed in this study.

Table 3. Pearson correlation coefficients analysis.

	Variety	Rootstock	Interaction Variety/Rootstock	Treatment	C	H	N	C/H	GCV	Moisture Content (%)	Ash (%)	Volatile Compounds (%)
Variety		0	0.9869 *	0	−0.4053 *	−0.1831	0.027	−0.1983 *	−0.6465 *	−0.2160 *	0.2163 *	−0.1180
Rootstock	0		0.1612	0	−0.072	0.3213 *	−0.4055 *	−0.4346 *	−0.4769 *	−0.3373 *	−0.2213 *	0.6926 *
Interaction variety/rootstock	0.9869 *	0.1612		0	−0.4116 *	−0.1289	−0.0387	−0.2658 *	−0.7149 *	−0.2675 *	0.1778	−0.0049
Treatment	0	0	0		−0.1932	−0.3167 *	−0.4427 *	0.1606	0.0797	0.1503	0.2787 *	0.1605
C	−0.4053 *	−0.072	−0.4116 *	−0.1932 *		0.5593 *	0.1868 *	0.3581 *	0.4168 *	−0.0617	−0.0048	0.2528 *
H	−0.1831	0.3213 *	−0.1289	−0.3167 *	0.5593 *		−0.1143	−0.5722 *	0.0851	−0.2663 *	−0.1574	0.4891 *
N	0.0270	−0.4055 *	−0.0387	−0.4427 *	0.1868 *	−0.1143		0.3199 *	0.2552 *	0.2706 *	0.2334 *	−0.4033 *
C/H	−0.1983 *	−0.4346 *	−0.2658 *	0.1606	0.3581 *	−0.5722 *	0.3199 *		0.3118 *	0.2426 *	0.1688	−0.3013 *
GCV	−0.6465 *	−0.4769 *	−0.7149 *	0.0797	0.4168 *	0.0851	0.2552 *	0.3118 *		0.2781 *	0.2354 *	−0.0938
Moisture content (%)	−0.216 *	−0.3373 *	−0.2675 *	0.1503	−0.0617	−0.2663 *	0.2706 *	0.2426 *	0.2781 *		0.0584	−0.4015 *
Ash (%)	0.2163 *	−0.2213 *	0.1778	0.2787 *	−0.0048	−0.1574	0.2334 *	0.1688	0.2354 *	0.0584		0.2146 *
Volatile compounds (%)	−0.118	0.6926*	−0.0049	0.1605	0.2528 *	0.4891 *	−0.4033 *	−0.3013 *	−0.0938	−0.4015 *	0.2146 *	

* Pairs of variables with *P*-values lower than 0.1; C: carbon (%); H: hydrogen (%); N: nitrogen (%); GCV: gross calorific value ($J \cdot g^{-1}$).

3.1. The Variety Factor

Plants show a huge amount of similarities in properties between species, yet with significant specific variations such as the ones related to combustion properties. In this research, we focused on contrasting whether the differences were also notable between varieties within the same species. Marcona and Vayro varieties were statistically compared by means of variance analysis. The results obtained are shown on Figure 1. As we can see, the gross calorific value of Marcona (18858.70 J·g^{-1}) was statistically higher than the one obtained by Vayro (18148.20 J·g^{-1}). The same results were reached in the evaluation of the carbon and the hydrogen contents, while no differences were found for the nitrogen content. Marcona has been described in previous research as a variety with very high residue productivity (13.91 kg dry matter/tree) [20], this variety could be of interest in order to obtain not only a higher level of biomass in weight per tree but also a higher energetic potential because of its superior gross calorific value.

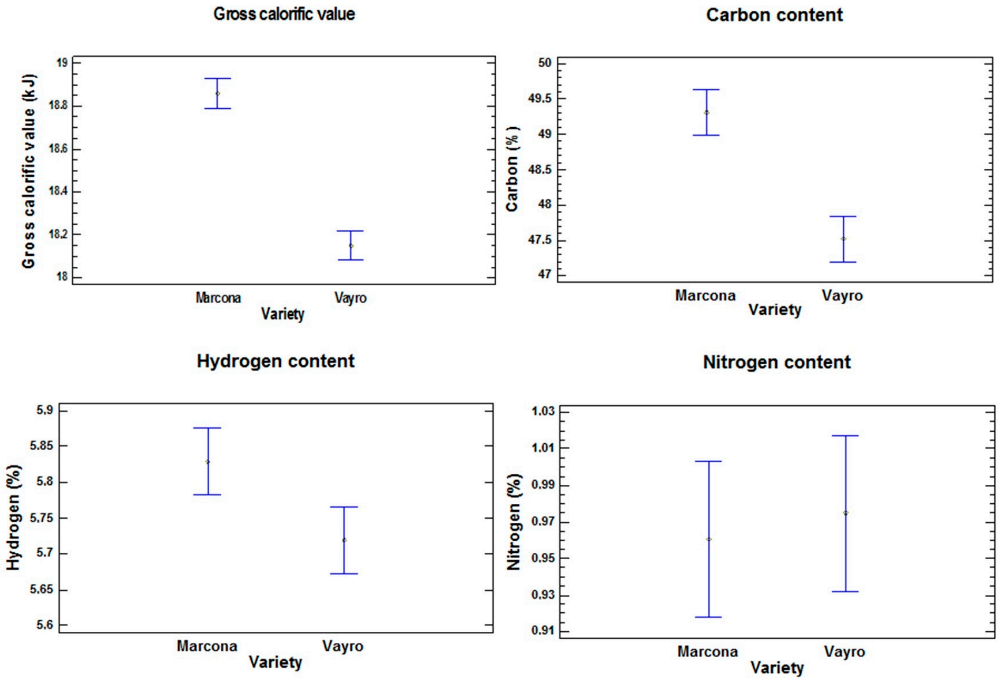

Figure 1. LSD intervals at 90% confidence level in the influence of the variety factor in residual biomass characteristics. (n = 108).

3.2. The Rootstock Factor

It has been widely studied that the selection of an appropriate rootstock is a key factor in fruit trees, as rootstocks can confer better adaptability to climatic and edaphic conditions and have influence on the plant mineral uptakes, the yield properties and efficiency, and the vigor of the grafted variety [54]. Figure 2 presents the statistical comparison of rootstocks by means of variance analysis. The rootstock with the highest gross calorific value was GF 305 (18850.70 J·g^{-1}) and the lowest gross calorific value was presented by rootstock GN Garnem (18148.20 J·g^{-1}). These differences between rootstocks cannot be attributed in our study to differences in carbon content, as we can see in Figure 2. This result would not correspond to those published by Demirbaş [55], in which the heating power was linked to the oxidation phase of the fuels, and in which carbon commonly dominated and overshadowed small changes of the hydrogen content. Although the contents of nitrogen in almond trees are low compared to other species [50], we can see that the rootstock factor affects nitrogen levels. In this study, the rootstock with the lowest nitrogen content is GN Garnem (0.84%) followed by GF 677 (0.95%). Nox and N_2O gases are generated when obtaining energy through biomass and are highly polluting [56]. These emissions directly depend on the nitrogen content of the biomass and must be as limited as possible.

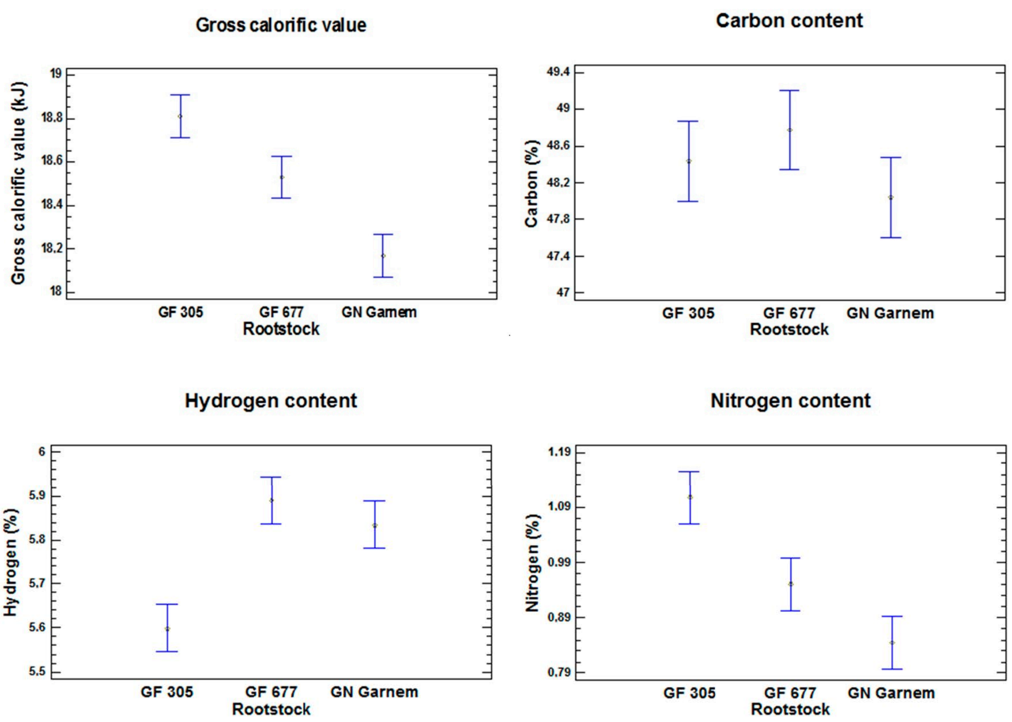

Figure 2. LSD intervals at 90% confidence level in the influence of the rootstock factor in residual biomass characteristics. (n = 108).

3.3. The Combination Variety-Rootstock Factor

Regardless of the rootstock on which they were grafted, Marcona samples presented greater values of gross calorific value than Vayro samples. The rootstock that induced the highest calorific value in both cultivars was GF 305. As seen in Table 4 and Figure 3, the gross calorific value of Marcona on GF 305 is superior to Marcona on GF 677 and Marcona on GN Garnem respectively. This same pattern of behavior is repeated in Vayro variety. The highest carbon contents were found in the combination variety-rootstock Marcona-GF 305 (50.45%) followed by Marcona-GF 677 (48.80%), Marcona-GN Garnem (48.68%) and Vayro-GF 677 (48.73%). As shown in Table 3, which contains the Pearson correlation coefficients, the carbon content is much more influenced by the grafted variety than by the rootstock. All variety-rootstock combinations presented similar values on the hydrogen content except for Vayro-GF 305, which was lower. This result coincides with the one obtained in the individualized study of the rootstocks (Figure 2). The lowest nitrogen content was identified in the Vayro-GN Garnem combination.

Table 4. Influence of the interaction variety-rootstock factor in residual biomass characteristics (n = 108; mean value ± standard deviation; mean values with different minor letters (a–e) differ significantly).

Interaction Variety-Rootstock	GCV (J)	Carbon (%)	Hydrogen (%)	Nitrogen (%)
Marcona 305	19270.66 ± 266.47 e	50.45 ± 1.77 c	5.77 ± 0.19 b	1.10 ± 0.14 d
Marcona 677	18807.45 ± 204.93 d	48.80 ± 1.07 b	5.87 ± 0.20 b	0.83 ± 0.30 ab
Marcona GN	18497.88 ± 375.76 c	48.68 ± 0.83 b	5.83 ± 0.24 b	0.94 ± 0.15 bc
Vayro 305	18349.76 ± 90.33 bc	46.41 ± 3.50 a	5.41 ± 0.39 a	1.11 ± 0.16 d
Vayro 677	18255.92 ± 238.11 b	48.73 ± 1.77 b	5.90 ± 0.14 b	1.06 ± 0.34 dc
Vayro GN	17839.02 ± 565.84 a	47.40 ± 0.80 a	5.83 ± 0.26 b	0.74 ± 0.19 a

In the same column, mean values with different minor letters differ significantly ($P < 0.1$).

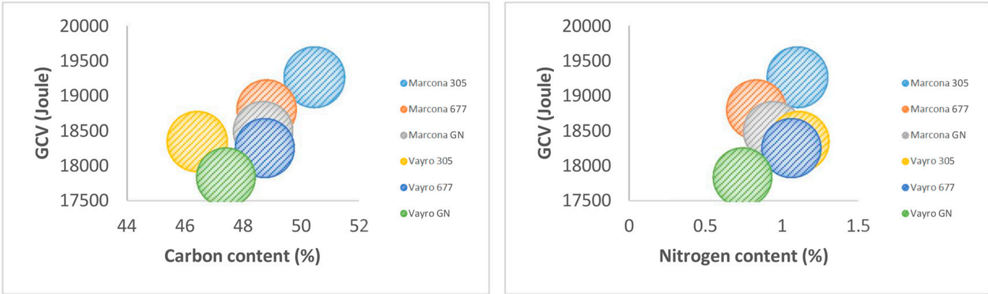

Figure 3. Dispersion diagram for gross calorific values (J·g^{-1}) according to the carbon and nitrogen content for each variety-rootstock combination.

3.4. The Treatment Factor

The direct uptake of organic compounds from the soil solution by the root system constitutes an important base of nutrients for the plant [57]. The purpose of this essay is to obtain greater knowledge on how the composition of fertilizers can modify the characteristics of residual biomass obtained in almond trees. No significative differences were found in terms of carbon content between the tested biostimulants and the control test; however, biostimulant 1 showed a higher carbon content than biostimulant 2. (Figure 4). The gross calorific value of the samples was not affected by fertilization, either. These results are in agreement with those obtained by Ercoli [58], in which the supply of N fertilizers in Miscanthus translated into a higher biomass yield, but did not affect the calorific value of the crop. Results obtained for biostimulant 1 regarding hydrogen and nitrogen contents are higher than those of biostimulant 2. These outcomes could be partially explained by the composition of the products, since biostimulant 1 mainly provides saccharides and amino acids (tryptophan, glutamic and GABA), which are very fast absorbing molecules [57]. Similar conclusions were reached by Mantineo [59], where extra doses of nitrogen fertilization led to a higher nitrogen concentration in the studied biomass. As high N produces a negative impact on the environment, due to nitrogen dioxide emissions [19], fertilizers that promote an increase of the percentage of N may be considered to be environmentally disadvantageous for biomasic uses.

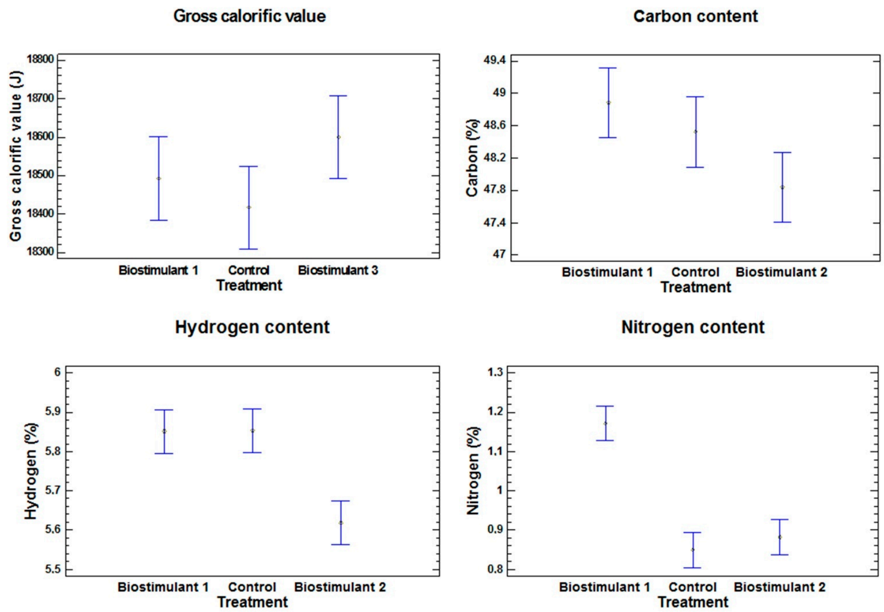

Figure 4. LSD intervals at 90% level of confidence in the influence of the treatment factor in residual biomass characteristics. (n = 108).

4. Conclusions

From a chemical point of view, almond tree pruning has suitable characteristics for use as a possible source, of biofuel as almond trees present high average gross calorific value with low moisture contents and low concentration of nitrogen.

The proposed hypothesis at the beginning of the trial is confirmed. In our study, the gross calorific value of the samples was more dependent on the varity and on the rootstock than on the C, H, N composition. It will therefore be advisable in the future to find those practices that maximize the yields of this crop both at the level of fruit production and at the level of biomass obtaining.

Among the studied varieties, Marcona presented higher energetical potential than Vayro. Important differences were found between the studied rootstocks. Although GF 305 presented the best results in terms of calorific power, it was also the rootstock with the highest nitrogen content. In contrast, GN had the lowest energy potential but also the lowest nitrogen content.

No significative differences were found in the gross calorific value of the samples according to the fertilization. However, percentage of nitrogen depended highly on the fertilization treatment applied, the saccharides and aminoacid fertilization accumulated a higher level of nitrogen than the humic and fluvic fertilization.

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