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Full Length Research Paper

Chemical composition and antioxidant capacity in edible biomass of tagasaste (*Chamaecytisus proliferus* var. *palmensis*)

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Tagasaste (*Chamaecytisus proliferus* (L. fit.) Link var. *palmensis* (Christ) Hansen and Sunding is a perennial fodder shrub naturalized in a range of environments for its valuable forage traits. This research was aimed at evaluating the seasonal variation in chemical composition, bioactive compounds and antioxidant properties in edible biomass components (leaves, twigs, flowers, and pods) of tagasaste grown in a Mediterranean environment of Sardinia (Italy). Season influenced the composition of edible biomass and chemical composition and antioxidant capacity of leaves and twigs. On average, crude protein concentration was 200, 97 and 66 g kg^{-1} dry matter, in leaves, twigs and mature pods, respectively. Neutral and acid detergent fibre were high in twigs. In late summer, antioxidant capacity of leaves reached 44 mmol/100 g dry weight of Trolox equivalent and was correlated with the levels of total phenolics and flavonoids. Research highlights that the edible biomass of tagasaste is a high quality feed source available during the most critical forage shortage for rainfed farming systems of Mediterranean climatic areas.

Key words: Bioactive compounds, forage quality, plant parts, polyphenols, tree lucerne.

INTRODUCTION

Browse forages and multipurpose trees are widely used in Southern Europe, Eastern Mediterranean region and African savannas, to alleviate feed shortages and supply vital nutrients for growth and reproduction for ruminants, during long and dry periods (Assefa et al., 2008; Franzel et al., 2008; Papanastasis et al., 2008; Kökten et al.,

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2012; Fomum et al., 2015). The indigenous *Chamaecytisus* from the Canary Islands comprises a taxonomic complex (Francisco-Ortega et al., 1992); the form that is endemic to La Palma is commonly known as "tagasaste" (*Chamaecytisus proliferus* (L. fit.) Link var. *palmensis* (Christ) Hansen and Sunding), which is cultivated as a fodder tree and has achieved agricultural importance around the world, particularly in areas of Australia and New Zealand (Chinea et al., 2013). Tagasaste has become naturalized in Java, the Hawaiian Islands, California, Chile, North Africa, Ethiopia, Kenya, Tanzania and South Africa (Francisco-Ortega et al., 1991). Since 1879, it has been grown as a drought-resistant fodder plant in Australia (Dann and Trimmer, 1989).

It has been reported that this fodder tree also appears to be well suited to the drained sandy soils in both the winter and summer rainfall regions of South Africa (Meissner, 1997; Lindeque and Rethman, 1998). According to Assefa (1998), tagasaste could be the best browse tree for the highlands of East Africa. A marked feature of this shrub species is its ability to retain evergreen leaves and hence maintain a relatively high nutritive value during the dry season (González-Rodrıguez et al., 2005). Edible biomass of tagasaste is represented by leaves and other parts such as twigs (that is, fine stems) and pods (Becholie et al., 2005). Based on its favourable chemical composition, tagasaste may have a role as a high quality feed source and/or supplement for ruminants, which could be used to correct the critical seasonal shortage of forage caused by prolonged dry periods and harsh environmental conditions (Varvikko and Khalili, 1993; Assefa, 1998; Kumara Mahipala et al., 2009a; Kitaw et al., 2012).

Increasing attention is being paid to forage secondary compounds such as polyphenols and condensed tannins, which can reduce intestinal infections in grazing animals (Marley et al., 2003; Piluzza et al., 2013). Polyphenols are among the most significant compounds related to the antioxidant properties of plant materials. This information could be very helpful in establishing relationships such as between forage polyphenol contents and their feeding values for ruminants. Unfortunately, there is limited knowledge regarding the antioxidant capacity of tagasaste biomass in spite of its potential.

In the last decade, tagasaste germplasm has been introduced to Sardinia, Italy, where a private breeder has also started a selection program.

As different plant organs constitute the edible biomass of tagasaste, a study of their relative contribution and chemical composition is necessary in view of the local exploitation of this valuable leguminous shrub. We hypothesized that growth seasonal conditions affect biomass components ratios and chemical composition of plant organs in tagasaste. Therefore, our specific objectives were to investigate the relative contribution of leaves, twigs, flowers and pods to the total edible biomass of tagasaste and their seasonal chemical composition, bioactive compounds content (total polyphenols, non-tannic phenolics, tannic phenolics, flavonoids, condensed tannins) and antioxidant capacity.

MATERIALS AND METHODS

The field experiments were conducted during 2013 to 2014 in Southern Sardinia (39° 31' N, 8° 51' E, Italy), where the climate is Mediterranean with mild winter. The area has a long-term average annual rainfall of 446 mm received mainly in the autumn and winter months, and a mean annual air temperature of 17.6°C. The total rainfall from September 2013 to August 2014 did not substantially differ from climatic data. The soil at the experimental site, classified as *Typic Fluvaquents*, is sandy-clay-loam, with pH 7.8, sufficient average nitrogen content of 1.1‰ and phosphorous 16.2 ppm.

Plant material, sampling and measurements, chemical composition

Tagasaste used in the study was grown on experimental plots of the Council for Agricultural Research and Economics (Sanluri, Italy). Plots size consisted in tagasaste plants (three year old) each spaced 2.5 m between rows and 2 m apart within rows, under a randomised block design with three replicates. The trees had never been pruned, with the exception of eliminating the branches on the basal part of the plant (up to 50 cm in height). No mechanical intervention on the soil, fertilisation, herbicide application or irrigation was applied. At bi-monthly intervals, starting from November 2013 and ending in September 2014, three undisturbed plants per plot were pruned at a cut height of 50 cm. Sampling months corresponded to late autumn (November), winter (January), early spring (March), late spring (May), summer (July) and late summer (September), respectively. Plant aerial biomass was separated into branches, lignified stems and edible biomass. Edible biomass was then subdivided in leaflets (thereinafter referred as leaves), young twigs (thin stems < 3 mm of diameter), flowers, green pods, and mature (that is, brown-dry) pods. Tagasaste leaves, twigs and reproductive organs were immediately weighted to determine fresh weight and the contributions of each plant component. Phytomass sub-samples were oven dried at 65°C for 48 h, then ground to 1 mm screen to be analysed for quality traits. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Neutral and acid detergent fibres (NDF and ADF) and acid detergent lignin (ADL) were determined by using the procedure of Van Soest et al. (1991) and ether extract (EE) using Soxhlet extraction.

Total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_l) of edible biomass were estimated according to the following equations adapted from Lithourgidis et al. (2006) and Sadeghpour et al. (2014):

$TDN = (-1.291 \times ADF) + 101.35$
$DMI = 120 / \%NDF$ dry matter basis
$DDM = 88.9 - (0.779 \times \%ADF$, dry matter basis)
$RFV = \%DDM \times \%DMI \times 0.775$
$NE_l = (1.044 - (0.0119 \times \%ADF) \times 2.205$

Antioxidant capacity and bioactive compounds

Harvested subsamples of edible biomass components were kept on

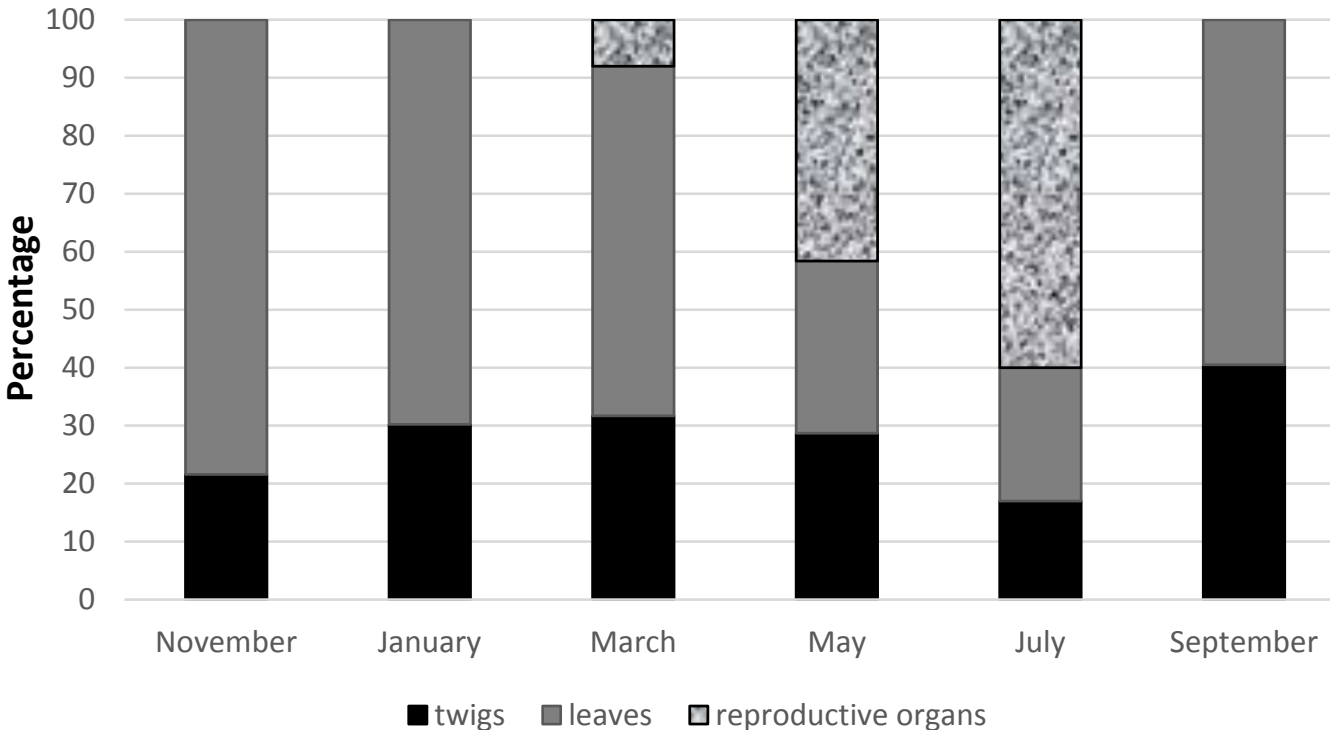


Figure 1. Contribution of twigs, leaves and reproductive organs to the edible biomass of tagasaste.

ice, freeze dried and ground to a fine powder for chemical analysis. The powdered material was then used for extract preparation following Piluzza et al. (2014). Antioxidant capacity was determined by means of the improved ABTS ((2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)) and by DPPH (1,1-diphenyl-2-picrylhydrazyl) (Surveswaran et al., 2007; Piluzza and Bullitta, 2011) assays. Trolox, a water-soluble analogue of vitamin E was used as the reference standard. The results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC), as mmol Trolox equivalents per 100 g dry weight of plant material (mmol TEAC/100 g DW).

Total phenolics (TotP), non-tannic phenolics (NTP) and tannic phenolics (TP) were determined using the Folin Ciocalteau colorimetric assay according to procedures previously described by Sulas et al. (2016). Results were expressed as g gallic acid equivalent (GAE) kg^{-1} dry weight of plant material (g GAE kg^{-1} DW).

Total flavonoids (TotF) were quantified by colorimetric assay using Aluminium trichloride, following procedures previously reported (Piluzza and Bullitta, 2011). Catechin was used as a standard and the flavonoid content was expressed as g catechin equivalent kg^{-1} dry weight of plant material (g CE kg^{-1} DW).

For each bi-monthly sampling, three subsamples of each available plant organ were analysed and all the assays were performed in triplicate.

Statistical analysis

The data were subjected to analysis of variance, using Statgraphics Centurion XVI version (StatPoint Technologies Inc., 2009), considering month of sampling as independent variable and forage quality parameters, antioxidant capacity and bioactive compounds as dependent variables. Differences between means were assessed by the Fisher's least significant difference (LSD) procedure for means separation. Coefficients of determination (R^2) were calculated using Microsoft Excel 2000.

RESULTS

Edible biomass and its chemical composition

The contribution of leaves to the total edible biomass exceeded 60% from September to March, representing the most important component, whereas the contribution of twigs ranged from 17 to about 40% (Figure 1). Flowering started in March (early spring) and mature pods represented about 60% of edible biomass in July, making them a relevant component in summer.

The time of sampling significantly affected chemical composition of leaves and twigs (Tables 1 and 2). Dry matter content in leaves ranged from 268 (January) to about 400 g kg^{-1} fresh sample in July and September (Table 1). Leave dry matter content recorded in January, March and May was significantly lower than in the remaining months. Crude protein concentration peaked in January (254 g kg^{-1} DM), followed by March and significantly decreased in May and July to about 170 g kg^{-1} DM, with intermediate values in September. Except for January, when NDF reached the highest value (522.3 g kg^{-1} DM), the remaining NDF values did not significantly differ. ADF showed a quite similar trend. ADL content ranged from 70 (May) to 149 (January) g kg^{-1} DM, the

Table 1. Bi-monthly trend of chemical composition (g kg^{-1} DM) in tagasaste leaves: Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ether extract (EE) and ash.

Cutting	DM	CP	NDF	ADF	ADL	EE	Ash
	(g kg^{-1} DM)						
Nov	362.9^{a}	191.0^{cd}	370.6^{b}	193.9^{c}	86.8^{b}	30.7^{cd}	70.1^{b}
Jan	267.7^{b}	254.7^{a}	522.3^{a}	296.9^{a}	149.6^{a}	35.2^{b}	72.4^{b}
Mar	287.5^{b}	229.5^{b}	387.0^{b}	203.0^{bc}	100.6^{b}	34.8^{bc}	57.8^{c}
May	304.8^{b}	173.6^{d}	396.1^{b}	219.9^{bc}	69.9^{b}	41.7^{a}	81.9^{a}
Jul	392.9^{a}	172.0^{d}	390.4^{b}	231.5^{b}	110.3^{ab}	38.7^{ab}	62.0^{c}
Sep	395.6^{a}	195.2^{c}	338.0^{b}	209.4^{bc}	74.8^{b}	29.3^{d}	44.9^{d}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 2. Bi-monthly trend of chemical composition (g kg^{-1} DM) in tagasaste twigs: Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ether extracts (EE) and ash.

Cutting	DM	CP	NDF	ADF	ADL	EE	Ash
	(g kg^{-1} DM)						
Nov	394.7^{d}	96.9^{a}	623.8^{b}	409.6^{c}	122.7^{b}	34.4^{a}	54.2^{a}
Jan	288.3^{f}	112.1^{a}	675.2^{a}	463.2^{ab}	151.9^{a}	22.1^{d}	38.0^{cd}
Mar	325.8^{e}	97.8^{a}	630.9^{b}	448.9^{b}	101.3^{c}	13.5^{e}	46.3^{b}
May	425.0^{c}	72.3^{b}	664.5^{a}	465.5^{ab}	126.0^{b}	22.9^{cd}	37.1^{cd}
Jul	485.6^{a}	99.9^{a}	630.1^{b}	481.3^{a}	126.3^{b}	26.1^{bc}	33.6^{d}
Sep	445.5^{b}	100.8^{a}	680.2^{a}	485.2^{a}	126.1^{b}	39.8^{b}	38.6^{c}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 3. Total digestible nutrients (TDN), digestible dry matter (DDM), digestible dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_l) of tagasaste leaves.

Cutting	TDN (g kg^{-1} DM)	DDM (g kg^{-1} DM)	DMI (g kg^{-1} of body weight)	RFV (%)	NE_l (Mcal kg^{-1})
Nov	763^{a}	738^{a}	32	185^{a}	1.793^{a}
Jan	630^{c}	658^{c}	23	117^{b}	1.523^{c}
Mar	751^{ab}	731^{ab}	31	182^{a}	1.769^{ab}
May	730^{ab}	718^{ab}	30	171^{a}	1.725^{ab}
Jul	715^{b}	709^{b}	31	174^{a}	1.695^{b}
Sep	743^{ab}	726^{ab}	35	200^{a}	1.753^{ab}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

latter significantly higher than the remaining values, except for July. Ash content ranged from 45 to 82 g kg^{-1} DM.

In twigs, dry matter content significantly increased from January to July, reaching 486 g kg^{-1} fresh sample (Table 2). Crude protein concentration ranged from 72 to 112 g kg^{-1} DM and the value recorded in May was significantly lower than the remaining. Significant but slight variations were found in the values of NDF. ADF values also showed significant but limited variations. ADL content ranged from 101 (March) to 152 (January) g kg^{-1} DM. Ether extract varied from 14 to 34 g kg^{-1} DM and ash content from 34 to 54 g kg^{-1} DM. Overall, chemical composition of tagasaste twigs showed little variation across the year.

The chemical composition of flowers was similar to that of leaves at the same cutting time (March); the CP concentration of green pods reached 126 g kg^{-1} DM, whereas it decreased to 66.6 g kg^{-1} DM in mature pods. The values of NDF of both green and mature pods were within the range recorded for twigs.

In leaves, the lowest values of TDN, DDM, DMI, and NE_l were recorded in January (Table 3). TDN ranged

Table 4. Total digestible nutrients (TDN), digestible dry matter (DDM), digestible dry matter intake (DMI), relative feed value (RFV) and net energy for lactation (NE_l) of tagasaste twigs.

Cutting	TDN (g kg^{-1} DM)	DDM (g kg^{-1} DM)	DMI (g kg^{-1} of body weight)	RFV (%)	NE_l (Mcal kg^{-1})
Nov	485^{a}	570^{a}	19^{a}	85^{a}	1.227^{a}
Jan	416^{bc}	528^{bc}	18^{b}	73^{cd}	1.087^{bc}
Mar	434^{b}	539^{b}	19^{a}	80^{ab}	1.124^{b}
May	412^{bc}	526^{bc}	18^{b}	74^{cd}	1.080^{bc}
Jul	392^{c}	514^{c}	19^{a}	76^{bc}	1.039^{c}
Sep	387^{c}	511^{c}	18^{b}	70^{d}	1.028^{c}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 5. Bi-monthly trend of trolox equivalent antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of tagasaste leaves.

Cutting	TEAC(mmol/100 g DW)		TotP	NTP	TP	TotF
	ABTS	DPPH	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)
Nov	14.4^{b}	22.2^{b}	76.8^{b}	65.4^{b}	11.3^{b}	43.5^{b}
Jan	8.6^{c}	9.5^{d}	48.5^{d}	45.2^{d}	3.3^{c}	21.3^{d}
Mar	11.5^{bc}	10.2^{d}	65.6^{c}	54.1^{c}	11.5^{b}	36.6^{bc}
May	7.0^{c}	7.1^{d}	49.7^{d}	40.3^{d}	9.4^{bc}	20.5^{d}
Jul	9.1^{bc}	15.2^{c}	75.4^{b}	56.4^{c}	19.6^{a}	30.2^{c}
Sep	34.2^{a}	44.3^{a}	112.1^{a}	95.6^{a}	16.5^{ab}	67.7^{a}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

Table 6. Bi-monthly trend of trolox equivalent antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of tagasaste twigs.

Cutting	TEAC (mmol/100 g DW)		TotP	NTP	TP	TotF
	ABTS	DPPH	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)
Nov	5.0^{b}	3.5^{bc}	23.0^{ab}	17.3^{bcd}	5.8^{a}	5.7^{d}
Jan	3.9^{bc}	3.2^{c}	18.2^{cd}	16.4^{cd}	1.8^{b}	7.7^{c}
Mar	2.8^{c}	2.7^{c}	16.6^{d}	14.0^{d}	2.6^{b}	5.4^{d}
May	3.9^{bc}	2.6^{c}	21.2^{bc}	18.4^{abc}	2.8^{b}	9.0^{b}
Jul	4.8^{b}	4.4^{b}	23.3^{ab}	21.4^{a}	1.9^{b}	7.7^{bc}
Sep	6.9^{a}	5.9^{a}	25.8^{a}	21.0^{ab}	4.8^{a}	11.3^{a}

Values with different letters in a column are significantly different at $p \leq 0.05$ (Fisher's test).

from 630 (January) to 763 (November) g kg^{-1} DM and variations were related to the ADF concentration. DDM ranged from 738 (November) to 658 (January). DMI, which is negatively correlated with NDF, ranged from 23 to 35. Except in the January sampling, RFV exceeded 151 value, which is indicative of a prime forage, and a similar trend was recorded for NE_l. In twigs, the most favourable values of TDN, DDM, RFV and NE_l were recorded in November. The value of RFV was on average 80 (Table 4).

Trolox equivalent antioxidant capacity (TEAC) and phenolic contents

The content of phenolics and the antioxidant activities detected by means of the two *in vitro* assays (ABTS, DPPH) on the tagasaste leaves and twigs for each cutting date are shown in Table 5 and 6. Statistically significant differences among sampling months where found for total antioxidant capacity, TotP, NTP, TotF. In leaves, both ABTS and DPPH assays exhibited wide

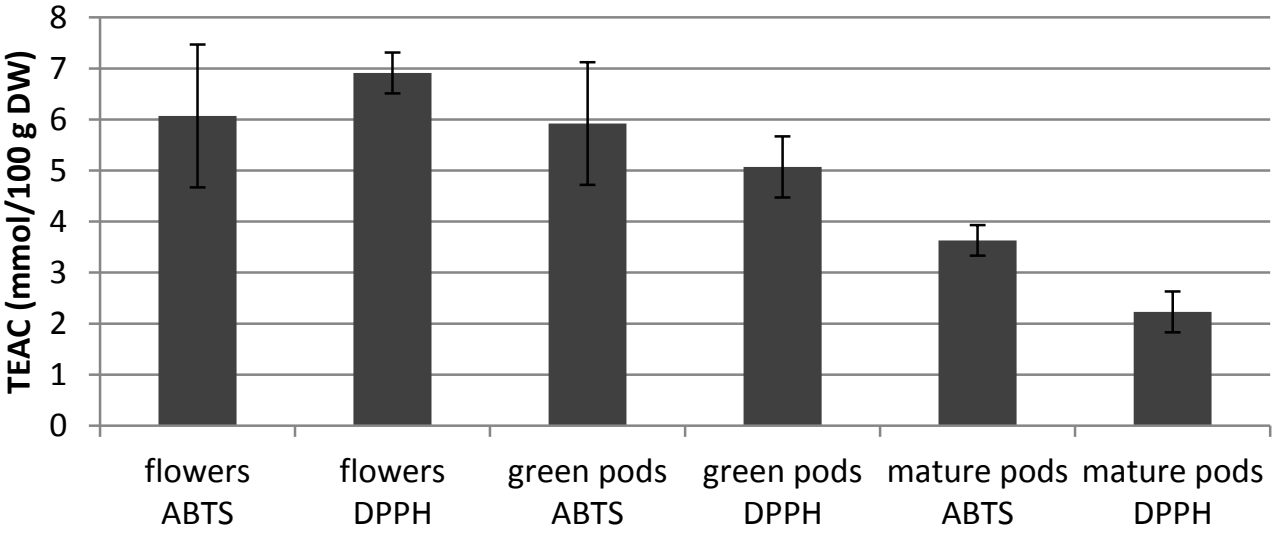


Figure 2. Antioxidant capacity in flowers and pods of tagasaste (Vertical bars indicate standard deviations of means).

seasonal variations of antioxidant capacities from May to September (Table 5). The highest antioxidant capacities were observed in late summer, and the values (34.2 and 44.3 mmol TEAC/100 g DW, respectively) were five to six-fold higher than those recorded in late spring. Total phenolics (TotP) ranged from 48.5 (January) to 112.1 (September) g GAE kg^{-1} DW. High TEAC values corresponded to high TotP contents, and low TEAC values to lower TotP contents. The seasonal trend also showed differences for the contents of Non-tannic phenolics (NTP) from 40.3 (May) to 95.6 (September) g GAE kg^{-1} DW, Tannic phenolics (TP) from 3.3 (January) to 19.6 g GAE kg^{-1} DW (July) in leaves. Total flavonoids (TotF) of leaves ranged from 20.5 (May) to 67.7 (September) g CE kg^{-1} DW.

In twigs, both ABTS and DPPH assays exhibited wide seasonal variations of antioxidant capacities from March/May to September, when again the highest values (6.9 and 5.9 mmol TEAC/100 g DW, respectively) were recorded (Table 6). However, they were about twice as high as the early spring values. Total phenolics (TotP) ranged from 16.6 (March) to 25.8 (September) g GAE kg^{-1} DW, whereas the contents of NTP from 14.0 to 21.1 g GAE kg^{-1} DW at the same cutting times. The values of TP varied from 1.8 (January) to 5.8 g GAE kg^{-1} DW (November) and TotF from 5.4 (May) to 11.3 (September) g CE kg^{-1} DW.

The antioxidant capacity of flowers and green and mature pods of tagasaste is shown in Figure 2. Both ABTS and DPPH assays evidenced high TEAC values in flowers and green pods compared to mature pods. The concentrations of TotP, NTP and TP in flowers were high (Figure 3).

The correlations between the antioxidant activity and TotP, NTP, TP and TotF (Table 7) were highly significant for the different months, except for TP in January, May and September (with ABTS), and in September (with DPPH), when no significant correlations were found.

DISCUSSION

The six consecutive samplings, carried out at bi-monthly intervals across a complete productive year, indicated quantitative and qualitative variations occurred in the edible biomass of tagasaste. In particular, leaves resulted the predominant component of the edible biomass during the vegetative growth, whereas the relative contribution of pods in summer was important. Season affected the chemical composition of edible biomass and the lowest CP values of leaves were recorded in May (late spring) and July (summer). However, the above-mentioned CP values, coupled with moderate levels of NDF, indicated a high quality feed source, which is also available during the most critical forage shortage for Mediterranean farming systems.

Overall, results indicate that quality of tagasaste remained high during all the year. Regarding seasonal chemical composition of tagasaste, our data if expressed as averaged means of leaves and twigs are quite similar to those reported from South Africa, Canary Islands, Ethiopia, Australia and New Zealand (Borens and Poppi, 1990; Lindeque and Rethman, 1998; Ventura et al., 2002; Becholie et al., 2005; Assefa et al., 2012). However, only few authors have reported a separate analysis for each component of edible biomass, as in this study. In addition, less pronounced changes in phenological stages (that is, no pod production) were noticed under different environmental conditions (Assefa et al., 2012) and different methodological approaches were used sometimes, by including stems up to 6 mm diameter in the edible biomass. As regard Mediterranean conditions

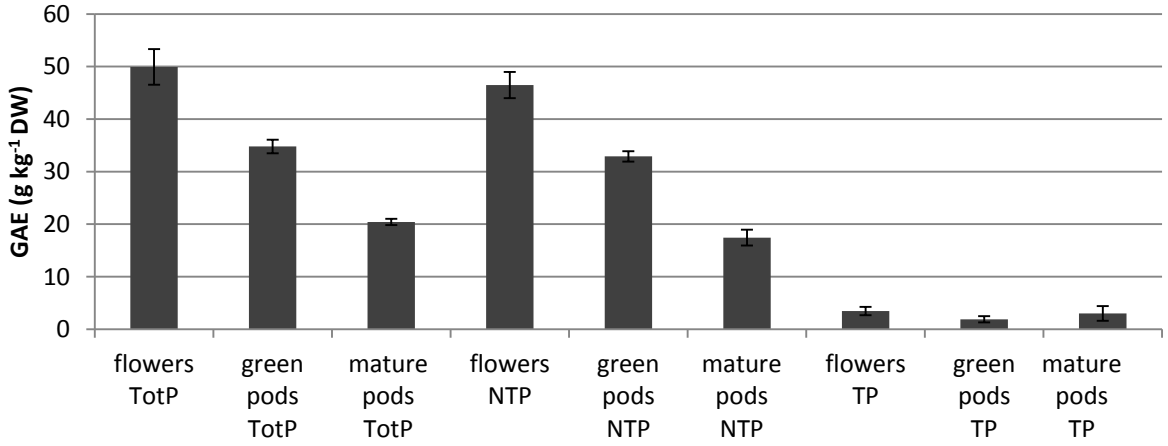


Figure 3. Polyphenolic contents in flowers and pods of tagasaste (Vertical bars indicate standard deviations of means).

Table 7. Correlations (R^2) established between total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), flavonoids (TotF) and antioxidant capacity (ABTS, DPPH) in tagasaste.

Parameter	ABTS						DPPH					
	Nov	Jan	Mar	May	Jul	Sep	Nov	Jan	Mar	May	Jul	Sep
TotP	0.918**	0.855**	0.784**	0.746**	0.892**	0.987**	0.991**	0.880**	0.836**	0.886**	0.940**	0.971**
NTP	0.902**	0.855**	0.697**	0.809**	0.886**	0.961**	0.989**	0.860**	0.793**	0.889**	0.925**	0.995**
TP	0.914**	0.451ns	0.789**	0.271ns	0.869**	0.634ns	0.868**	0.710*	0.634*	0.445*	0.934**	0.489ns
TotF	0.919**	0.849**	0.917**	0.830**	0.810**	0.956**	0.981**	0.914**	0.766**	0.804**	0.849**	0.984**

** Significance level at P ≤0.001, * Significance level at P ≤0.05.

of Sardinia, crude protein level of standing hay (that is, ungrazed dry residuals) decreased to 4% in summer, with relevant reduction in nutritive value (Sulas et al., 1995). It is worth nothing that the lowest CP values recorded in tagasaste leaves and twigs during summer were 17.2 and 7.2%, respectively. Moreover, only in January, the RFV of tagasaste leaves was less than 151 value, which is indicative of a prime forage. In addition, our study evidenced that tagasaste pods represent a complement instead of an alternative to green leaves and twigs on offer. In contrast to herbaceous plants, browse has the advantage of maintaining a sufficient nutritional level of protein, digestible fibre and minerals during critical periods of the year (Decandia et al., 2008). Another weak point linked with annual forage crops management deals with the possible delay in their establishment and subsequent first utilization by ruminants (Ligios et al., 2000) that are both affected by the occurrence of late summer rains. Based on the obtained results, tagasaste can be regarded as a strategic and inexpensive green fodder and protein source for ruminants.

Our study has indicated variations regarding phenolics and antioxidant capacity in edible biomass and sampling times (Tables 5 and 6). The highest antioxidant capacity of leaves was recorded in late summer and it was 2 to 5 times higher than in the other sampling times. The highest antioxidant capacity found in tagasaste leaves was twice as high as in leaves of forage chicory (Piluzza et al., 2014), but similar to values found in leaves of *Mirtus communis* L. populations (Melito et al., 2016). On the other hand, leaves of other Mediterranean perennial shrubs, such as *Cistus creticus* L., and *Pistacia lentiscus* L., showed higher values of antioxidant capacity (Piluzza and Bullitta, 2011), but the latter with known limitations in terms of intake and digestibility (Decandia et al., 2000). As late summer is a very critical period for animal feeding reared in rainfed Mediterranean farming systems, the above mentioned peaks of antioxidant capacity from tagasaste represent a valuable trait of this species.

Ver Elst and Pieterse (2006) studied the biomass composition as a factor influencing the possible utilization as mulches of eight legume species, including tagasaste. They evaluated parameters such as lignin, polyphenols, cellulose and hemicellulose contents; the content of polyphenols was 3.47% and similar levels were also indicated by Ventura et al. (2012). Our results showed a higher content in leaves during the different cutting times. Kumara Mahipala et al. (2009b) found a content of total

polyphenols of 44 g kg^{-1} and total tannin of 8.9 g kg^{-1} in tagasaste grown in Western Australia. The present study reported similar results for total polyphenols in the cutting of January (48.5) and May (49.7) in leaves; for tannic phenolics similar results were at the May cutting (9.4) in the leaves.

The levels of TotP, NTP and TP in tagasaste leaves were 3 to 6 times higher than values found in a range of Mediterranean annual grass, legume and herb species such as *Lolium rigidum* Gaud., *Medicago polymorpha* L., *Medicago arabica* L., *Plantago lanceolata* L., *Trifolium cherleri* L. and *T. resupinatum* L. (Cabiddu et al., 2013). The levels of TotP, NTP found in Mediterranean populations of forage chicory (Piluzza et al., 2014) were half than those reported for tagasaste. Karimi et al. (2013) reported TotP and TotF contents of 37 and 12.6 mg g^{-1} DM, respectively in leaves of *Medicago sativa* L., investigated as medicinal plant; these values were markedly lower than our results, but they were obtained with a methanol extraction.

Overall, our findings agree with previous studies that document the relationship between antioxidant activity and total phenolic compounds in different plants (Salama et al., 2012; Hariprasanna et al., 2015; Sulas et al., 2016). To the best of our knowledge, this study provides new insights into quality and antioxidant activities in all edible biomass organs of tagasaste. The results represent basic information not available so far, and could be useful for a proper exploitation of tagasaste, for complementing forage resources already available in the local context.

Conclusions

Season affected composition of edible biomass, chemical composition and antioxidant capacity of leaves and twigs. The highest antioxidant capacities of both leaves and twigs were detected in late summer. At each sampling, antioxidant capacity was correlated with TotP, NTP and TotF. Results highlight that tagasaste has remarkable potential for improving animal nutrition and welfare and the overall high quality of its fodder may be exploited for compensating typical seasonal feed shortages associated to drought seasons. Such results encourage further investigations dealing with the chemical characterization of its phenolic compounds for contributing to a full valorisation of this leguminous shrub as source of natural antioxidants. Additional research is required to set up a proper management of the tagasaste crop, taking into account the available forage resources and chains in each context.

Conflict of Interests

The authors have not declared any conflict of interests.

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