Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: www.elsevier.com

Research paper

Valorising faba bean residual biomass: Effect of farming system and planting time on the potential for biofuel production

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ARTICLE INFO

Keywords:
Vicia faba L.
Greenhouse
Harvest index
Cellulose
Pretreatments
Saccharification

ABSTRACT

Research was carried out in southern Italy with the aim to assess the quality of faba bean residual biomass and its potential for biorefinery application. Faba bean is a sustainable crop, due to its ability to fix atmospheric nitrogen, and a large amount of biomass remains after harvest which can be valorised for energy production. Greenhouse and early planting are known to affect pod yield and, in this respect, even the residual biomass quality needs to be assessed. For this purpose, the effects of five planting times (i.e. the dates of transplants ranging from 27 September to 22 November at two-week interval, earlier and later than the common planting date of 25 October in Naples province) on pods yield, residual biomass, and saccharification potential were evaluated in faba bean grown in open field and in greenhouse. The third planting time resulted in the highest fruit and residual biomass yield under greenhouse, whereas the fourth was the best in open field. Harvest index was best affected by the third and fourth planting times in open field. Greenhouse grown biomass showed higher values of lignin, hemicellulose and pectin, compared to open field, whereas the opposite trend was recorded with cellulose. Lignin content showed a gradual decrease from the first to the last planting time (17.7%–13.7% biomass fraction respectively), as well as pectin (from 14.1 to 11.5% biomass fraction); conversely, cellulose increased from the first to the last planting time (from 41.1 to 48.7% biomass fraction). Glucose was the most represented monosaccharide (46.7 mol%), followed by xylose (27.4 mol%) and galactose (9.9 mol%). Overall, the potential of faba bean residual biomass for energy production was best affected by open field growing, the latest planting time and alkali pre-treatment, the latter giving the highest value of saccharification (60.7 g kg^{-1} h^{-1} compared to 27.6 relevant to hot water pre-treatment).

1. Introduction

Faba bean (*Vicia faba* L.) is a food crop grown in several world areas such as the Mediterranean basin, UK, and China. Italy is a major European producer, with a total area of 49,000 ha devoted to this crop in 2014 [1]. Faba bean is mostly grown under field conditions, but greenhouse culture allows for crop growth and productivity enhancement in mild climate areas with a rainy early spring season [2], as well as for plant protection against environmental unbalances [3]. Planting time is also crucial in faba bean management, as it affects the growth cycle in terms of possible occurrence of abiotic and/or biotic stresses [4].

Large amounts of faba bean biomass residues are left on the field, which can be employed as a feedstock to valorize the overall production process. These residues can be employed for alternative uses, as they are characterized by high polysaccharide content and low lignin (48.8–56.5% biomass fraction and 13.1–14.4% biomass fraction, respectively) [5,6]. Some research has been conducted to investigate the potential of producing fuel from residual biomass of faba bean due to the large amounts of biomass residues, ranging from 3.7 to 5.7 t ha^{-1}

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https://doi.org/10.1016/j.biombioe.2017.10.019
Received 7 February 2017; Received in revised form 18 October 2017; Accepted 20 October 2017
Available online xxx
0961-9534/

and representing 42.3–50.5% of the total crop biomass [5,7,8]. The uses for faba bean biomass can be diverse, from biofuels and biogas [9], to higher value chemicals, but for any of these applications the yield and composition of the biomass are critical to determine the viability of a commercial application.

In this work, we assessed the effects of farming system and planting time on the residual biomass productivity and composition, the saccharification potential, as well as the fruit yield.

2. Materials and methods

2.1. Plant material and growth conditions

Faba bean (*Vicia faba* L.) cultivar Aguadulce supersimonia was grown in Naples, southern Italy (40°50′ N, 14°15′ E, 17 m a.s.l.), in Mediterranean climate, in 2011–12 and 2012–13 on a sandy-loam soil field (81% sand, 8% silt, 11% clay, 2% organic matter). The ten-day means of temperature (day/night) and photosynthetically active radiation (P.A.R.) recorded at the plant level are shown in Table 1.

Comparisons were made among ten experimental treatments, obtained by the factorial combination of two farming systems (open field, greenhouse) and five planting times (27 September, 11 October, 25 October, 8 November, 22 November). A split plot design with three replicates was arranged, where each elementary plot was 6.00 m^2; plants were transplanted in single rows spaced by 85 cm from each other and the spacing was of 13 cm along the rows, with an areal density of 9 plants per m^2.

Planting times were chosen in order to assess the effects of earlier and later transplant dates, referred to the common planting date of 25 October in Naples province, on pod yield as well as on residual biomass amount and quality.

Greenhouse consisted of a three-span polytunnel, each span being 30 m long, 5 m wide, 2 and 3.5 m high at wall and at roof respectively. Both in open field and greenhouse, the following practices were performed. Each year the plants were supplied with 90 kg ha^{-1} of N, 75 kg ha^{-1} of P_2O_5 and 200 kg ha^{-1} of K_2O. A 30% fertilizers dose was given just before transplanting and the remaining 70% on dressing, by using the 6-8-18 Timac fertilizer, integrated with ammonium sulphate (20–21% N) and potassium sulphate (50% K_2O). Drip irrigation was activated when the soil available water capacity (AWC) decreased to 80%.

Pod harvest was performed every time the seeds had completed their growth, visually assessed through the complete swelling, starting with the first planting time and ending under the fifth transplant, from 6 February to 10 June in greenhouse and from 18 February to 16 June in open field, as an average of the two research years.

2.2. General analytical methods

Plant samples were random selected to assess the maximum leaf surface extension using a bench top LI-COR leaf area meter. Harvests of pods were performed to coincide with maximum seed. Growth and determinations were made in each plot of-weight and number of ripe undamaged pods classified as marketable, and also fruit mean weight on 50 unit samples. At the end of each crop cycle, assessment was performed of residual biomass, including leaves, shoots, stems, and not marketable pods. Harvest index was calculated as a ratio between marketable pods and total plant weight and it was expressed as a percentage (the residual biomass was harvested cutting it off at ground level). After harvest, residual biomass showed no fungal symptoms, therefore

Table 1
Temperatures and P.A.R. values in Naples (Italy): means of 2011–12 and 2012–13.

Time of year		Open field			Greenhouse		
		Temperature (°C)		P.A.R. ($MJ \cdot m^{-2} \cdot d^{-1}$)	Temperature (°C)		P.A.R. ($MJ \cdot m^{-2} \cdot d^{-1}$)
		min	max		min	max	
October	1–10	13.1	29.1	5.8	16.2	34.1	4.6
	11–20	11.4	26.3	5.6	16.6	28.7	4.3
	21–31	11.2	25.0	5.3	14.6	27.2	4.0
November	1–10	10.5	23.2	3.6	13.8	25.6	2.8
	11–20	9.3	21.4	3.3	9.5	23.9	2.5
	21–30	9.0	19.2	2.9	10.2	21.3	2.2
December	1–10	6.4	17.2	2.8	5.7	19.3	2.1
	11–20	6.6	16.5	2.4	5.8	18.1	1.9
	21–31	5.6	16.2	2.3	5.4	17.6	1.6
January	1–10	5.2	16.0	3.0	5.5	17.4	2.2
	11–20	4.0	14.8	3.2	3.7	16.7	2.5
	21–31	4.1	15.2	3.6	5.5	16.7	2.7
February	1–10	2.6	11.3	3.4	4.5	14.5	2.5
	11–20	1.9	13.2	4.2	3.8	18.3	3.3
	21–28 (29)	5.0	15.5	4.8	9.2	21.2	3.9
March	1–10	6.5	16.4	6.8	10.8	25.6	5.4
	11–20	7.2	17.8	7.4	11.6	27.7	6.0
	21–31	7.4	19.6	8.1	12.8	29.4	6.5
April	1–10	8.3	20.3	8.6	15.2	29.9	7.1
	11–20	9.5	22.0	9.1	17.4	30.4	7.4
	21–30	9.8	26.9	9.4	15.6	31.9	7.7
May	1–10	13.0	27.0	10.8	16.5	31.7	9.1
	11–20	11.6	26.2	10.9	16.0	31.4	9.1
	21–31	13.5	26.5	11.1	16.9	31.5	9.3
June	1–10	15.1	29.5	11.4	17.7	32.8	9.6
	11–20	17.6	32.7	12.2	19.3	34.7	10.4

samples were random collected in each plot and immediately transferred to laboratory, where they were dried in an oven at 70 °C under vacuum, at 15 kPa pressure, until they reached constant weight. After assessing the dry residue, the whole samples were carefully milled using a laboratory mill, in order to avoid segregation of materials belonging to different plant organs. The final material, composed of particles ≤1 mm diameter, was stored in air-tight bags at −20 °C and further dried just before being processed.

2.3. Lignin determination: acetyl bromide method

Biomass powder was weighed out (4 mg) into 2 cm^3 tubes. The biomass was heated at 50 °C for 3 h after adding 250 mm^3 of acetyl bromide solution (25% acetyl bromide and 75% of glacial acetic acid in volume). After the samples were cooled to room temperature, the content was transferred into 5 cm^3 volumetric flasks. A further 1 cm^3 of NaOH (2 mol dm^{-3}) was used to rinse the 2 cm^3 tubes, and then added to the 5 cm^3 flasks. 175 mm^3 of hydroxylamine HCl (0.5 mol dm^{-3}) was added to the volumetric flasks and, after vortexing, the latter were filled up to 5 cm^3 with glacial acetic acid and mixed several times. Finally, in order to measure the 280 nm UV absorbance by spectrophotometer, 100 mm^3 of each sample were diluted in 900 mm^3 of glacial acetic acid. The amount of lignin was calculated using the following formula: [absorbance/(coefficient pathlength)] · [(total volume · 100%)/ biomass weight], where coefficient = 15.69, pathlength = 1 cm, total volume = 5 cm^3, biomass weight = 4 mg.

2.4. Cellulose, hemicellulose and pectin determination

2.4.1. Holocellulose

A mixture of 240 cm^3 of water, 0.75 cm^3 of glacial acetic acid and 2.25 g of sodium chlorite were added to 7.5 g of extracted and dried sample and kept at 75 °C for 3 h.

At hourly intervals, a volume equivalent to the initial amounts of glacial acetic acid and sodium chlorite was added to the biomass. The sample obtained was filtered and washed - first with cold water, then with warm water and finally with acetone. The residue was oven-dried at 105 °C for 24 h and then weighed to calculate the content of holocellulose.

2.4.2. Pectin

1.3 g of the resulting holocellulose was treated with 26 cm^3 of potassium acetate (0.6 mol dm^{-3}) and incubated at 75 °C for 3 h before adding 26 cm^3 of ammonium oxalate (0.04 mol dm^{-3}). The suspension was kept at 75 °C for 3 h. Then, the sample was filtered and washed - with excess of water before the residue was oven-dried at 105 °C for 24 h. The pectin content was calculated as the difference between the holocellulose fraction and the above residue.

2.4.3. Cellulose and hemicellulose

A sample of holocellulose (3.8 g) was treated with 100 cm^3 of sodium hydroxide (4.4 mol dm^{-3}) at room temperature for 30 min and filtered. Then, it was washed sequentially with warm water (200 cm^3), 5 cm^3 of acetic acid (2 mol dm^{-3}) and 500 cm^3 of water. Next, the residue was oven-dried at 105 °C for 24 h and weighed, providing the cellulose fraction. The hemicellulose content was calculated by subtracting the cellulose and pectin amount from that of holocellulose.

2.5. Non cellulosic monosaccharide determination

Biomass dry powder (4 mg) was partially hydrolyzed by adding 0.5 cm^3 of trifluoroacetic acid (2 mol dm^{-3}). The vials were flushed with dry argon, mixed and heated at 100 °C for 4 h, mixing periodically. The vials were then cooled to room temperature and dried in centrifugal evaporator with fume extraction. 500 mm^3 of 2-propanol was added to the samples, and they were vortexed before drying in centrifugal evaporator. This was then repeated. Finally, the samples were resuspended in 200 mm^3 of deionised water, and the supernatant was filtered with 0.45 μm PTFE filters, and analyzed by HPAEC.

2.6. Crystalline cellulose

Biomass dry pellets after TFA hydrolysis were washed once with 1.5 cm^3 of water, and three times using 1.5 cm^3 of acetone. The dried pellets were left to air dry overnight before complete hydrolysis by adding 90 mm^3 of 72% mass fraction of sulfuric acid, incubating at 25 °C for 4 h 1.89 cm^3 of water was subsequently added and the sample was heated for 4 h at 120 °C. The glucose content of the supernatant was assessed by the colorimetric Anthrone assay, using a glucose standard curve.

2.7. Saccharification assay

Loading of plant powder into 96-well plates, using a custom-made robotic platform (Labman Automation, Stokesley, North Yorkshire, UK), and saccharification assays were performed according to Gomez et al. [10] after water, acid or alkali pretreatment. Enzymatic hydrolysis was carried out using an enzyme cocktail with a 4:1 ratio of Celluclast and Novozyme 188.

2.8. Statistical analysis

Data were processed by analysis of variance and mean separations were performed through the Duncan multiple range test, with reference to 0.05 probability level, using SPSS software version 21. Data expressed as percentage were subjected to angular transformation before processing. Correlations were performed with all pairs of chemical parameters using the software mentioned above.

3. Results

3.1. Fruit and residual biomass production

Open field fruit production was higher than in greenhouse and the fourth planting time resulted in the highest yield in open field (23.9 t ha^{-1}), whereas the highest production in greenhouse was recorded under the second to the fourth transplants (13.0 t ha^{-1}). Yield differences depended on the fruit number per plant (5.7 and 5.0 in open field and in greenhouse respectively) while the mean pod weight did not significantly change (22.1 g on average). Moreover, in the first research year it was recorded a 7.6% and 8.3% higher production of pods and of crop residual biomass respectively, compared to the second year.

As revealed by harvest index (Fig. 1), residual biomass attained high percentages of the whole crop biomass under all the experimental treatments. Notably, the lowest values of harvest index were recorded with the first planting time (8.8% of the maximum biomass yield as an average between open field and greenhouse) and the highest levels with the fourth transplant in open field (19.9% of the maximum biomass yield) and the fifth one in greenhouse (17.8% of the maximum biomass yield).

The interaction between farming system and planting time was significant on faba bean residual biomass yield (Fig. 2), which attained higher values in open field than in greenhouse, with the exception for the latest transplant when it did not vary. In open field, residual bio-

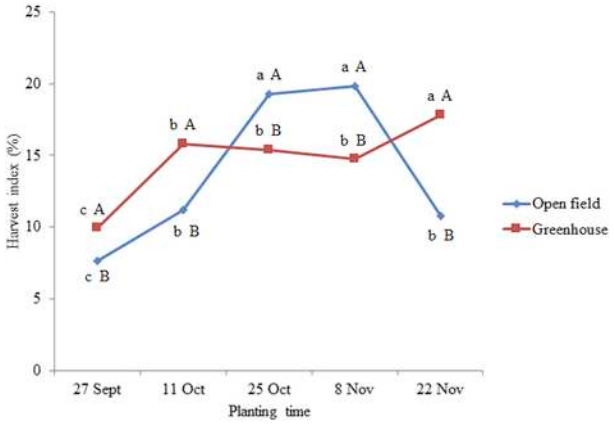

Fig. 1. Effect of the interaction between farming system and planting time on faba bean harvest index. Lowercase letters refer to comparison among planting times, whereas capital letters refer to comparison between farming systems; means followed by different letters are significantly different according to the Duncan test at $p < 0.05$.

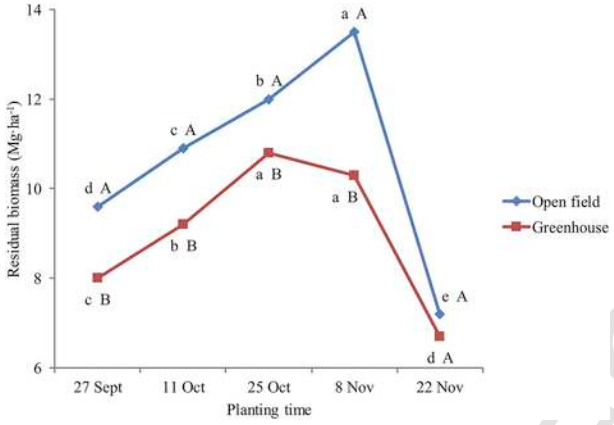

Fig. 2. Effect of the interaction between farming system and planting time on faba bean residual biomass. Lowercase letters refer to comparison among planting times, whereas capital letters refer to comparison between farming systems; means followed by different letters are significantly different according to the Duncan test at $p < 0.05$.

mass showed an increasing trend from the first to the fourth planting time, whereas in greenhouse the highest residual biomass was recorded with the third and the fourth transplant.

Table 2
Elemental composition of faba bean residual biomass as affected by farming system and planting time.

Treatment	Nitrogen		Phosphorus		Potassium	
	mass fraction of dry biomass (%)					
Farming system						
Open field	2.64		0.52		1.72	
Greenhouse	2.67		0.53		1.32	
	n.s.		n.s.		*	
Planting time						
27th September	3.18	a	0.60	a	1.71	a
11th October	2.94	b	0.56	a	1.65	a
25th October	2.54	c	0.51	b	1.49	b
8th November	2.39	cd	0.48	bc	1.40	bc
22nd November	2.23	d	0.46	c	1.34	c

n.s. not significant; * significant difference at $p \leq 0.05$. Within each column, means followed by different letters are significantly different according to the Duncan test at $p < 0.05$.

3.2. Elemental and cell wall composition of residual biomass

As reported in Table 2, no significant differences were recorded in nitrogen and phosphorus concentration between faba bean residual biomass grown in open field and under greenhouse, whereas potassium accumulation was better affected by open field conditions (+30.3% compared to the protected environment). Moreover, all the three elements showed a gradual decrease from the first to the fifth planting time, the difference magnitude being as much as - 29.9, - 23.3 and - 21.6% for nitrogen, phosphorus and potassium respectively.

The cell wall composition of faba bean residual biomass in open field and greenhouse growing conditions with five planting times is shown in Table 3. Notably, compared to open field, greenhouse crops showed significantly ($p \leq 0.05$) higher content of lignin (16.4 vs 15.3% of total biomass), hemicelluloses (15.1 vs 13.9% of total biomass) and pectin (13.4 vs 12.5% of total biomass). Open field grown biomass showed higher cellulose content (46.0 vs 44.5% of total biomass), whereas no difference in crystalline cellulose was detected between the two farming systems.

The biomass composition varied among different planting times. Lignin content showed a gradual decrease from the first (17.7% of total biomass) to the last transplant (13.7% of total biomass); similar trend

Table 3
Chemical composition of faba bean residual biomass as affected by farming system and planting time.

Treatment	Lignin		Total cellulose		Crystalline cellulose		Hemicellulose		Pectin	
	mass fraction of dry biomass (%)									
Farming system										
Open field	15.3		46.0		12.0		13.9		12.5	
Greenhouse	16.4		44.5		12.0		15.1		13.4	
	*		*		n.s.		*		*	
Planting time										
27th September	17.7	a	41.1	d	10.9	d	14.9		14.1	a
11th October	17.2	a	42.7	c	11.3	cd	14.6		13.7	ab
25th October	16.1	b	45.9	b	12.0	bc	14.5		13.2	b
8th November	14.5	c	47.8	a	12.8	ab	14.3		12.4	c
22nd November	13.7	c	48.7	a	13.0	a	14.2		11.5	d
							n.s.			

n.s. not significant;* significant difference at $p \leq 0.05$. Within each column, means followed by different letters are significantly different according to the Duncan test at $p < 0.05$.

was recorded for pectin (from 14.1 to 11.5% of total biomass). Conversely, cellulose and crystalline cellulose percentage increased with the planting delay (from 41.1 to 48.7% biomass fraction, and from 10.9 to 13.0% biomass fraction for cellulose and crystalline cellulose respectively), whereas hemicelluloses were not affected by the planting time.

In the present work, lignin showed positive correlation with pectin ($r = 0.61$ at $p < 0.01$), but a negative correlation with total cellulose ($r = -0.73$ at $p < 0.01$) and with crystalline cellulose ($r = -0.68$ at $p < 0.01$). Total and crystalline cellulose, in turn, were positively correlated to each other ($r = 0.72$ at $p < 0.01$), but negatively correlated with pectin ($r = -0.59$ at $p < 0.01$ and $r = -0.63$ at $p < 0.01$ respectively). Finally, no examined component showed statistically significant correlation with hemicellulose.

The monosaccharide composition in the hemicellulosic fraction is showed in Table 4. Glucose is the most represented monosaccharide (44.6%), followed by xylose (26.0%) and galactose (9.6%), whereas glucuronic acid and fucose attained the lowest values (0.29 and 0.27% respectively). The farming system showed a significant effect on most hemicellulosic monosaccharides, as arabinose, fucose and mannose attained higher levels under greenhouse, whereas galacturonic acid and mannose contents were higher in the open field conditions. With regard to planting times, different trends were recorded: some sugars decreased from the first to the last planting time, such as arabinose (from 6.8 to 6.2%), fucose (from 0.32 to 0.21%), glucose (from 45.9 to 43.2%) and rhamnose (from 2.8 to 2.3%); galacturonic acid and mannose increased with the planting delay (from 4.5 to 6.4% and from 3.9 to 5.5% respectively); galactose, glucuronic acid and xylose were not significantly affected by planting time.

3.3. Effects of farming system, planting time, and different biomass pretreatments on the saccharification potential of residual biomass

Saccharification potential, a determination of how easily a biomass feedstock can be hydrolyzed to fermentable sugars, provides a parameter to evaluate how different agricultural practices impact on biomass quality. The saccharification potential was found to be adversely correlated with lignin ($R^2 = 0.59$), but positively correlated with total cellulose ($R^2 = 0.55$) and crystalline cellulose ($R^2 = 0.60$). Hemicellulose content and pectin content are also inversely correlated with the rate of saccharification ($R^2 = 0.50$ and $R^2 = 0.62$, respectively).

No significant effects of farming system on saccharification rate of residual crop biomass was recorded. Otherwise, planting time showed a significant effect on this variable (Table 5), as the glucose yield increased from the earliest transplanting performed on 27 September ($41.0\ g\ kg^{-1}\ h^{-1}$) to the latest one carried out on 22 November ($46.7\ g\ kg^{-1}\ h^{-1}$).

Since saccharification is deeply determined by the type of pretreatment applied, we choose three different types of pretreatments (alkaline, acid and water) to determine the saccharification potential across our experiments. The type of pretreatment significantly affected the saccharification rate (Table 5). Alkaline pretreatment showed the best glucose yield of $60.7\ g\ kg^{-1}\ h^{-1}$ (based on dry biomass), whereas water pretreatment provided the lowest saccharification rate ($27.6\ g\ kg^{-1}\ h^{-1}$ of glucose).

4. Discussion

In southern Italy, transplant carried out prior to mid-October is conditioned by high temperature, exceeding faba bean tolerance threshold, which caused 20%–30% plant failure in open field and greenhouse respectively, as well as a decrease in pod production. In Australia, Loss and Siddique [11] reported a decreasing trend of faba bean yield as a function of planting delay, conversely to the increase described in the present work from the first to the fourth planting time in open field. Lower *Vicia faba* biomass yields than the values recorded in the best treatments of our trial have been previously published, showing that our trial covers near optimal growth conditions [12,13]. Moreover, we observed a higher residual biomass production in open field than in greenhouse, with a maximum in the third and fourth transplant for greenhouse and in the fourth planting time for open field; the latter experimental treatment also resulted in the highest harvest index value.

In our research, the residual biomass composition showed statistically significant differences between greenhouse and open field as well as among planting times. Consistently with our findings, Mahmood et al. [14] reported a decrease in biomass lignin and hemicellulose content with the planting delay in spring grown sorghum, and Gatta et al. [12] found similar hemicellulose values to those recorded in the present work. Additionally, compared to Pakarinen et al. reports [9], the composition of faba bean residual biomass detected in our analyses shows a higher amount of lignin and hemicellulose, but the same prevalence of glucans and arabinoxylans in hemicellulose.

The differences in biomass composition observed in our experiments had a crucial impact on the saccharification potential of the residual biomass. Our results show that biomass enzymatic digestibility is affected by plant cell wall composition and the type of pretreatment employed. In sugarcane [15], reduction in total lignin by 6% improved saccharification efficiency by 19%–23% with no significant difference in biomass yield. However, 8% and 12% lignin reduction compromised biomass yield, but increased saccharification efficiency by 28%–32% compared with control plants. Lima et al. [16] reported a positive linear correlation between glucose production and cellulose crystallinity index in Eucalyptus, whereas in the same species an opposite correlation was found by Sun et al. [17]. However, in spite of the above contrasting reports, in our research we correlate the percentage of crystalline cellulose as a biomass basis and not as an index [18]. In sweet sorghum, enzymatic hydrolysis was more effective for forage sorghums with a low crystalline cellulose index and easily transformed crystalline cellulose to amorphous cellulose, despite initial cellulose content [19].

Previous work has shown [20] that glucose yield obtained by enzymatic hydrolysis following alkaline pretreatment was adversely correlated with hemicellulose content. However, other authors [21] reported that increase in total hemicellulose content resulted in enhanced biomass digestibility in Miscanthus after acid and alkaline pretreatments. Our results in faba biomass show that higher hemicellulose content reduces saccharification (Table 3).

Alkaline pretreatment produced the highest levels of saccharification in faba bean biomass, irrespective of the growth condition. The action of alkaline pretreatment is the saponification and linkages cleavage in lignin and in lignin-polysaccharides complex [22]. This increases the cell wall structure porosity, internal surface area and swelling, decreases the cellulose polymerization and crystallinity, and facilitates the hydrolysis of cellulose [23]. We have previously shown that alkaline pretreatment enhances the enzymatic digestibility of tomato residual biomass, compared to acid or hot water pretreatment [24]. Both faba bean and tomato are annual dicots with uncondensed lignin that can be removed by alkali pretreatment.

As above described, faba bean residual biomass valorization for biofuel production is a current worthy prospect of this resource, though it has got a good fertilization potential due to the remarkable content of nitrogen, phosphorus and potassium as observed in the present research. However, the use as a green manure for supplying nutrients to the next crop is neither usually practised in wealthy Countries, due to the higher use efficiency of chemical fertilizers than the legume derived extra N, nor in the poor ones such as African areas [25]. In addition, the nitrogen supply from legume residual biomass is strictly dependent on the variety, i.e. atmospheric nitrogen fixation may not re-

Table 4
Hemicellulose monosaccharide composition in faba bean residual biomass as affected by farming system and planting time.

Treatment	Ara		Fuc		Gal	Gal A		Glc		Glc A	Man		Rha		Xyl
	mass fraction of hemicellulose (%)														
Farming system															
Open field	6.3		0.22		9.5	5.7		44.3		0.29	4.3		2.9		26.4
Greenhouse	6.8		0.32		9.7	5.0		44.9		0.28	5.0		2.3		25.5
	*		*		n.s.	*		n.s.		n.s.	*		*		n.s.
Planting time															
27th September	6.8	a	0.32	a	9.8	4.5	d	45.9	a	0.29	3.9	d	2.8	a	25.5
11th October	6.8	a	0.31	a	9.8	4.8	d	45.3	ab	0.28	4.1	cd	2.8	a	25.8
25th October	6.5	ab	0.27	b	9.6	5.2	c	44.5	bc	0.28	4.6	bc	2.6	ab	26.1
8th November	6.4	ab	0.23	c	9.5	5.7	b	44.0	cd	0.28	4.9	ab	2.5	bc	26.2
22nd November	6.2	b	0.21	c	9.3	6.4	a	43.2	d	0.28	5.5	a	2.3	c	26.3
					n.s.					n.s.					n.s.

Ara, arabinose; Fuc, fucose; Gal, galactose; Gal A, galacturonic acid; Glc A, glucuronic acid; Glc, glucose; Man, mannose; Rha, rhamnose; Xyl, xylose.
n.s. not significant; * signicant difference at $p \leq 0.05$. Within each column, means followed by different letters are significantly different according to the Duncan test at $p < 0.05$.

Table 5
Saccharification rate in faba bean residual biomass as affected by pretreatment, farming system and planting time.

Treatment	Saccharification rate (glucose from dry biomass) g kg^{-1} h^{-1}	
Pretreatment type		
Water	27.6	c
Acid	43.6	b
Alkaline	60.7	a
Planting time		
27th September	41.0	c
11th October	41.9	c
25th October	44.5	b
8th November	45.7	ab
22nd November	46.7	a

n.s. not significant; * significant difference at $p \leq 0.05$.
Within each column, means followed by different letters are significantly different according to the Duncan test at $p < 0.05$.

sult in net positive contribution to soil nitrogen, either due to a high nitrogen harvest index of the varieties available or when the plant-N derived from atmospheric nitrogen is much lower than nitrogen harvest index [25]. Finally, soil nitrogen is available for uptake by a subsequent crop if there is no nitrate leaching downwards out of the next crop root expansion.

Sustained biomass supply represents one of the main constraints for the development of biorefineries. Seasonal variations in biomass availability are one of the reasons that hold the developments in this area in Europe. The present work determined the differences that planting time and growth system produce in the yield and saccharification potential in faba residual biomass, as a step to explore a biorefinery model where the biomass supply could be achieved by using different crop residues and managing practises to maximise yield of both, bean and residues.

5. Conclusions

Management of the farming system and planting time in faba bean crops produced in Mediterranean environment represents an interesting way to valorize residual biomass. In comparison to greenhouse management, open field growing resulted in better performance, achieving the highest fruit and residual biomass outcome with early November transplanting. Moreover, open field grown biomass showed higher cellulose fraction, leading to a higher saccharification rate. Overall, our results suggest that the choice of farming system and planting time can affect the processability of faba bean residual biomass which may affect agricultural practises and potential valorization of the faba bean residual biomass.

Acknowledgements

A short-mobility grant was provided by the University of Naples "Federico II" for carrying out analyses at Center for Novel Agricultural Products, University of York (United Kingdom). The authors wish to thank Dr. Carlo Borrelli, Mr. Antonio Cuciniello, Mr. Roberto Maiello, Dr. Giuseppe Melchionna, Mr. Rosario Nocerino and Dr. Silvano Somma for their assistance with the field and laboratory equipment.

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