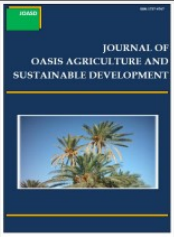

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Polygenic Olive by-products to Silage production by Anaerobic Digestion : a new alternative of sustainable agriculture security and socio-economic advantage impact

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Abstract

Olive by-products consisting of 40 % wood pruning and leaves, 40 % Olive pomace and 20 % OMW were ground and combined with preserving them by anaerobic fermentation (wet silage) for 4 months. The pH and total nitrogen/dry matter silage sample were 5.46 and 45 mg/g, respectively; the values comply with standards norms. After the silage process, a decreasing of phenol compound concentration of 67.85% was revealed and among 37 fatty acids compounds were identified with a valuable richness in mineralnutrients. The silage was devoid of *total coliforms* and *fecal coliforms* and yeast number doesn't exceed 489.9510^4 by gram of the silage. Correlation studies were done between the Silage and *silage by-products* parameters to evaluate the fermentation silage process. They showed different significant trends. A social-economic challenge was studied and discussed. Compared to that of using barley, the cost was 3 times less, with better quality for silage.

1. INTRODUCTION

The olive and olive oil industries represent several economic and social assets in the Mediterranean region, affecting most agricultural and food-processing activities (Weinberg et al. 2008). Olive culture and the olive oil industry represent the lively hood of a million people and generate 34 million work days per year, equivalent to more than 20 % of the Farming sector employment (Karray 2012). Tunisia was classified among the top countries in olive production. Indeed, the culture of olives represents a total of 15 % of the value of the final agricultural production (Abichou 2011). However, the Olive sector generates several amounts of by-products, which have harmful environmental effects (Souilem et al. 2017). By-products, e.g. Olive pomace, Olive mill wastewater, wood pruning's represent 90 % of the biomass produced by the olive tree (Souilem et al. 2017). They can provoke fatal effects on the environment. However, the by-products exploitation in fodder sources for goats represents a new alternative to reduce the

environmental risks in the arid zone. However, the valuation of these by-products remained limited to the quantitative plan, and they were the object only of traditional operation methods. Academic research and attempts to develop this biomass of the olive tree, representing both economic and environmental benefits, has been initiated since the 1970s by many organizations, and have produced convincing results (Abichou 2011).

In this context, new strategies turned to the treatment, and use of these coproduced as a source of local food for breeding and other biotechnological use. Therefore, the by-products will be used as raw material to obtain the new products with higher benefits such as manufacturing the silage for the animal's feeds. Moreover, sheep and goat production has an important social and economic asset (Paraskevopoulou et al. 2020). In fact, the use of the typical conventional feed stuffs as animal feeds remain inefficient and very expensive (Alcaide-Molina and Yanez-Ruiz 2008). Consequently, several works were studied into the utilization of these by-products for animal feeding (Falola et al. 2013). The appropriate utilization of by-products as ruminant feed can enhance agricultural and industrial animal production systems.

Furthermore, it has a social and environmental advantage, especially in semi-arid ecosystems where available pastures and forages are limited (Alcaide-Molina and Nefzaou 1996). These by-products contain energy, protein and fiber, which could be useful by ruminants (Falola et al. 2013). However, the utilization of these by-products for animal feeding was limited due to low digestibility and the high polyphenol content, which decreased the protein availability and microbial protein synthesis (Martin-Garcia and Alcaide 2008). Indeed, the silage of olive trees by-products by anaerobic fermentation could benefit ruminant feed and limit environmental pollution (Weinberg et al. 2008;Habeeb et al. 2017). Ensiling is a preservation method of wet biomass based on decreased pH values and anaerobic conditions. An objective of silage is to preserve the green food in the wet state with a minimum of dry matter loss and nourishing elements, and without toxic products fermentation for the animal (Demarquilly 1969). Good silage was defined by different characteristics namely, preserving the nutritional value minimize DM losses and inhibition of molds (Muck 1988). In addition, it was necessary to maintain the anaerobic pH stability, which most microorganisms are inhibited and destroyed (Muck 1988). This work joins the research of the sustainable development of the olive sector, namely the biomass sustainable valorization of olive by-products. This study is qualified as quality controls that will allow us a product silage estimation made from olive tree by-products. An evaluation of the olive by-products by the silage technique was made. The silage represents an economical and efficient technique to obtain well-preserved and not toxic animal feeds. To achieve this objective, microbial and biochemical quality control of silage as well silage by-products was realized. In addition, a socio-economic impacts study of the Silage technique in the arid zone were presented and discussed.

2. MATERIAL AND METHODS

2.1. Field experiment description

The Olive by-products, such as Olive pomace (OP), olive mill wastewater (OMW), wood pruning's and leaves, were ground and combined with preserving them by anaerobic fermentation (wet silage) during four months in pits. The pits dimensions were 5x2x1 m. The contents are covered by tarpaulin. This method represents an efficient economic technique that allows storing very variable quantities of some tons in several hundred tons. The amounts of Olive by-products were the following: (40 % wood pruning's and leaves, 40 % Olive pomace and 20 % OMW).

2.2. Sampling

The silage product sample was taken from three various depths of the swath 20, 40 and 60 cm. The silage products sample was a pool of three different swath silage points. The OMW and OP were taken from a three-phase discontinuous extraction factory. The wood pruning's and leaves were collected from the olive tree (*Olea europaea L*) Field. The Olive tree field and Extraction Olive factory were located in the region of Chemmakh-Zarzis (Southern Tunisia 33° 36'N, 11° 02'E).

2.3. Physiochemical analysis

The pH and Electrical Conductivity (EC) of silage and olive by-products were determined with a pH meter *XP, pH50 lab* model, and Conductivity *into Labcond 730* model. Total organic carbon (TOC) was determined by the *Walkley–Black* method and then multiplied by 1.724 to obtain the organic matter (OM) value (APHA 2012)

multiplied by the TOC. The Dry matter (DM) was determined according to the standard method described by APHA (2012). Total nitrogen was determined by the Kjeldahl Standard method (APHA 2012).

2.4. Biological Silage analysis

The biological activity of the silage (Silage respiration) was achieved by measuring CO_2 evolution during its incubation in a closed system (24 hours at 28°C). CO_2 was trapped in a NaOH solution which was titrated with HCl(APHA 2012). The total bacterial and yeast counts were determined after incubation on PCA at 30 °C and GELOSE glucose at 25 °C, respectively. VRBL agar medium was used to detect the *Total coliform* and *fecal coliform* at 44°C and 37°C during 24 h hours, respectively.

2.5. Silage test phytotoxicity

The Silage phytotoxicity test was applied according to Zucconi et al. (1981) method. This technique consists of germinating 10 tomato seeds in a Petri dish with the silage studied added. The Petri dish was incubated for one week in the darkness at 25°C. The distillate water was used as positive control. After incubation, the results were determined by counting the number of germinated seeds. The length of the roots of seeds having germinated was measured in mm. The germination index "GI" is calculated according to the following formula:

$$GI\ (\%) = \left[\frac{(Nbre\ of\ seed\ germinated * root\ lenght)}{Control\ Nbre\ of\ seed\ germinated * Control\ root\ length}\right] \times 100$$

2.6. Phenol Compounds Analysis

Phenolic compounds were quantified by high-performance liquid chromatography (HPLC 2010 PLUS SHIMADZU). The mobile phase was (A) 0.1% of formic acid in water and (B) 0.1% of formic acid in methanol with a flow of 0.4 ml/min. The column TSK (TSK gel with length and inner diameter of 30 cm and 7.8 mm, respectively) was used (Kemal 1994; Perez et al. 1990). The extraction of phenols compounds was realized according to the FOLIN-DENIS method by using ethanol (Ranalli 1997).

2.7. Fatty acid analysis

Free fatty acids were analysed with a Shimadzu Gas Chromatograph system (GC-MS GP2010ULTRA) adapted for capillary columns. A fused silica capillary column, 30m x 0.25 mm x 0.25 µm film thickness, was used. The injector and detector temperatures were set at 200°C with an interface of 220. The column temperature was set at 50°C, then raised to 250°C with rate of 50/minute, then increased from 200 °C to 230 °C at the rate of 50 °C/min. Peak heights were determined by integration software (D'Annibale et al. 1998). The fatty acid extraction was performed using hexane.

2.8. Silage Mineral Compounds analysis

The Silage mineral compounds were determined by Atomic Absorption by atomic absorption spectrometry *(Avanta, GBC spectrometer, Australia),* using air and acetylene as the mode of oxidation.

2.9. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Version 20.0). Data are presented as means ± SD. Values were obtained from triplicate determinations, and the differences were examined using one-way analysis of variance (ANOVA) followed by the Fischer's LSD (Least Significant Difference) *post hoc* test. Statistical significances of the correlations between data sets were calculated using Pearson's r-values. At least three replicates were performed for each laboratory measurement. The Silage analysis properties were run in replicate.

3. RESULTS AND DISCUSSION

3.1. The physical-chemical characteristics of silage sample and by-products

The physical-chemical properties are reported in Table 1. The silage pH was 5.46 and was within the conformity of the world standards (Muck 1988; Slim and Ben Jeddi 2012). In fact, the silage pH value must be on the average range between 3.9 and 5.5. Indeed, this pH range could improve the feed's anaerobic stability (Slim and Ben Jeddi 2012). Knowing that olive by-products used to silage products were characterized by an acid pH (Rajhi et al. 2018). Indeed, we can deduce that these by-products' acid nature facilitated the acidity pH maintenance of the silage process. However, the silage sample showed a low EC value of 3.69 $ms.cm^{-1}$ compared to the primary by-products. OMW as well as OP, which showed a significant value of EC, 9.66 $ms.cm^{-1}$ and 40.36 $ms.cm^{-1}$, respectively (Table 1.a). In fact, i EC during ensiling may indicate decrease in polyphenol content (Rajhi et al. 2018). We can deduce a degradation of polyphenols compounds and silage fermentation. The dry material DM of silage by-

Table 1a. The physic-chemical properties of Silage (S), wood pruning's (WP), Olive pomace (OP) and Olive mill wastewater (OMW).

Sample	pH	EC	DM	OM	N	N/DM	Phenol C
S	5.46 $^{\$\alpha\pounds}$±0.25	3.69$^{\alpha\pounds}$±0.09	0.35 $^{\$\alpha\pounds}$±0.02	29.39±50.75	2.6$^{\$\alpha\pounds}$±0.15	7.45$^{\$\alpha\pounds}$±0.92	0.61$^{\$\alpha\pounds}$±0.001
OP	6.28$^{*\alpha\pounds}$±0.01	40.36$^{*\alpha\pounds}$±1.58	8.37$^{*\alpha\pounds}$±0.16	6.37±0.15	5.5$^{*\alpha\pounds}$±0.43	0.65$^{\$\alpha}$±0.06	0.89$^{*\alpha\pounds}$±0.01
WL	6.63$^{*\$\pounds}$±0.11	3.84$^{\alpha}$±0.38	4.31$^{*\$\pounds}$±0.07	2.01±0.005	15.23$^{*\$\pounds}$±0.28	3.52$^{*\$\pounds}$±0.12	0.02$^{*\$\pounds}$±0.003
OMW	5.01$^{*\$\alpha}$±0.03	9.66$^{*\$\alpha}$±0.29	104.65$^{*\$\alpha}$±0.13	60±44.21	1.4$^{*\$\alpha}$±0.01	0.01$^{\$\pounds}$±0.0001	4.86$^{*\$\alpha}$±0.15

*Data are presented as means ± SD. Values were obtained from triplicate determinations and statistical significance was examined by one-way analysis of variance (ANOVA) followed by the Fischer's LSD (Least Significant Difference) post hoc test. * p<0.05 as compared to **S**; $ p<0.05 as compared to **OP**; $^{\alpha}$p<0.05as compared to **WL**; $^{\pounds}$ p<0.05 as compared to **OMW**.*

Table 1b. Degradation and increments of the silage by-products.

Silage characteristics	pH	EC	DM	OM	N	N/DM	Phenol Compounds
Increasing (%)	-	-	-	22.03	-	81.22	-
Decreasing (%)	8.51	79.31	99.09	77.96	64.48	18.77	67.85

(-): data not determined

products was higher than the silage products (Table 1.a). Thus, the DM content of the fresh by products was higher than that of the silage (99.09%) during the raw by-products silage fermentation that explains the anaerobic fermentation efficiency. Slim and Ben Jeddi (2012) suggested that dried material silage decreasing from 38 % could stimulate lactic fermentation, while the acidity of the silage decreased, and thus reduced the matter losses by drying.

Moreover, a critical decreasing of OM after silage process of 77.96 was observed (Table 1.b). A strict anaerobic fermentation explained this OM degradation. Previous studies suggested that a higher degradation of initial OM exceeding 56 % corroborated an efficient feeds products obtained after the silage process (Slim and Ben Jeddi 2012). The relationship total of nitrogen/DM revealed the silage quality; indeed, an actual dry martial content suggested the excellent quality of the silage sample. The value found for our sample was equal to 7.45 mg/g. This value complies with the values found in the international model developed by Slim and Ben Jeddi (2012) that the nitrogen total / DM relationship could be between 1.3 and 22.0. The phenol compounds of OMW and Olive were known by their biochemistry complexity due to their important polyphenols compounds (Leouifoudi et al. 2014). A decrease of the phenol compound concentration to 67.85% was revealed after the Silage process (Table1.b). This decrease of phenol compounds values may be due to their degradation during the silage process (Kuppusamy et al. 2020).

3.2. Silage identification: Fatty acids, phenol and nutriments characterization

3.2.1. Fatty acids Compounds identification

Different fatty acids compounds were detected in the silage. 37 compounds were identified (Table 2.a). These acids have various importance among which we quote the most important following ones: Butanoicacid was produced as an end-product of a fermentation process (Rajhi et al. 2013). Octanoicacid and Capricacid were naturally found in the milk of various mammals (Merck 1989). Lauricacid or dodecanoic acid was the main fatty acid in coconut oil and palm kernel oil and was believed to have antimicrobial properties (Stedman 1995). Tetradecanoic acid was found naturally in palm oil, coconut oil and butter fat (Merck 1989). Pentadecylic acid constitutes 1.05% and 0.43% of milk and meat ruminant fat, respectively (Merck 1989). Palmitic acid was a common saturated fatty acid found in fats and waxes, including olive oil, palm oil, and body lipids (Merck 1989). Heptadecanoic acid was a fatty acid of exogenous (primarily ruminant) origin. It constitutes 0.61% of milk fat and 0.83% of ruminant meat fat (Merck 1989). In addition, Heptadecanoic acid is a tra1ce component of ruminants' fat and milk fat; Oleic acid is a fatty acid that occurs naturally in various animal and vegeTable fats and oils

Table 2a. Silage Fatty acids Contents Identification

Lipid Compounds		
1	Butanoicacid ($C_4H_8O_2$)	1.796
2	Hexanoicacid ($C_6H_{12}O_2$)	2.785
3	Octanoicacid ($C_8H_{16}O_2$)	3.455
4	Decanoicacid	3.801
5	Undecanoicacid ($C_{11}H_{22}O_2$)	1.908
6	Dodecanoicacid (C12H24O2)	4.098
7	Tridecanoicacid ($C_{13}H_{26}O_2$)	2.049
8	Tetradecanoicacid ($C_{14}H_{28}O_2$)	4.314
9	cis-9 methylmyristoleate (C19H36O2)	2.001
10	Pentadecanoicacid ($C_{15}H_{30}O_2$)	2.132
11	cis-10 pentadecenoicacid (C15H28O2)	2.008
12	Hexadecanoicacid C16H32O2 =(palmiticacid)	6.946
13	9-Hexadecenoic acid $C_{16}H_{30}O_2$=(palmiticacid)	2.084
14	Heptadecanoicacid ($C_{17}H_{34}O_2$) = margaicacid)	2.184
15	cis-10 heptadecenoicacid)$C_{17}H_{32}O2$)= margaicacid)	2.112
16	Methylstearate ($C_{19}H_{38}O_2$)	4.583
17	9-Octadecenoic acid ($C_{18}H_{34}O_2$)	2.142
18	9-Octadecenoic acid($C_{18}H_{34}O_2$)	4.488
19	9,12-Octadecadienoic acid (E,E) (C18H32O2) =Linoleicacid	1.943
20	9,12-Octadecadienoic acid (Z,Z)($C_{18}H_{32}O_2$)=Linoleicacid	1.948
21	6,9,12-Octadecatrienoic acid (C18H30O2)= Linoleicacid	1.745
22	9,12,15-Octadecatrienoic acid (C18H30O2)= Linoleicacid	1.756
23	Eicosanoicacid=Arachidicacid $C_{20}H_{40}O$	4.669
24	11-Eicosenoic acid $C_{20}H_{38}O_2$=(gondoicacid)	2.187
25	Heneicosanoicacid C21H42O2	2.571
26	11,13-Eicosadienoic acid C20H36O2	1.675
27	8,11,14-Eicosatrienoic acid C20H34O2	1.776
28	5,8,11,14-Eicosatetraenoic acid $C_{20}H_{32}O_2$	1.707
29	11,14,17-Eicosatrienoic acid $C_{20}H_{34}O_2$	1.795
30	Docosanoicacid $C_{22}H_{44}O_2$=Behenicacid	4.734
31	13-Docosenoic acid C23H44O2	2.247
32	5,8,11,14,17-Eicosapentaenoic acid C22H34O2	1.545
33	Tricosanoicacid $C_{23}H_{46}O_2$	2.294
34	cis-13,16-Eicosadienoic acid C20H38O2=Gadoleicacid	2.082
35	Tetracosanoicacid$C_{24}H_{48}O_2$ Lignoceric acid	4.680
36	15-Tetracosenoic acid C24H46O2 =selacholeicacid	2.341
37	4,7,10,13,16,19-Docosahexaenoic acid C22H32O2	1.418

(Merck 1989). 9,12-Octadecadienoic acid (E, E) occurring widely in plant glycosides. It was an essential fatty acid in mammalian nutrition and is used in the biosynthesis of prostaglandins and cell membranes (Merck 1989). α-Linolenic acid (ALA) was an n−3 fatty acid. It was one of two essential fatty acids (Merck 1989). Indeed, it was suggested that the acids detected present a significant energy animal contribution.

3.2.2. Phenol Compounds

An important decreasing of phenol compound concentration was observed (Table 1b). However, eight phenol compounds were identified, as shown in Table 2.b. Quinic acid was an acid that was found in plants. Previous studies suggested that this phenolic compound constitutes a valuable compound that supports nutrition rather than originating from (Rajhi et al. 2020). Protocatechuic acid was antioxidant and anti-inflammatory (Gessner et al. 2017). Syringic acid was the least toxic phenol compound (Rajhi et al. 2018).

Phloretic acid was found in the rumen of sheep fed with dried grass (Gessner et al. 2017). Rutin showed anti-inflammatory activity in some animal and in vitro models (Gessner et al. 2017). Kaempferol acts as an antioxidant by reducing oxidative stress (Kashyap et al. 2017). Apigenin as well Kaempferol were natural products; they were an aglycone of several naturally occurring glycosides (Holland et al. 2020). As a result, the several phenol compounds detected in silage cannot induce any serious clinical damage for the animals. In contrast, the different compounds which were detected have an animal's clinical benefits.

Table 2b. Silage Phenol compounds,

Phenol compound (mg/L^{-1})	
Quinic acid($C_7H_{12}O_6$)	231.317
Protocatchuic acid($C_7H_6O_4$)	370.780
Syringic acid($C_9H_{10}O_5$)	10.057
p-coumaricacid	4.623
Rutin ($C_{27}H_{30}O_{16}$)	0.113
Luteolin-7-o-glucoside ($C_{21}H_{20}O_{11}$)	0.417
Kampherol ($C_{15}H_{10}O_6$)	1.043
Apegenin ($C_{15}H_{10}O_5$)	0.065

3.2.3. Mineral nutriments

The silage mineral nutriments rates were presented in Table 2c. Generally, mineral salts were very essential in the smooth running of the body of animals and in their development. The silage potassium rate was 2.77 mg/L. It was an important mineral to represent in the cell (Arthington et al. 2014).

In addition, it allows the action of the nerve and muscular tissues as potassium deficiency affect the neuromuscular system (Arthington et al. 2014). The magnesium rate was 0.29 mg/L^{-1} that

have a major role in residues in the regulation of the transmission of the nerve impulse in the nervous system and in all the organs (Chizzotti et al. 2009). In addition, the excess of magnesium does not present any toxic risk (Chizzotti et al. 2009). The iron rate was 3.2 mg/L^{-1}, which constitutes the most mattering mineral to improve the performance of the cattle, due to their important role in the body, it is the central constituent of the haemoglobin (Prache 1994). The zinc rate was 0.122 mg/L^{-1} that have an essential role in all the stages of protein synthesis. A deficiency in Zn can cause immunizing problems (Hagmeyer et al. 2014). Thus, Zn establishes a mineral mattering in the silage to improve the performances of animals by food (Hagmeyer et al. 2014). The sodium rate was 0.95 mg/L^{-1}, which is positive for the animal's hydroelectric balance (Khelil-Arfa et al. 2014). Thus, we can suggest that the silage obtained was nutritionally well-balanced.

Table 2 c. Sillage nutriments identification.

Nutriments Compounds	mg/L
Iron	3.2
Potassium	2.77
Magnesium	0.29
Manganese	0.018
Sodium	0.95
Zinc	0.122

Table 2d. Silage phytotoxicity and Respiration.

Phytotoxicity (%)	Silage Respiration (mgCO2/g 24 h)
146.44%	0.069

3.2.4. Microbial distribution and Silage biological proprieties

The comparative analysis of microbiological distribution between the different samples such as OMW, OP, leaves and silage was shown in Table 2e. A difference in Yeast and Bacterial distribution were noted. According to the results obtained, we notice that the samples of OMW, leaves, OP and silage revealed a significant number of total counts and in yeasts, but they were devoid of total and *fecal coliforms*.

These results corroborate with those found by Kung et al. (2018) and Tyrolová et al. (2017). No sanitary risk that can be contributed to animal diarrhea. In contrast to previous works, the microorganisms represented by bacteria that were widely dominant in qualities and in quantities during the silage such as *total coliforms* (Muck 2010). In addition, the silage yeast number found in this study doesn't exceed 489.95 ^{104} by gram of the silage. A yield very lower compared with the norms. A study was showed that the number of yeasts can reach 1000 to one billion by gram of the silage in three days only in aerobic conditions; in this case the silage becomes little palatable or is even totally rejected by animals and its entrained ingestion diarrhea (Ogunade et al. 2018). Among the co-products used in the silage production, we note that the OMW has very high yeast content as well an important total germ (TG) content by comparing with that found at OP leaves. It is well known that OMW contains all essential elements for microbial growth (Rajhi et al. 2018). However, we showed a rising in silage yeast and TG number. This increase can be explained by the effect of acidification as well as by contact with air a leak of oxygen during the wet fermentation process (the silage). Indeed, the insufficient sealing and the too slow advance of the silage front constitute important factors for the multiplication of yeast growth (Muck 1988). However, the CO_2 quantity released during the respiration test was in the order of 0.069 (Table 2.d). Silage aeration was deficient. In fact, suitable anaerobic fermentation silage was shown. Revealed to the phytotoxicity test result in Table 2.d, we notice that the fermentation applied in this study can't present any risk

Table 2e. The Microbial distribution :Yeast Y, *total counts* (TC), *Total coliform* (TC), *Fecal coliform* (FC) of Silage (S), wood prunings (WP) , Olive pomace (OP) and Olive mill wastewater (OMW).

Sample	Yeast (CFU/100ml)	TC(CFU/100ml)	TC	FC
S	$489.95 10^{4\ \$\alpha\pounds}$±0.05	$17.98\ 10^{4\ \$\alpha\pounds}$±0.02	-	-
OP	-	-	-	-
WL	-	-	-	-
OMW	$380.34\ 10^{4\ *\$\ \alpha}$±0.056	$10.04 10^{4\ *\$\ \alpha}$±0.003	-	-

effects for animals and therefore can't contribute to animal toxicity.

3.3. Correlation with silage and silage by-products parameters

A Correlation study was done between the Silage and *silage by-products* parameters to evaluate the fermentation silage process. The correlations showed different significant trends, which the most relevant results can be drawn. Negative correlations were found between pH and different parameters. The negative correlation with pH and DM, OM, Phe, Y and B were: (r=-,710**), (r=-,703*), (r=-,783**), (r=-,876**) and (r=-,775**) respectively. In fact, the correct pH for the degradation of organic matter was close to neutrality. Likewise, a pH close to neutrality was a pH widely known for the oxidation of phenol compounds (Rajhi et al. 2018). As well as microorganisms, bacteria and yeasts coexist in a pH close to neutrality (Rajhi et al., 2020). Y and B (r =, 979 **), these plead in favor of the hypothesis that these microorganisms coexist in the same microhabitat. In addition, a negative correlation between nitrogen and the microorganisms such as Yeast and Bacteria. The correlation between N and Y, and N and B were (r = -, -,743**)and (r = -, 684*), respectively. In fact, the nitrogen alkalizes the pH. pH and N (r=858**), previous studies suggested that biological nitrification processes can increase the pH of the medium (Amatya et al. 2013) and consequently, the alkaline pH can inhibit the proliferation of certain microorganisms. In addition, it was necessary to reach also the anaerobic pH stability, which all the microorganisms were inhibited and destroyed and consequently made good silage preservation of the silage (Muck, 1988). Nitrogen generation inhibits the release of phenol compounds (r = -, 624*), previous studies suggested this observation (Aude 2015). Both positive correlation with Dry and organic matter and Phenol compounds DM were (r=, 987**) OM and Phe (r= ,597*), respectively, it's not surprising, previous studies suggested the higher contents of the olive by products well known by their higher contents in phenol compounds (Leouifoudi et al. 2014).

3.4 Silage Socio-economic impact study

Several experimental works have been carried out to enhance these by-products and assess this silage process's socio-economic and environmental benefits. The experience initiated by the mutual agricultural service companies (SMSA) of the governorate of Mednine in collaboration with the Olivier Institute of Zarzis and the Pasture Office. Typical proportions for the mixture are in the order of 40% Olive pomace (OP), 20% olive mill wastewater (OMW), 40% wood pruning's and leaves. The mixture should be stored for 4 months before use. In this study we will propose to evaluate the interest of olive tree by-products as fodder resources and to enhance their socio-economic benefits, by making a comparison in terms of the cost of production of feed for livestock by the ensilage and barley process, as nutritional value for cattle and as a socio-economic asset due to its lower production cost. The Table 3 summarizes the calculation of the various costs in USD. Thus, compared to barley which has a nutritional contribution and a relatively high selling price on the market (between 141.4 USD and 151.5 USD per ton of product), the selling price of a tone of silage offered on the market (as a substitute for a tone of barley) was in the order of 80.8 USD per ton; which represents about 55% of the selling price of barley on the market. This, therefore, indicates the significant economic interest in this project. The process consists of calculating the cost of producing animal feed by mixing OP, OMW and WL and

Table 3. Estimated cost of olive tree by-products.

		Price Per Ton in USD	Share in the mixture (%)	Unit cost per Ton in USD
Leaves and wood pruning's	10 Kg to 1.01 USD	101	40%	40.4
Olive pomace (OP)	25 Kg to 0.84 USD	33.6	40%	13.44
Olive mill wastewater	only the cost of transport / m^3	33.7	20%	6.74
Total Mix			100%	60.58
Barley price		141.4	50% [a]	70.7

a- This result is obtained knowing that the nutritional value of silage compared to barley is half (50%). (according to analyzes made in the forage analysis laboratory (National School of Veterinary Medicine of Sidi Thabet, Tunisia)

comparing it with that of barley.

The Table 4 shows that the production of silage mixture has a lower unit cost per kg than that of barley. An economic cost more advantageous than barley, which showed the economic benefit of silage. We estimated the cost of the by-products based on the composition of the raw material and the labor required. This was a very rough approach, the aim of which was to locate the cost levels compared with traditional products. The study consists of formulating a feed in silage intended for livestock to fill the feed deficit at a competitive cost. The process was based on the use of a mixture composed of: 40% WL, 40% OP and 20% OMW. This cost should be compared to that of using barley, knowing that, from a nutritional stand point, 2 Ton of silage was equivalent to 1 Ton of barley. 2 Ton of silage was 50.5 USD, and 1 Ton of barley was 151.5 USD. As a result, the cost was 3 times less, with a better level of quality for silage. Nonetheless, it remained to consider the comparison in terms of acceptability to livestock.

Table 4. Estimation of the cost of olive tree by-products based on the composition of the raw material and the labor required.

Composition	Unit cost	In USD/ Ton
40% Olive pomace (OP)	33.67 USD/Ton	13.5
40% Leaves and wood pruning's (WP)	6.73 USD/Ton	2.7
20% Olive mill wastewater (OMW).	1.68 USD/Ton	0.34
Packaging (bags)	0.101 USD / 50 kg bag	2.02
Labor and miscellaneous	~20%	5.05 [a]
Total		~23.61 USD/Ton

a- This labor cost was estimated from the market selling price of one kg of silage, which is equivalent to 0.025 dollars per kilogram (i.e. 25.25 dollars per ton), therefore ((20% x 25.25 = 5.05) dollars per ton of silage produced.

4. CONCLUSION

With this silage technique, it was possible to reduce further the cost of feeding livestock and derive maximum profit and socio-economic interest. There was a real opportunity in the activity of pruning olive trees, which requires good training of pruners. Today, a mechanized (chainsaw) pruning technique lends itself well in areas where the olive tree is old. It reduces the cost of pruning, which can drop from 1.683 USD per tree currently with manual pruning to 0.673 USD per tree. Generally, the OP was left in the open air for several days without storage in a shelter. This was able to degrade the oil it contains and make it inedible. Therefore, it was necessary to promote the silage industry by quickly using the OP in the mixture with the OMW and producing a compound for silage. This silage technique made it possible to create a certified label for livestock, such as "*fattened with natural silage*". We have to master the grinding technique for olive leaves to retain their antioxidant power. Indeed, this characteristic is volatile and can deteriorate if an unsuitable process is used. However, the use of olive leaves and twigs in animal feed is faced with several difficulties, including (I) The dispersal of leaves and twigs on the plots, (II) The need to transport these sub-products, which increases the final cost, (III) The existence of a pathogenic risk and (IV) the problem of conservation of the leaves and risk of degradation of the food value. Finally, the experiences carried out in the region and presented in this study show that the outlook is encouraging and that silage is presented as an alternative to the traditional nutritional method that is economically more profitable and less degrading on the environment. The Silage product bioprocess was a subject of a patent being fled: Patent Deposit in the Tunisian Agency of Patent and Marks under the following number N° TN 2022/0103.

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