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Chemical, nutritive, fermentation profile and gas production of citrus pulp silages, alone or combined with maize silage

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Abstract

Quality attributes of citrus pulp silages that were ensiled alone and combined with maize silage were determined. Fresh samples of lemon, orange and tangerine pulps, maize plants and their combinations were fermented in glass jars for 90 days at about 20 - 25 °C. Treatments included i) 100% maize silage as control (MS); ii) 100% lemon pulp silage (LPS); iii) 100% orange pulp silage (OPS); iv) 100% tangerine pulp silage (TPS); v) 50% LPS and 50% maize silage (LPS + MS); vi) 50% OPS and 50% MS; and vii) 50% TPS and 50% maize silage (TPS + MS). The pH differed among treatment groups. The highest and lowest pH values were recorded for MS group and the OPS + MS group, respectively (3.84 vs. 3.51). The highest dry matter (DM), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose (HEM) were observed for MS (P <0.01). Citrus pulp silages alone had significantly greater total digestible nutrients (TDN), organic matter (OM) and non-fibre carbohydrate (NFC) values than MS and the combined silages (P <0.01). Maize silage had higher lactic acid (LA) (101.2 g/kg DM) and acetic acid (AA) (49.3 g/kg DM) concentrations than the citrus groups (P <0.05). In vitro gas production (TG), methane (CH_4), metabolizable energy (ME) and organic matter digestibility (OMD) of the silages were similar (P >0.05). Thus, citrus pulps can be ensiled in spite of their moisture content. However, to increased DM and nutrient content, the citrus pulps should be ensiled with 50% maize.

Keywords: fermentation metabolites, methane production, organic matter digestibility
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Introduction

Citrus is a genus of flowering trees and shrubs including the economically valuable species sour orange, sweet orange, tangerine, grapefruit and lemon. Of these species, orange *(Citrus sinensis),* tangerine *(Citrus reticulata)* and lemon *(Citrus limon)* are commonly grown in Turkey. Low-quality fruits and processing wastes of these fruits may be a significant source of environmental pollution and create serious economic losses. World annual citrus production is about 70 million tons (USDA/FAS, 2003). This quantity is composed mostly of sweet orange (*C. sinensis* at 67.8%) (USDA/FAS, 2003), tangerine (*C. reticulata* at 17.9%) and lemon (*C. limon* at 6.3%) (Kale & Adsule, 1995). The major citrus-producing countries are the Mediterranean countries of Turkey, Italy, Spain, Greece, Morocco and Egypt (24%), Brazil (24%), and the USA (21%). Pulp is a by-product of facilities that process the fruit into juice. The citrus (orange, tangerine, lemon, grapefruit and orange) production of Turkey was 4 769 726 tons in 2017 (TUIK, 2017). Despite the studies about ensilage of fruit pulps and potential use of ensiled pulps in animal feeding (Ashbell, 1994; Yalçınkaya *et al*., 2012; Canbolat *et al*., 2014; Ülger *et al.*, 2018a) there are large differences between the amounts of fruits used in the fruit juice industry and the amount of pulp released from them (Yalçinkaya *et al.*, 2012). Since some fruits are rich in antioxidants, carotenoids, anthocyanins, pectin, fatty acids, flavonoids, phenolics, vitamins and minerals, which are quite significant for human health and nutrition (Velioglu *et al*., 1998). Their pulps could be good sources of feed for livestock and their attributes may contribute to silage quality (Ülger *et al*., 2015; Koc, 2019). Previous studies revealed that these sources reduced feed costs and increased profitability in livestock-raising activities. Such studies also stressed the significance of alternative feed

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sources for livestock. In this sense, fruit pulps are regarded as an important alternative feed source in ruminant nutrition (Filya *et al*., 2006; Duru & Kaya, 2015; Ülger *et al*., 2018b).

The goal of this study was to determine the chemical, nutritional and fermentative values of citrus pulp as a silage material. The characteristics of ensiled citrus pulp were compared with the qualitative parameters of maize silage.

Materials and Methods

Citrus pulp materials were obtained from a commercial fruit juice factory in Kayseri, Turkey. Citrus pulp DM varied between 201 and 263 g/kg. Its chemical composition is provided in Table 1. Materials were chopped into 1.5 - 3 cm pieces with a silage machine. A total of 42 (7 samples × 6 replications) samples [1 maize + 3 pulp (lemon, orange and tangerine) + 3 mixture (maize + pulps)] were ensilaged in vacuum bags (1 kg capacity) with a vacuum machine in six replications without treatment. Silage material was filled into bags by hand before the bags were heat-sealed (without melting the plastic bag) and the air evacuated. After sealing, the vacuum machine cut the plastic bag automatically with 5 mm remaining above the seal. Then, ensiled materials were kept at room temperature (about 20 - 25 °C) for 90 days.

The experimental treatments consisted of i) maize silage (MS); ii) lemon pulp silage (LPS); iii) orange pulp silage (OPS); iv) tangerine pulp silage (TPS); v) lemon pulp silage+maize silage (LPS+MS, 50/50 w/w); vi) orange pulp silage+maize silage (OPS+MS, 50/50 w/w); and vii) tangerine pulp silage+maize silage (TPS+MS, 50/50 w/w).

At the end of the 90-day ensilage period, silage samples were taken for chemical and nutritional analyses, which were performed as three replicates. For pH measurements, 25 g of silage sample was put in a beaker and 100 mL distilled water was added. The sample was then mixed in a blender for five minutes, the resultant mixture was filtered through Whatman filter paper, and the pH of this filtrate was measured (Akyildiz, 1986). Dry matter (AOAC, 2000) (method 934.01**),** crude ash (CA) (AOAC, 2000) (method 942.05), crude protein (CP) (AOAC, 1996) (method 954.01), crude fibre (CF) (AOAC, 1996) (method 978.10) and ether extract (EE) (AOAC, 2000) (method 920.39) were determined. Neutral detergent fibre was analysed with heat-stable amylase and without Na-sulphite. Acid detergent fibre and acid detergent lignin (ADL) were determined according to the sequential method of Van Soest *et al.* (1991) with an ANKOM fibre analyser ($ANKOM_{220}$ Technology, Macedon, NY, USA) and expressed inclusive of residual ash. Hemicellulose was defined as NDF–ADF.

The Fleig point (FP) was calculated with the equation of FP = 220 + (2 × DM% – 15) – 40 × pH (Akyildiz, 1986). The total digestible nutrient (TDN) was calculated according to the equation proposed by Chandler (1990), where TDN% = 105.2-0.68 × NDF%. The non-fibre carbohydrates (NFC) were calculated with the equation proposed by Weiss *et al*. (1992): NFC% = 100 – (NDF% + CP% + EE% + CA%). Total carbohydrates (TC) were determined as: TC% = 100 – (CP% + EE% + CA%) (Sniffen *et al*., 1992).

To determine water-soluble carbohydrates (WSC), liquid extractions were prepared with 40 g silage. Samples were placed in a beaker, and 360 mL distilled water was added and mixed in a blender. The resultant slurry was filtered through Whatman 54 filter paper and then centrifuged. Supernatant liquid samples were stored at -20 °C until analysis. The WSC of samples were determined by the phenol sulphuric acid method (Dubois *et al*., 1956).

Lactic acid (LA) was determined by the colorimetric method of Barker and Summerson (1941) and by gas chromatography. The volatile fatty acids (VFA): acetic acid (AA, Chem Service O-4), propionic acid (PA, Chem Service O-25) and butyric acid (BA, Chem Service O-5)] were measured with a gas chromatograph (Shimadzu GC-2010+, Kyoto, Japan) with a capillary column (30 m × 0.25 mm × 0.25 μm) (Restek, Bellefonte, PA, USA) and with flame ionization detector (FID) over a temperature range of 45 - 230 °C (Fussell & McCalley, 1987).

Citrus pulp that had been milled through a 1 mm sieve was incubated in vitro in rumen fluid in 100 mL calibrated glass syringes according to the method described by Menke *et al*. (1979). Rumen fluid was obtained from a slaughterhouse from two cows that had been fed a diet of at least 60% roughage (Chaudhry, 2006). Before the rumen fluid was mixed with buffer solution, it was filtered through four layers of cheesecloth under flushing with CO_2 and combined with buffer solution in the ratio of 1:2. Approximately 0.200 g citrus pulp samples were incubated with 30 mL rumen fluid-buffer mixture in calibrated glass syringes in a water bath at 39 °C. Gas production was determined at 24 hours after incubation and corrected for blank and hay standard (University of Hohenheim, Germany).

Metabolizable energy (MJ kg^{-1} DM) and OMD of citrus pulp samples were estimated with the equations described by Menke and Steingass (1988):

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 2.20 + 0.136\ \text{GP} + 0.057\ \text{CP}$$

$$\text{OMD (\%)} = 14.88 + 0.889\text{GP} + 0.45\text{CP} + 0.0651 \times \text{A}$$

Where: GP = 24 hours net gas production (ml/200 mg),
CP = crude protein (%),
EE ether extract (%), and
A = ash (%).

Methane was measured with an infrared methane analyser (Sensor Europe GmbH, Erkrath, Germany) (Goel *et al*., 2008). Gas samples were then transferred into the inlet of an infrared methane analyser with a plastic syringe, which displays methane as a percentage of total gas. Methane production (mL) was calculated as: Methane production (mL) = total gas production (mL) × percentage of methane (%)

Data were analysed using the general linear model procedure of SPSS (1997, Chicago, Illinois, USA). Differences between reported means were determined using Duncan's multiple range tests with a 5% level of probability. The results were presented as means and standard error of the means (SEM).

Results and Discussion

The chemical composition of silage raw materials is provided in Table 1. Citrus pulps had lower DM ratios, and greater OM than the maize plants. Although the low DM content of citrus by-products means they may be regarded as difficult ensiling products for anaerobic fermentation, their lower cellulose may compensate for energy deficits that result from lower DM ratios. On the other hand, citrus pulp generally shows low pH owing to the acidic structure (the presence of organic acids, mainly citric, malic, ascorbic and tartaric acid) (Falade *et al.*, 2003). Therefore, the low pH is thought to have the potential to affect fermentation positively during the ensiling process, especially during the fermentation phase.

Table 1 Chemical composition of silage raw materials

	Maize	Lemon pulp	Orange pulp	Tangerine pulp
DM, g/kg	263.2	237.9	201.3	214.5
OM, g/kg DM	899.8	952.8	964.5	961.6
CP, g/kg DM	62.6	75.6	46.3	48.1
EE, g/kg DM	18.4	28.4	8.1	9.8
CF, g/kg DM	261.5	115.2	68.3	75.3
ADF, g/kg DM	376.3	194.5	144.4	131.5
NDF, g/kg DM	590.5	216.1	155.1	148.4
ADL, g/kg DM	114.8	79.3	76.1	56.2
HEM, g/kg DM	214.2	21.6	10.7	16.9

DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fibre, NDF: neutral detergent fibre, ADF: acid detergent fibre, HEM: hemicellulose, ADL: acid detergent lignin

The chemical compositions of citrus pulp silage alone and with maize silage are given in Table 2. pH is one of the significant indicators of silage quality (Kiermeier & Renner, 1963). The pH value of the silages (between 3.51 and 3.84) was close to the optimum silage pH value (between 3.8 and 4.2) (Coskun *et al.,* 1998). The pH of the citrus pulp silage was not improved with the maize silage combination. But this is related mostly to DM, which varied between 158.7 g/kg in the orange group and 369.7 g/kg in the maize silage group and the values were lower than the expected DM values (between 250 and 350 g/kg) (Demirel & Yıldız, 2000). Ergül *et al*. (2001) reported that the pH values of the silages prepared by adding 0, 15, 30 and 45% broiler litters to fruit juice and wet sugar beet pulp were between 4.1 and 4.2. Similar to the present findings, Deniz *et al*. (2001) reported pH values of between 3.72 and 4.30 for the silages with 20% DM. In another study, Avcı *et al.* (2005) reported pH values of between 3.64 and 4.33 for silage containing 17% DM and between 3.96 and 4.34 for silages containing 20% DM. On the other hand, Ülger *et al*. (2015) reported a lower pH value (3.76) for sugar beet pulp as compared with the present results.

The DM of citrus pulp silage (between 158.7 and 212.2 g/kg as fed) in the present study was in agreement with the DM that was previously reported as being between 123.7 and 149.2 g/kg (Yalçınkaya *et al*., 2012; La Van Kinh & Phuong, 1997). However, in the present study, combined citrus pulp and maize silage (50:50, on weight basis) had DM of between 230.9 and 289.4 g/kg, which is close to that of a high-quality silage (Ergül, 1988). Thus, the present results indicate that the DM of citrus pulp should be increased to produce good silage. A desirable DM can be achieved by adding 50 - 60% maize silage to the citrus pulp (Gurbuz & Kaplan, 2008). Combining citrus pulp with maize (50:50) in the current study increased the DM of the silage (between 230.9 and 289.4 g/kg), but it was still far from being high-quality silage. The DM of maize silage (369.7 g/kg) of the present study was parallel with the findings of those authors who reported similar values of DM from maize silage (Idukut *et al*., 2009; Arslan & Çakmakçı, 2011).

Organic matter levels of citrus pulp silages were close to each other and greater than the OM values of the maize silages. Citrus pulp thus reduced the OM of the combined silages. The greatest CP was observed in tangerine silage. Silage CP varied between 59.9 and 112.7 g/kg. The CP of citrus pulp silages was consistent with the findings of Bath *et al*. (1980). With regard to cellulose fractions, citrus pulp silages had lower ADF and NDF ratios than the maize silages, and citrus pulps generally had lower cellulose than the roughages. The ADF and NDF of the citrus pulp silages was increased with the maize silage combination, so citrus pulps are more suitable for inclusion in ruminant diets because of the ability of ruminants to ferment high fibre feeds in the rumen. In a previous study, the ADF of orange pulp silage and citrus pulp silage were reported as 220 g/kg and 200 g/kg, respectively (Bath *et al*., 1980). The NDF and ADF of citrus lemon were consistent with the findings of Nazem *et al.* (2008), who reported the NDF and ADF of citrus lemon as 213 and 179 g/kg, respectively. However, the NDF and ADF of *Citrus sinensis* were considerably lower than those reported by Nazem *et al*. (2008). The number of studies about citrus pulp silages is limited. In a study on lemon varieties, the differences were found to be significant and the mean values were OM: 94.73%, CP: 7.4%, EE: 5.60%, NDF: 20.05% and ADF: 17.16% (Özkan *et al*., 2017).

The nutritive values and fermentation metabolites of citrus pulp silage alone and with maize silage are given in Table 3. The total digestible nutrient (TDN) values varied between 654.6 (MS) and 857.6 (TPS) g/kg, and the differences in TDN values of the silages were found to be significant (P <0.01). Citrus pulp silages generally had greater non-fibre carbohydrate (NFC) than single maize silage and combined (P <0.01). Contrarily, fermentation metabolites of citrus pulp silages were less than the maize silages. While the lowest LA was observed in lemon and orange pulp silages, the greatest values were observed in maize silage (P <0.01). (The values varied between 29.77 and 101.2 g/kg DM.) The present LA productions were greater than the values reported in previous studies, probably because the proportion of material that remained in the pulp during the production of fruit pulps did not vary with the sugar. Citrus pulp generally generates more LA than sugar beet pulp, corn grain and sorghum grains (Cullen *et al.*, 1986). In the previous studies, the mean values of LA, AA, PA and BA in orange silage were reported as 21.9 g/kg, 29.8 g/kg, 2.9 g/kg and 0.5 g/kg, respectively (Martinez & Fernandez, 1980; Lanza, 1984; Miron *et al.*, 2001).

Table 2 Chemical composition of citrus pulp silages alone and when combined with maize silage 50:50 on a weight basis

Parameters	Treatments							SEM	*P*-value
	MS	LPS	OPS	TPS	LPS + MS	OPS + MS	TPS + MS		
pH	3.84^{a}	3.63^{c}	3.61^{c}	3.73^{b}	3.58^{cd}	3.51^{d}	3.57^{cd}	00.3	<0.001
DM, g/kg	369.7^{a}	212.2^{e}	158.7^{f}	162.3^{f}	289.4^{b}	245.6^{c}	230.9^{d}	15.3	<0.001
OM, g/kg	901.3^{d}	942.3^{a}	945.0^{a}	944.0^{a}	908.7^{c}	926.7^{b}	912.3^{c}	39.0	<0.001
CP, g/kg DM	65.5^{e}	83.7^{c}	90.5^{b}	112.7^{a}	78.0^{d}	59.9^{f}	84.0^{c}	3.6	<0.001
EE, g/kg DM	24.1^{abc}	17.7^{d}	16.4^{d}	28.8^{a}	26.3^{ab}	19.5^{cd}	20.6^{bcd}	1.1	0.003
CF, g/kg DM	246.4^{a}	104.4^{d}	123.3^{d}	117.2^{d}	195.2^{b}	166.0^{c}	189.3^{bc}	10.9	<0.001
NDF, g/kg DM	584.5^{a}	288.8^{d}	287.6^{d}	285.8^{d}	435.0^{b}	411.2^{c}	448.0^{b}	23.3	<0.001
ADF, g/kg DM	387.7^{a}	232.7^{e}	231.2^{e}	260.3^{d}	333.7^{b}	312.5^{c}	323.0^{bc}	12.2	<0.001
ADL, g/kg DM	141.2	128.3	107.9	143.1	138.4	146.5	133.7	4.2	0.19
HEM, g/kg DM	196.8^{a}	26.1^{d}	16.4^{d}	25.5^{d}	101.3^{c}	98.7^{c}	125.0^{b}	11.9	<0.001

MS: maize silage; LPS: lemon pulp silage; OPS: orange pulp silage; TPS: tangerine pulp silage; DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fibre, NDF: neutral detergent fibre, ADF: acid detergent fibre, HEM: hemicellulose, ADL: acid detergent lignin SEM: pooled standard error of means

[a, b, c, d] Within a row values with a common superscript are not different

Table 3 Nutritive value and fermentation metabolites of citrus pulp silage alone and when combined with maize silage 50:50 on a weight basis

Parameters	Treatments							SEM	*P*-value
	MS	LPS	OPS	TPS	LPS+MS	OPS+MS	TPS+MS		
TDN, g/kg DM	654.6[d]	855.6[a]	856.4[a]	857.6[a]	756.2[c]	772.4[b]	747.3[b]	15.8	<0.001
NFC, g/kg DM	227.3[e]	552.1[a]	550.5[a]	516.7[b]	369.3[d]	436.1[c]	359.8[d]	25.0	<0.001
TC, g/kg DM	811.7[c]	840.9[ab]	838.2[b]	802.5[d]	804.3[cd]	847.3[a]	807.8[cd]	4.1	<0.001
FP	125.33[a]	102.22[e]	92.20[f]	88.26[f]	119.80[b]	113.85[c]	108.52[d]	2.89	<0.001
WSC, g/kg DM	44.6[b]	56.5[ab]	34.5[b]	40.0[b]	81.2[a]	55.4[ab]	44.7[b]	4.2	0.03
LA, g/kg DM	101.21[a]	37.51[c]	29.77[c]	73.77[b]	41.64[c]	39.68[c]	51.64[b]	5.79	0.00
AA, g/kg DM	49.36[a]	29.48[c]	43.74[ab]	38.47[abc]	35.29[bc]	32.82[bc]	28.83[c]	2.03	0.03
PA, g/kg DM	28.27[ab]	33.32[a]	20.84[bc]	20.11[bc]	19.49[bc]	13.77[c]	14.99[c]	1.77	0.01
BA, g/kg DM	ND	0.61	ND	ND	ND	ND	ND	0.08	0.24
LA/AA	2.11[a]	1.43[abc]	1.95[bc]	0.69[c]	1.19[bc]	1.83[ab]	1.18[bc]	0.13	0.03

MS: maize silage; LPS: lemon pulp silage; OPS: orange pulp silage; TPS: tangerine pulp silage TDN: total digestible nutrients; OM: organic matter; NFC: non-fibre carbohydrates; TC: total carbohydrates; FP: Fleig point; WCS: water soluble carbohydrate; LA: lactic acid; AA: acetic acid; PA: propionic acid; BA: butyric acid; ND: not detected; SEM: pooled standard error of means;
[a, b, c, d] Within a row values with a common superscript are not different

The in vitro gas and methane production of citrus pulp silage alone and with maize silage are given in Table 4. The TG values of silage materials varied between 65.33 mL (MS) and 77 mL (OPS) and the differences in TG values of the silages were not found to be significant (*P* =0.07). The 24-hour methane production levels of the silage groups ranged from 9.7 to 12.38 and the lowest and highest values for ME were determined as 11.08 (MS) and 12.67 (OPS) MJ/kg DM. The greatest net energy lactation (NEL) (8.49 MJ/kg DM) was obtained from the orange group and the lowest value (7.15 MJ/kg DM) was obtained from the maize group. The OMD values varied between 76.96% (MS) and 87.33% (OPS). In a study carried out with different types of lemon pulp silage, total gas production was reported as between 68.7 and 77.6 mL. Methane production ranged from 10.1 to 13.6 mL. ME values ranged from 12.0 to 13.2 MJ/kg, and OMD values ranged from 82.7 to 91.5% (Özkan *et al*., 2017).

Table 4 In vitro gas and methane production of citrus pulp silage alone and with maize silage

Parameters	Treatments							SEM	P
	MS	LPS	OPS	TPS	LPS+MS	OPS+MS	TPS+MS		
TG, mL	65.33	74.00	77.00	74.67	68.00	66.67	68.00	1.31	0.07
CH_4, mL	10.79	12.38	11.73	11.77	9.70	11.52	10.63	0.27	0.12
CH_4, %	16.53	16.78	15.24	15.77	14.31	17.26	15.65	0.32	0.19
ME, MJ/kg DM	11.08	12.26	12.67	12.35	11.45	11.27	11.45	0.18	0.07
NEL, MJ/kg DM	7.15	8.14	8.49	8.22	7.45	7.30	7.45	0.15	0.07
OMD, %	76.96	84.66	87.33	85.26	79.33	78.15	79.33	1.17	0.07

MS: maize silage; LPS: lemon pulp silage; OPS: orange pulp silage; TPS: tangerine pulp silage; LPS+MS: lemon pulp silage+maize silage (50/50 by weight basis); OPS+MS: orange pulp silage+maize silage; TPS+MS: tangerine pulp silage+maize silage; SEM: pooled standard error of means; P: probability; *:*P* <0.05; **:*P* <0.01; NS: not significant; TG: total gas production; ME :metabolizable energy; NEL: net energy lactation; OMD: organic matter digestibility
[a, b, c, d:] Values with different superscript in a line differ significantly between treatment groups

Conclusion

By-products of the fruit juice industry can be used as silage materials in spite of their high moisture content. The citrus pulp silages generally had good silage quality in terms of OM, OMD and ME. However, the DM content of the citrus pulps should be increased with the addition of maize. The nutrient content (especially TDN and NFC) of maize silage may also increase silage quality when ensiled with citrus pulps. Thus, it is recommended that citrus pulp be combined with chopped combined with maize to control the freshness and management of these silages.

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Authors' Contributions

Conflict of Interest Declaration

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