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RESEARCH ARTICLE

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Improve nutritive value of silage based on prickly pear peel by-products

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ABSTRACT

Prickly pear (*Opuntia ficus-indica* L. Mill) is widely cultivated in arid regions of every continent for its nutritious fruits and various commercial applications, cactus pear cladodes are used as forage in the most arid regions, while recently prickly pear by-products are studied as ruminant feed. They are characterised by a high moisture and sugar content; therefore, it is necessary to study suitable conservation methods. The aim of this study was to verify the effectiveness of silage to preserve prickly pear peels (PP) in a mixture with other by-products at different inclusion levels (5% straw and 6% or 12% wheat bran). For each treatment, six under vacuum micro-silos were prepared and, after 40 d of storage, the state of preservation was evaluated. Subsequently, the aliquots were analysed for chemical composition and incubated with sheep rumen fluid to evaluate the fermentation kinetics. The use of wheat bran and straw added to PP resulted in an increase in dry matter (DM); PP silage with wheat bran was found to have higher protein content and nutritional value. The greater presence of lactic acid was found in PP, such as acetic acid, therefore the lactic/acetic ratio was 2.33, significantly lower than silages with the addition of wheat bran. Silage with 5% straw showed the lowest level of organic matter disappearance (OMD) and the cumulative volume of gas (OMCV) produced during the incubation. The PP showed the fastest fermentation rate achieved after 12 h of fermentation, while those containing wheat bran, presented a higher maximum fermentation rate (R_{max}), which need longer time to be achieved. Canonical discriminant analysis (CDA) showed clear discrimination of different silages based on their chemical characteristics and parameters detected during *in vitro* incubation. Overall, the silages with the addition of wheat bran can be considered the best and, of the two, those produced with the addition of 12% bran. Finally, ensilage represents a conservation technique suitable for preserving the nutritional characteristics of PP.

HIGHLIGHTS

- Ensiling is a good way to storage the PP by-product.
- Adding 12% wheat bran to PP had the best effects on silage.
- Simultaneous ensiling of several by-products is a good method for preserving agri-food by-products.

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Introduction

Nowadays the topic of the sustainability is a much debated and the livestock sector is considered an important player in global warming (Leip et al. 2015). An additional environmental impact of the livestock sector is ascribable to feed production.

The use of by-products as animal feed has been explored (Vastolo, Calabrò, et al. 2022; Manju Wadhwa et al. 2015) and could represent socio-economic and ecological benefits (e.g. recycling, cost reduction and feed-to-food competition). Indeed, the use of local feed resources such as by-products is considered a feasible strategy to reduce water and land consumed at a global level (Makkar and Ankers 2014; Flachowsky et al. 2017) and limit the transport greenhouse gas emissions (Bronts et al. 2023).

The by-products are often used for feeding ruminants, and there is an increasing interest in exploiting new ones due their nutritional characteristics and potential nutraceutical role (Buffa et al. 2020; Branciari et al. 2021; Luciano et al. 2020; Helkar et al. 2016). Considering the composition, novel by-products could be used as feed supplements to improve the quality

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of formulated diets and animal performance (Salami et al. 2019). Furthermore, several by-products have indeed considerable amounts of bioactive compounds, especially polyphenols, such as proanthocyanidins (tannins) or flavonoids (Castrica et al. 2019). These compounds, included at low or moderate levels in the diets of animals, have positive effects on productive performance and animal health (Correddu et al. 2020).

Among the by-products used to feed ruminants, waste from the processing of the prickly pear (*Opuntia ficus-indica* L. Mill.) is increasingly present in Sicily: the prickly pear peels (PP) obtained following peeling of the fruit intended for fresh consumption and the prickly pear 'pastazzo' (PPP) obtained from the residue of grinding the whole fruit for the extraction of the juice, consisting of the peel, pulp and seeds (Todaro et al. 2020).

The Cactaceae family, with about 1600 species, is cultivated worldwide for fruits, forage, fodder and even as a vegetable. Cacti are well adapted to arid and semi-arid regions where food and fodder crops are limited, and are naturalised in several areas over the world, including the Mediterranean basin, Middle East, South Africa, Australia and India (Osuna-Martinez et al. 2014). Prickly pear (*Opuntia ficus-indica* L. Mill) is a versatile plant that is widely cultivated in arid regions of every continent for its nutritious fruits and various commercial applications. Italy is the third-largest producer in the world of the *Opuntia* fruits, after Mexico and the United States. Sicily is the Italian region which produces most prickly pear fruits (97.82% of national production), with a total production of 151,257 tons/year of fresh fruits (ISTAT 2022). The prickly pear fruit is mainly eaten as a fresh fruit after the peel has been removed. This results in a large availability of this agro-industrial by-product, a source of digestible dietary fibre and rich in bioactive compounds (Melgar et al. 2017; Amaya-Cruz et al. 2019). The prickly pear by-products become available at the end of the summer, when fresh forage resources are practically non-existent and could represent a source of water and digestible fibre for ruminants (Morshedy et al. 2020). PP accounts for about 30% of the fruit weight (Melgar et al. 2017). However, prickly pears by-products, PP and PPP, due to the high level of moisture and fermentable carbohydrates, it cannot be stored as such for long periods. It is therefore necessary to find alternative solutions for its conservation. Ensilage, a widely practiced preservation technique, is a promising solution for the preservation and utilisation of prickly pear by-products due to the anaerobic fermentation process it involves. This conservation technique has also been suggested to conserve both cladodes (Matias et al. 2020) and PPP (Vastolo et al. 2020). Moreover, PP contains more moisture than PPP, so the addition of other by-products with a high dry matter (DM) content, such as straw or wheat bran, could solve the problem and, at the same time, balance the water-soluble carbohydrates (WSCs) and nitrogenous fractions (Kordi and Naserian 2012; Abidi et al. 2013; Vastolo et al. 2020).

The PP could be a valid nutrition source for ruminants and their silage with other by-products could guarantee their use along the year. The aim of this study was to evaluate the effectiveness of silage as a method of preserving PP in mixture with other by-products (straw or wheat bran) at different inclusion levels (5% straw and 6% or 12% wheat bran on fresh weight basis). For this purpose, the nutritional characteristics and the *in vitro* fermentation characteristics and kinetics parameters of the silage have been studied.

Material and methods

Preparation of microsilos

The laboratory-scale ensiling process was employed according to the model proposed by Johnson et al. (2005). A commercial chamber vacuum machine (Lavezzini device; Fiorenzuola d'Arda, Piacenza, Italy) equipped with an automatic heat-sealing mechanism that seals the bag after the air is removed was used to remove the air from the bag.

At the end of September 2022, 15 kg of PP were used approximately 48 h after peeling the prickly pear fruits grown in the province of Palermo (Sicily), Italy, and transferred to the experimental laboratories of the Department of Agricultural, Food and Forestry (University of Palermo, Italy). Four theses were prepared: control with only PP, PP with 5% wheat straw on fresh weight, PP with 6% wheat bran on fresh weight and PP with 12% bran on fresh weight. For each of them, six microsilage (polyethylene bags) were filled with 500 g of feed.

The polyethylene bags (400 × 500 mm) were made of polyamide bioriented (OPA) and polypropylene (P) (15 μm OPA/75 μm P) and were characterised by an oxygen permeability of 30 cm^3; the air vacuum pump draws air at 10 m^3 h^{-1} at 25 °C (Alpak srl, Taurisano, Italy). The vacuum bag silos were stored in a conditioned room (at 18 °C) for 40 days; subsequently each bag was opened, and chemical, physical and *in vitro* gas production analysis were performed.

Evaluation of silage quality and chemical composition

The 24 bags, after 40 d of storage, were opened and the fresh silages were analysed for the determination of ammoniacal nitrogen (N-NH_3) following the method proposed by Martillotti et al. (1987). Organic acids were determined on fresh silage samples using high performance liquid chromatography (HPLC), according to the methodology proposed by Martilotti and Puppo (1985). The sulphuric acid was of analytical grade for chromatographic use. The water used was ultrapure. A mixed standard stock solution was prepared containing 4.5 mg/mL lactic acid, 5.4 mg/mL acetic acid, 6.66 mg/mL propionic acid (Merck and Sigma-Aldrich). The organic acids in the sample test solution were separated by reversed phase chromatography on a 250 mm × 4.6 mm, 5 μm particle column (Shimadzu, Asia Pacific), of which were detected by absorbance and quantified with external calibration graphs. For the detection of the analytes, the detector was set at $\lambda = 220$ nm. The HPLC analysis was performed with a HPLC Ultimate 3000 (Thermo Scientific, Germany) system comprising UltiMate 3000 Series SD, BM and RS Pump Series, UltiMate 3000 Series ACC-3000 Autosampler Column Compartment and UltiMate 3000 Series VWD-3100 and VWD-3400 RS Variable Wavelength Detectors. Integration, data storage and processing were performed by Thermo Scientific Dionex Chromleon Chromatography Data System version 7.2.7 (Waltham, Massachusetts, USA). The determinations were made in isocratic conditions, at 25 °C, using a mobile phase made of sulphuric acid 0.005 N. The flow rate of the mobile phase was 0.6 mL/min for all the chromatographic separations. The volume injected was 5 μL for either prepared sample or standard solution.

The pH was measured on fresh silage samples using a pH-meter for solids (HI 9025 142) equipped with a spear electrode FC 200 (Hanna Instruments Inc., Woonsocket, RI).

The silage samples, after freeze-drying, were analysed according to the procedures of the Association of Official Agricultural Chemists (AOAC) to determine DM (934.01), ether extract (EE, 920.39), crude protein (CP, 2001.11) and ash (942.05). The fibre fractions: Neutral Detergent Fibre on organic matter basis (NDFom, 2002.04), Acid Detergent Fibre on organic matter basis (ADFom, 973.18) and Acid Detergent Lignin (ADL; 973.18) were determined in accordance with AOAC and Van Soest et al. (1991) and expressed exclusive of residual ash. The WSCs content was measured using the anthrone method (Martin et al. 2017) with modifications. In brief, the freeze-dried and ground samples (1 mm sieve) were weighed (0.2 g) into a screw-cap Pyrex tube, added with 10 mL of water and incubated at 100 °C for 30 min. After cooling, the samples are centrifuged at 5000 x g for 10 min, filtered (Whatman 4 filter paper) in a 100 mL volumetric flask and diluted to volume with water. The diluted sample (1 mL) was placed in a Pyrex tube with a screw cap and added with 5 mL of anthrone solution (0.2% anthrone in concentrated sulphuric acid) and placed at 105 °C for 20 min. After 30 min of cooling in the dark, the absorbance at 625 nm was measured with a spectrophotometer (DR 3900 Spectrophotometer, Hach-Lange). The content of WSCs was calculated according to the calibration curve (R^2 0.9979) obtained with glucose-standards.

The nutritional value of the silages was determined according to the INRA method (2018), by estimating the net energy per lactation (NE_L) and the metabolisable energy (ME) of the silages. The qualitative evaluation was carried out by calculating the FP index, determined according to the formula proposed for the first time by Kilic (1986):

$$FP = 220 + (2 \times \%DM - 15) - 40 \times pH$$

When the FP was below 20, the quality of a silage was very bad; 21–40, poor; 41–60, medium; 61–80, good and 81–100, very good.

The other quality index used is the one proposed by Vanbelle (1985): the silage is assigned a score based on the quantity of lactate, acetate and butyrate present and also based on the presence of ammonia nitrogen (N-NH_3/N %). The more lactic acid there is, the higher the score will be. While the score drops if the levels of acetic acid, butyric acid and ammonia nitrogen increase.

In vitro *gas production*

The *in vitro* fermentation characteristics were determined according to the method reported by Vastolo, Matera, et al. (2022), briefly substrates were incubated at 39 °C under anaerobic conditions with an inoculum consisting of a pool of three sheep ruminal fluid in order to limit the donor effect. Specifically, one run was carried out to allow for a large batch of bottles in a single incubation run and it has been proven not to affect the rate or extent of gas production respect to higher inoculum proportions (Amanzougarene and Fondevila 2020). Specifically, in one run, for each substrates the six polyethylene bags were incubated in double replication ($n = 12$; 1.0244 $g \pm$ 0.020 on DM

basis) into 120 mL serum bottles to which medium (75 mL) and a reducing solution (4 mL) was added. The medium used consists of a bicarbonate-phosphate buffer, a reducing agent, a macromineral solution, a micromineral solution and resazurin as redox indicator (Theodorou et al. 1994). Rumen liquor was collected in a slaughterhouse authorised according to EU regulations (EC Regulation 882/2004) from three adult ewes fasted from the previous night and fed with a standard diet based on hay and concentrate. The collected material was placed inside pre-heated thermos to avoid exposure to the air and brought quickly to the Feed Analysis laboratory, of the Department of Veterinary Medicine and Animal Production (University of Naples, Federico II). The ruminal inoculum was insufflated with CO_2 and filtered through cheesecloth added to each bottle (10 mL to obtain 1:7.5 inoculum: medium ratio). Gas produced, expressed in pound/square inch (p.s.i.), was measured 24 times during 120 h of incubation using a manual system consisting of a pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA) connected through three-way valve with a graduated syringe.

After 120 h of incubation, the bottles were opened, and the pH of the fermentation liquor was measured using a pH-meter (ThermoOrion 720 A+, Fort Collins, CO) to check the efficiency of the buffer system and record any abnormal fermentation at the end of the incubation.

The extent of sample disappearance, expressed as organic matter degradability (OMD, %), was determined by weight difference of the incubated OM and the undegraded filtered (sintered glass crucibles; Schott Duran, Mainz, Germany, porosity # 2) residue burned at 550 °C for 3 h. The cumulative volume of gas produced after 120 h of incubation was related to incubated OM (OMCV, ml/g). As per protocol, three bottles were incubated in the absence of substrate (blank) to correct dOM and cumulative volume of gas related to incubated organic matter (OMCV).

Regarding the determination of volatile fatty acids (VFAs), the fermentation liquor was centrifuged at 12,000 g for 10 min at 4 °C (Universal 32 R centrifuge, Hettich FurnTech Division DIY, Melle-Neuenkirchen, Germany) and 1 mL of supernatant was collected and acidified with 1 mL of oxalic acid (0.06 mol) in order to analyse VFAs in liquid matrix. The VFAs were measured by gas chromatography (ThermoQuest 8000top Italia SpA, Rodano, Milan, Italy; fused silica capillary column 30 m, 0.25 mm ID, 0.25 m film thickness), using an external standard solution composed of acetic, propionic, butyric, iso-butyric, valeric and isovaleric acids (Vastolo et al. 2023).

For each bottle, the gas production profiles were processed with a sigmoid model described by Groot et al. (1996):

$$G = \frac{A}{1 + \left(\frac{B}{t}\right)^{c}}$$

where G is the total gas produced (ml/g of OM) at time t (h), A is the asymptotic gas production (ml/g of OM), B (h) is the time at which one-half of the asymptote is reached, and C is the switching characteristic of the curve. Maximum fermentation rate (R_{max}, ml/h) and the time at which it occurred (T_{max}, h) were also calculated according to the following formulas (Bauer et al. 2001):

$$R_{max} = \frac{A{*}B^{C} \times B \times {T_{max}}^{(B-1)}}{[1 + (C^{B} \times {T_{max}}^{-B})^{2}]}$$

$$T_{max} = C \times \left[\frac{B-1}{B+1}\right]^{1/B}$$

Statistical analysis

The data were analysed with the GLM and CANDISC procedures of the SAS software package version 9.2 (Cary, NC; SAS 2010). The chemical characteristics, silage quality and *in vitro* fermentation data were analysed with a one-way analysis of variance (ANOVA) model, with the effect of the substrate (PPS, PP + 5% wheat straw silage, PP + 6% wheat bran silage, PP + 12% wheat bran silage) as the main factor. When a significant effect was found ($p < 0.05$), Tukey's test was used for comparison of means.

Furthermore, a multivariate statistical approach was performed using Canonical Discriminant Analysis (CDA) according to the CANDISC procedure, in order to evaluate the ability of the chemical characteristics, silage quality indices and *in vitro* fermentation parameters to discriminate the substrates. Given the classification criterion (substrate), CDA derives a new set of variables, called canonical functions (CAN), which are linear combinations of the original variables. The coefficients of the linear combinations are the canonical coefficients (CC), which indicate the partial contribution of each original variable. In this study, two canonical functions (CAN1 and CAN2) were derived. Statistical significance between groups was assessed by Mahalanobis distance, visual inspection of CAN1 × CAN2 graph was also reported for better understanding of the phenomenon.

Results and discussion

Micro silos quality and composition

Table 1 shows the chemical composition of the raw materials and silages after 40 d of storage. As expected, DM content of the PP was found to be very low and lower than value reported for PPP by Todaro et al. (2020), despite this by-product having remained 48 h at room temperature before being used, highlighting how it is able to retain the waterfall. Compared to PPP, this by-product resulted lower in EE, protein and fibrous fractions less lignified. In fact, only 1% of lignin is present, compared to the 14.64% found in PPP (Todaro et al. 2020), this fact is due to the absence of seeds, present at 72%. Conversely, non-fibre carbohydrate (NFC) was found to be more than double that of PPP, which makes this by-product a highly fermentable feed. Finally, in accordance with Manzur-Valdespino et al. (2022) PP is a by-product with a high mineral content, more than PPP (Todaro et al. 2020) and more than double that of the other by-products used (bran and straw).

The chemical composition between silages was significantly different ($p < 0.001$) for all the chemical parameters considered, with the exception of the EE. The use of wheat bran and straw resulted in an increase in silage DM as expected. The DM of PP silage was significantly lower, while it increased in PP + straw silage and even more in PP + bran silages. As expected, the CP content was lower in PP silage and PP + straw silage, while it was higher with increasing levels of wheat bran due to its good protein content. As far as structural carbohydrates are concerned, for NDFom the highest value was found in silage with 5% straw, which however presented a higher non-digestible fibre fraction (ADL) than other silages.

Compared to the other by-products used, PP had the highest WSC content, rapidly fermented by microorganisms. In fact, under an anaerobic environment, WSC converted into organic acids and lactic acids with the help of lactic acid bacteria (LAB). The WSC content detected in the silages presented significantly higher values in the PP + wheat bran silages, probably due to the different microbiological activity of LAB. WSC is the primary substrate for the ensiling of silages, but many controllable and uncontrollable factors like temperature, moisture content, DM, additives, ensiling or storage time, etc. can affect the silage quality and influence the WSC concentration (Ali and Tahir 2021). The PP silage with straw showed the lowest WSC and NFC content.

The percentage of ash in the silages was high and significantly different from each other; as expected the PP silage presented the highest values, which should negatively affect digestibility and the ensiling process (Arieli et al. 1993). However, the use of these silages as feed should not cause problems for the animals, in fact it is known that ruminants are able to tolerate a high intake of minerals in their diet, increasing the intake of water to regulate the osmotic balance in the intestinal tract (Underwood and Suttle 1999).

Table 2 shows the organic acids, the energy value and the quality indexes of the silages. The higher presence of lactic acid was found in PP silage, probably due to the higher content of WSC and NFC used for bacterial fermentations. Moreover, PP silage showed a significantly higher presence of acetic acid than other silages. Furthermore, propionic acid levels were low and below 1.86 g/kg DM, while butyric acid remains below the threshold of detection. Overall, the organic acids detected in our silages, in particular lactic and acetic, had similar values than in silages

Table 1. Chemical composition (% DM) of raw materials (mean) and PP silages (least square means).

Items	Raw materials			Silages[1]					
	Peel	Straw	Bran	PP	PP + Straw (5%)	PP + Bran (6%)	PP + Bran (12%)	SEM	*p* Value
DM	8.55	92.7	89.1	8.71^{c}	13.5^{b}	14.8^{b}	17.3^{a}	0.449	0.001
EE	2.96	0.960	5.77	8.27	4.95	6.18	7.00	0.871	0.082
CP	3.83	2.87	16.2	5.79^{c}	4.32^{d}	9.73^{b}	12.1^{a}	0.327	0.001
aNDFom	17.5	84.7	31.9	20.1^{d}	40.1^{a}	24.0^{c}	27.9^{b}	0.998	0.001
ADFom	11.6	60.9	11.2	17.2^{b}	31.3^{a}	13.8^{c}	15.0^{c}	0.403	0.001
ADL	1.07	9.31	2.21	3.83^{ab}	5.33^{a}	2.23^{c}	3.65^{bc}	0.430	0.001
Cellulose	10.5	47.8	8.98	13.3^{b}	25.9^{a}	11.5^{c}	11.3^{c}	0.480	0.001
Hemicellulose	5.89	23.8	20.1	2.91^{c}	8.80^{b}	10.3^{ab}	12.9^{a}	0.988	0.001
WSC	29.1	0.480	8.01	3.19^{b}	2.32^{c}	5.94^{ab}	7.52^{a}	0.531	0.001
NFC	65.3	4.94	40.7	46.16^{a}	36.2^{b}	46.0^{a}	40.1^{b}	1.251	0.001
Ash	12.4	6.57	5.40	19.2^{a}	14.5^{b}	13.3^{c}	11.6^{d}	0.286	0.001

[1]Statistical analysis was carried out only on silages data.
PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage. DM: dry matter; EE: ether extract; CP: crude protein; NDFom: neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom: acid detergent fibre expressed exclusive of residual ash; ADL: acid detergent lignin; WSC: water soluble carbohydrate; NFC: non-fibre carbohydrate = 100 − (CP + ether extract + ash + NDFom); on the row different letter indicate significative differences; SEM: standard error of mean.

Table 2. Fatty acid content, energy value and quality indexes of silages.

	Silages					
Items	PP	PP + Straw (5%)	PP + Bran (6%)	PP + Bran (12%)	SEM	*p* Value
LA (g/kg DM)	39.6^{a}	24.7^{c}	30.3^{b}	27.1^{c}	0.816	0.001
AA (g/kg DM)	17.0^{a}	10.6^{b}	11.4^{b}	10.9^{b}	0.522	0.001
PA (g/kg DM)	0.99^{bc}	0.71^{d}	1.46^{ac}	1.86^{a}	0.134	0.001
BA (g/kg DM)	n.d.	n.d.	n.d.	n.d.	–	–
LA / AA	2.33^{b}	2.33^{b}	2.66^{a}	2.56^{a}	0.067	0.001
pH	3.97^{b}	3.84^{d}	3.90^{c}	4.03^{a}	0.014	0.001
N-NH_3/N (%)	0.810	0.548	0.686	0.604	0.390	0.967
ME (MJ/kg DM)	10.3^{c}	8.5^{d}	11.2^{b}	11.9^{a}	0.155	0.001
NE_L (MJ/kg DM)	6.26^{c}	4.90^{d}	6.89^{b}	7.33^{a}	0.107	0.001
FP	63.5^{a}	78.4^{b}	78.7^{b}	78.6^{b}	1.107	0.001
Vanbelle index	89.8	94.3	95.6	95.1	1.644	0.165

PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage. LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid; LA/AA: lactic/acetic acids ratio; Ammonia nitrogen expressed as Total Nitrogen; ME: metabolisable energy; NE_L: net energy for lactation; on the row different letter indicate significative differences; on the row different letter indicate significative differences; SEM: standard error mean; n.d.: non detected.

obtained with prickly pear cladodes (Matias et al. 2020) or with citrus by-products (Ulger et al. 2020; Grizotto et al. 2020). The LA/AA ratio was between 2.33 and 2.66, which demonstrates a good fermentative activity of the silage, lower values have been reported in other silages obtained with citrus by-products (Ulger et al. 2020; Grizotto et al. 2020) or fruit by-products (Mousa et al. 2019). Silages made with wheat bran added to PP had a significantly higher LA/AA ratio than PP + straw silage, probably due to the higher WSC content of bran compared to straw. While the higher LA/AA ratio in PP + bran silages compared to PP silages could be explained by the high AA content of PP silages, probably due to their higher fermentation temperature due to the lower DM and higher WSC content. It is in fact known that high ensiling temperatures reduce the number of some lactic bacteria and make fermentation less homolactic (Weinberg et al. 2001).

The pH of the silages was between 3.84 and 4.03 and the differences between them were statistically significant, probably due to different microbial development for different fermentation substrates; in any case the pH values found in our silages highlighted the high quality level of bacterial fermentation remaining below 4, 5 considered a threshold value reported in the literature (Collins et al. 2017).

Ammonia nitrogen showed a large variability, so the differences between silages were not statistically significant. As a percentage of total nitrogen, we found percentages below 0.81 which confirm the good ensiling process (Collins et al. 2017).

The nutritional value of the silages was interesting and comparable with other by-products silages reported in a recent review (Vastolo, Calabrò, et al. 2022); significant differences were found between the different theses both for the net energy content and for the ME. From an energy point of view, the best silages were those obtained with the addition of wheat bran while, as expected, the 5% straw silage had the lowest content of both ME and net (NE_L) energy content.

For a further evaluation of these silages, qualitative indices were determined, in particular the FP was determined according to Kilic (1986), a parameter still used and reported by several authors (Zhang et al. 2017; Dai et al. 2022). The silages obtained with PP alone or with the addition of other by-products have obtained a FPs which allows them to be placed in the range of good quality silages (61–80). However, significant differences were found between the different scores and PP silage scored lower. These results demonstrated that the addition of high DM by-products to PP before ensiling allows to positively modulate fermentations.

The other silage quality index reported is the one proposed by Vanbelle (1985), that ranges from 0 to 100 points; the results obtained with the silages tested in this study, although not significantly different, demonstrate the good quality of the silages and the success of the fermentation processes.

In vitro *fermentation characteristics*

Table 3 shows the results obtained with the *in vitro* gas production. Silage PP with 5% straw showed the lowest level of organic matter disappearance (OMD), while no significant differences were found between the other silages. The lower OMD value of silage with straw could be due to the higher ADL, despite the larger of cellulose of this substrate. The cumulative volume of gas (OMCV) produced during the

Table 3. *In vitro* parameters of tested silage.

	Silages					
Items	PP	PP + Straw (5%)	PP + Bran (6%)	PP + Bran (12%)	SEM	*p* Value
OMD (%)	80.2^{a}	64.7^{b}	81.8^{a}	82.8^{a}	0.644	<0.001
OMCV (ml/g)	319^{a}	286^{c}	311^{ab}	292^{bc}	5.30	<0.001
Tmax (h)	12.6^{b}	20.4^{a}	14.3^{b}	18.4^{a}	0.519	<0.001
Rmax (ml/h)	7.63^{a}	4.08^{d}	6.31^{b}	5.40^{c}	0.168	<0.001

PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage. OMD: organic matter disappearance; OMVC: cumulative volume of gas related to incubated organic matter; Tmax: time at which Rmax occurs; Rmax: maximum fermentation rate.

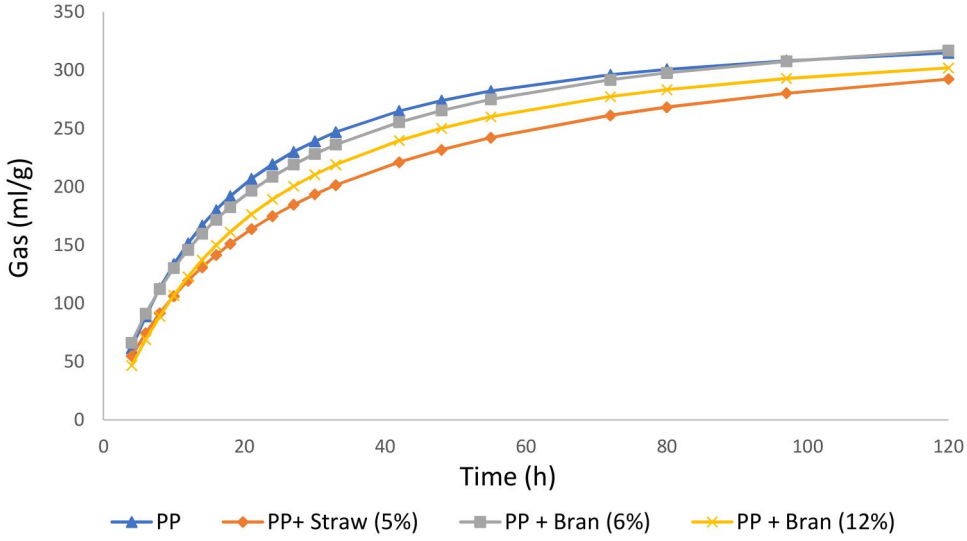

Figure 1. *In vitro* gas production of tested silage. PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage.

incubation of silage with 5% of straw was also significantly lower, both in terms of total gas production and as regards the production over the incubation time (Figure 1). We have found few studies on the prickly pear by-product in the literature, and it is difficult to compare the results obtained. When we employed the *in vitro* gas production with PP silage alone or with straw at different percentages (5% or 10%), the OMD and OMCV were lower than what we found with this study (Vastolo et al. 2020).

The best performances obtained from the fermentation process of PP are probably due to the different NFC content that characterises the two types of by-products, higher in PP than in PPP (Todaro et al. 2020). With regards to the OMD, Vastolo, Matera, et al. (2022) report that the highest digestibility of DM determined *in vitro* was found for mandarin pulp followed by bergamot pulp (80.44 and 78.38% OM, respectively), percentages similar to what we found on the by-product of the prickly pear.

Regarding the fermentation kinetics (Table 3 and Figure 2), PP silage showed the fastest fermentation rate achieved after 12 h of fermentation, among other silages, those containing wheat bran, presented a higher maximum fermentation rate (R_{max}), which need longer time to be achieved. Finally, straw silage had the lowest R_{max} achieved significantly later than the others.

The *in vitro* final products were reported in Table 4. PP silage with 5% straw showed the lowest level of total VFAs in contrast to silage produced with 12% of wheat bran which showed the highest level of VFA. The latter also presented the highest level of branched chain fatty acids (BCFA) and acetate as opposed to prickly pear silage with 5% straw. Similarly, silage with 5% straw produced the lowest level of propionate while silage with 12% bran showed the highest amount of propionate. PP + 6% wheat bran and PP + 5% straw silages showed the highest and lowest levels of butyrate, respectively; while the highest acetate/propionate ratio were detected in PP + 5% straw and PP + 12% wheat bran silages, respectively.

The production of VFA was mainly due to the sum of acetate, propionate and butyrate, contrary to the

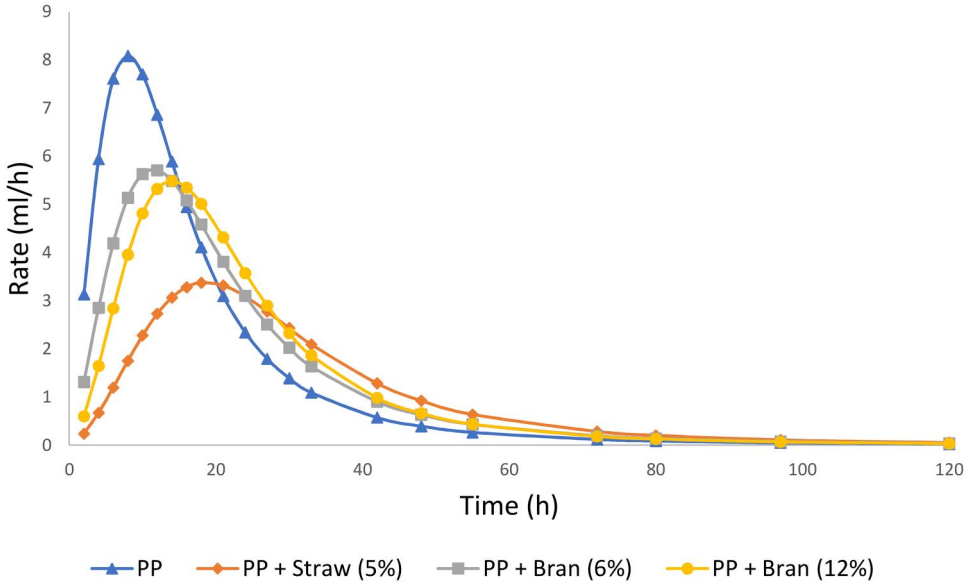

Figure 2. *In vitro* fermentation rate of tested silage. PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage.

Table 4. *In vitro* end-products of tested silage (mmol/g dOM).

	Silages					
Items	PP	PP + Straw (5%)	PP + Bran (6%)	PP + Bran (12%)	SEM	*p* Value
VFA	116^{ab}	101^{c}	112^{ab}	127^{a}	7.49	0.005
BCFA	3.81^{c}	3.51^{c}	4.63^{b}	5.05^{a}	0.144	<0.001
Acetate	65.2^{ab}	59.1^{b}	59.6^{b}	75.5^{a}	4.14	<0.001
Propionate	20.6^{ab}	19.5^{b}	21.2^{ab}	22.9^{a}	1.39	0.031
Iso-butyrate	0.692^{b}	0.611^{b}	0.894^{b}	0.931^{a}	0.097	<0.001
Butyrate	25.6^{ab}	15.6^{c}	26.2^{a}	22.3^{b}	1.94	<0.001
Iso-valerate	1.73^{bc}	1.35^{c}	1.93^{b}	2.52^{a}	0.154	<0.001
Valerate	2.26^{a}	1.39^{b}	2.17^{a}	2.65^{a}	0.217	<0.001
Acetate/Propionate	2.94^{ab}	3.07^{a}	2.81^{b}	3.02^{a}	0.086	<0.001

PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage; VFA: volatile fatty acids; BCFA: branched chain fatty acids; on the row different letter indicate significative differences; SEM: standard error mean.

Table 5. Canonical discriminant analysis: Mahalanobis quadratic distances.

	Silages			
Substrate	PP	PP + Straw (5%)	PP + Bran (6%)	PP + Bran (12%)
PP	0	3477(p = 0.0018)	1023(p = 0.0107)	1412(p = 0.0067)
PP + Straw (5%)	–	0	2310(p = 0.0032)	2393(p = 0.0031)
PP + Bran (6%)	–	–	0	117(p = 0.2026)
PP + Bran (12%)	–	–	–	0

PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage.

final products of the *in vitro* PPP fermentation (Vastolo et al. 2020) in our silages we found a higher production of butyrate. This fact is probably due to the higher WSC content of PP, in fact WSC can be immediately digested by the main *Phila* of the rumen flora (e.g. *Bacteroidetes* and *Firmicutes*) and converted anaerobically into organic acids such as succinate, propionate and butyrate (Dehority 2003). Among the different silages, the lowest VFA value was found in silages with the addition of straw, probably due to the lower fermentability of the lignified structural carbohydrate of straw, furthermore these silages have a lower concentration of butyrate due to the lower WSC content and the higher percentage of cellulose (Ma et al. 2022).

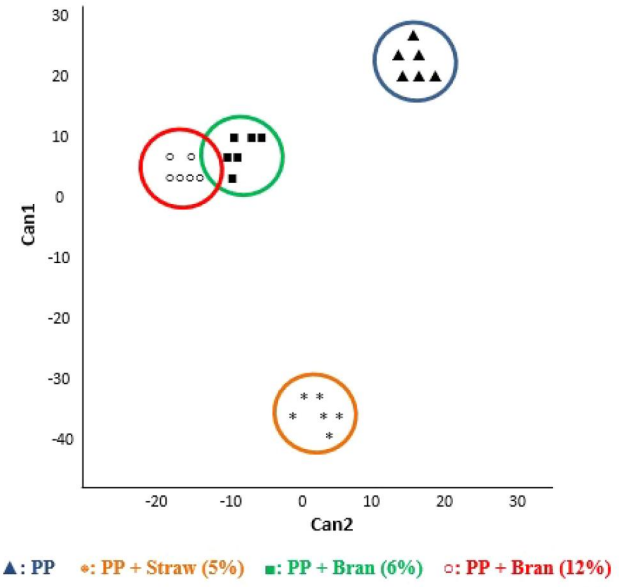

Figure 3. Plot of canonical 1 (Can 1) × canonical 2 (Can 2). Variable: by-products. PP: prickly pear peels silage; PP + Straw (5%): prickly pear peels with 5% of wheat straw silage; PP + Bran (6%): prickly pear peels with 6% of wheat bran silage; PP + Bran (12%): prickly pear peels with 12% of wheat bran silage.

Table 6. Total canonical structure: correlations between canonicals and original variables.

Variable	1st canonical variable	2nd canonical variable
Ash	0.259	0.940[1]
CP	0.484	−0.833[1]
EE	0.473	0.169
Cellulose	−0.918[1]	0.357
Hemicellulose	−0.212	−0.827[1]
Lignin	−0.354	0.485
pH	0.678[1]	−0.198
OMD	0.904[1]	−0.343
OMCV	0.603[1]	0.326
T_{max}	−0.732[1]	−0.280
R_{max}	0.841[1]	0.357
Acetate	0.123	−0.221
Propionate	0.254	−0.241
Butyrate	0.701[1]	−0.181
Iso-butyrate	0.340	−0.538
Valerate	0.570	−0.277
Iso-valerate	0.456	−0.545
BCFA	0.439	−0.736[1]
Explained variance (%)	*70.7*	*27.7*

[1]High loading values.

Multivariate analysis

The results obtained with the multivariate analysis make it possible to obtain a clear discrimination of the different silages based on their chemical characteristics and the parameters detected *during in vitro* incubation. The greatest distances of Mahalanobis (Table 5) were observed between the centroids of the silage with straw and the others, this is easily observable in Figure 3, where the plot of the canonical 1× canonical 2 is presented. Between the two silages with addition of bran (6% and 12%), the Mahalanobis distance was not statistically significant (Table 5), in fact an overlap of these two areas is evident in the graph. Finally, PP silage is statistically distant from the other ones, to a lesser extent with bran silage and much more with straw silage.

Table 6 shows the correlation coefficients between the canonical variables and the original variables. The first canonical variable explains more than 70% of the total variability, while the second explains more than 27%. The first canonical variable was positively correlated with pH, OMD, OMCV, R_{max} and Valerate organic acid, while was negatively correlated with cellulose and T_{max}. Figure 3 shows how the different types of silage differ mainly on the vertical axis (canonical 1), which explains most of the variability. Since the latter is positively and substantially correlated with the parameters that express the characteristics of good quality silage, such as pH, OMD and OMCV (Table 6), it is evident that the silages with the best qualitative performance are those which are higher up in the graphic, i.e. those with bran and that of peels only.

The second canonical variable was positively correlated with ash and negatively correlated with CP, hemicellulose contents and BCFA production. This means that moving to the left on the abscissa axis (Figure 3), we find those silages with the greatest protein and hemicellulose content and producers of the greatest quantity of branched-chain fatty acids. Isobutyrate and Isovalerate production are the result of branched-chain amino acids metabolism, such as valine, leucine and isoleucine; they can be hydrolysed and fermented to phenols, and biogenic amines (i.e. indole, skatole, 4-ethylphenol and *p*-cresol) (Davila et al. 2013). Moreover, anteiso-BCFA, as well as iso-series, are cytotoxic to the breast cancer cells, and the cytotoxicity of BCFA is comparable to that of conjugated linoleic acid, known as antitumoral fatty acid (Wongtangtintharn et al. 2004). Therefore, the silages with the addition of wheat bran, which are placed on the left of Figure 3, are to be considered the best and, of the two, those produced with the addition of 12% bran.

Conclusion

Despite the low DM content of silages, the quality level of the silage obtained allow us to state that ensiling is a suitable conservation technique for PP. The use of wheat straw or bran had a different effect on the chemical characteristics and fermentation kinetics. In fact, ensiling with bran, although certainly

more expensive from an economic point of view, has made it possible to reach the best quality levels from all points of view, more evident results have been observed in the thesis with 12% bran which was turned out to be the best. Further *in vivo* studies are needed to evaluate palatability and the effects on milk yield and quality and animal welfare, also evaluating the use of greater doses of straw and/or bran with the aim of increasing DM.

Ethical approval

Ethical review and approval were not required because this study did not involve animals for experimental or other scientific purposes.

Disclosure statement

No potential conflict of interest was reported by author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, Todaro M., upon reasonable request.

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