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Effects of adding cladode and epidermis extracts of *Opuntia ficus-indica* and *Opuntia atropes* to aerobic mesophilic bacteria and total coliforms in bovine raw milk

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ABSTRACT

The aim of this research was to evaluate the effect of methanolic extracts from cladodes and cladode epidermis of Opuntia ficus-indica and Opuntia atropes on bovine raw milk microbiology. Methanolic extracts were obtained by maceration and added to bovine raw milk. Five repetitions per treatment were performed. A microbiological analysis was carried out 2 h after methanolic extracts were added. Aerobic mesophilic bacteria and total coliforms CFU mL^{-1} in raw milk decreased ($p < 0.05$) when Opuntia ficus-indica and Opuntia atropes extracts were added ($\leq 3.1x10^5$ and $1.5x10^6$, respectively for aerobic mesophilic bacteria) and ($\leq 1.9x10^5$ and $1.2\ x10^6$, respectively for total coliforms). Adding this methanolic extracts to raw milk achieved Mexican quality standards for aerobic mesophilic bacteria; but not for total coliforms. However, both species reduced aerobic mesophilic bacteria and total coliforms CFU mL^{-1} counts improving microbiological quality of bovine raw milk.

***Key words**: quality of milk, Opuntia spp., secondary metabolites, polar extracts.*

INTRODUCTION

Being a nutritious food, Bovine (*Bos Taurus*) milk is recommended for human consumption by the United Nations Food and Agriculture Organization[1]. However, milk can be a breeding ground for pathogenic microorganisms and could act as a disease vector for consumers[1]. Mexican milk production systems follow food security and quality standard strategies used in many countries[2]. México has official regulations establishing sanitary and nutritional specifications that milk must fulfill to be suitable for human consumption[3]. However, due to idiosyncrasies, economy and poor technologies in some rural areas of the country, milk production and dairy processed products are marketed without taking into consideration the public health risks they can generate[4].

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In Mexico, bovine milk production approaches 12 million liters per year. Household livestock provides 9.4% of national production (3,000 kg per cow^{-1} per year)[5,6] and it supplies 50% of the milk used in artisan cheese[7]. However, in this type of family systems, milk production and processing is under poor automation and hygiene practices[4,8] representing a great risk for consumers, health, since milk obtained under these conditions is used for sale or susitenance[9]. In addition, in these production systems, investment for acquiring a cooling tank or for covering the costs represented by cooling or boiling milk are not viable[1]. For that reason, the use of raw milk (RM) with no refrigeration or pasteurization for consumption and for processing dairy products is a common practice in Mexico[9].

Under the actual economic, technological and productive circumstances at household level, it is difficult to fulfill the official norms established for production and consumption of milk and dairy products. For that reason, it is necessary to establish strategies that could help reduce milk and dairy products bacteria in order to approach quality standards under these production systems and to accomplish Mexican official regulations to reduce population health risks. It has been established that fruits and cladodes of *O. ficus-indica* contain phytochemicals with these functional properties[10,11], such as phenols[12], which, are considered as antimicrobial components in plants[13], they are part of plant resistance systems to pathogen attacks[14]. In addition, these molecules have antioxidant, antiviral and antibacterial biological functions[15]. Diverse aspects can affect quantity and phenol types in plant extracts, such as: age, genotype and bromatological characteristics[16]. In relation to the later it has been determined that both, protein and dry matter can affect the amount of total phenols in plant extracts[17], which modify effectiveness of their bactericidal or bacteriostatic properties. In this regard, it has been found that adding nopal cactus (*Opuntia spp.*) to dairy cattle diet (12 kg daily of *O. ficus-indica* per cow plus a conventional diet) increased milk yield as well as organoleptic and bacteriological qualities of RM and fresh cheese[18,19]. This improvement in milk might be due to bacteriostatic properties in nopal cactus, since a decrease in average colony forming units (CFU) mL^{-1} of aerobic mesophilic bacteria (AMB) and total coliforms (TC) in RM, has been found when cows are fed with a diet complemented with nopal cactus[20].

The same effect observed in RM bacteria counts by adding nopal cactus to cows, diet was found when nopal extracts were added directly to RM. Addition to RM of ground cladode, mucilage and nopal epidermis, at levels of 0.5, 1.0 and 2.0%, showed a significant reduction ($p<0.05$) of AMB and TC CFU mL^{-1}, 2 h after nopal cactus components were added (*O. ficus-indica*), compared to control[21]. These results are important for food safety and the public health problematic that small dairy cattle producers are facing, as well as for possible milk and fresh cheese consumers. For that reason, the objective of this research is to evaluate the effect of cladode and epidermis methanolic extracts of *O. ficus-indica* and *O. atropes* added to bovine RM microbiology.

MATERIAL AND METHODS

Cladode samples were obtained in Morelia Michoacán México, analyzed species were *O. ficus-indica (OFI)* and *O. atropes (OA)*. They came from two plots: the first plot had only *O. ficus-indica* cultivated with agronomic practices (PCP) for weed control, formation pruning, and cleaning; the second plot had both species, cultivated with no agronomic control practices (PWCP). Cladode harvesting was carried out during the month of March. The climate of the region is tempered, sub-humid, corresponding to Cw classification[22]; with annual average temperature and precipitation of 18.6 ºC and 786 mm respectively[23].

Cladode samples for total phenol extraction were cut from fourth plant level, according to Santos et al.[24] classification, as follows: 0 to the base of the nopal cactus, 1 to first cladodes (from the ground upwards) and so on. Plants of each species were identified in each plot to obtain a cladode sample at random per plant and three sample groups were formed: group 1 (27 cladodes of OFI in PCP), group 2 (17 cladodes of OFI in PWCP) and group 3 (29

cladodes of OA in PWCP). For each group five cladodes from third level were also collected, in order to perform bromatological analysis for both levels. Eighty-eighty cladodes were collected altogether. Each cladode was identified and its length was recorded with a measuring tape (cm).

Once the morphologic dimensions per cladode were obtained, the corresponding chemical composition analysis was made, following the methodology described by AOAC[25] for: dry matter (DM), moisture (M), crude protein (CP), crude fiber (CF), ether extract (EE), ashes (As) and free nitrogen extract (FNE). In addition, production of dehydrated mucilage was estimated per species, parcel and season; from 300 g samples of fresh matter (FM). Dehydrated mucilage was determinate using the methodology modified by Rodriguez[26].

Extraction methods

After cladodes were washed, half of the samples of the fourth level were cut into 2 cm^3 pieces in order to obtain the extracts. From the remaining half the epidermis was removed with a manual peeler. Cladode fragments and epidermis were dehydrated at 40 °C for 72 and 24 h respectively. Samples were grounded in a porcelain mortar, until obtaining dust for mixing and homogenizing. They were stored according to sample type (cladode or epidermis) and sample group (species and plot type), at - 4 °C in the dark. Extracts were obtained by maceration with successive extractions using hexane, chloroform and methanol, in that order. Five grams were macerated for three days at room temperature (25 °C) with 50 mL of each solvent in an orbital agitator (Thermo Scientific MaxQ). Between each solvent, extract was filtered and stored in a dark place at - 4 °C, the sample was treated with the next solvent[27]. There were three repetitions on each extraction group.

Total phenolic compounds quantification

Total phenolic compounds quantification (PT) was done only for methanolic extracts. The reaction mixture consisted of: 550 µL of distilled water; 50 µL of extract and 100 µL of Folin-Ciocalteu reagent (FC; Sigma Aldrich®). Eight minutes after the oxidation reaction initiated, 300 µL of Na_2CO_3 were added and was left it repose for 15 minutes at room temperature. Then an absorbance reading was taken at 760 nm in a spectrophotometer (UV-Vis Nanodrop 2000_C®), with its respective target[28].

In order to determine total phenol contents on extracts, a calibration curve of gallic acid was constructed (GA). Starting with 0.5 µg μL^{-1} GA solution, from which, volumes were taken, from 5 to 350 µL with intervals of 10 µL, adding reagents (100 µL of FC and 300 µL Na_2CO_3) and the necessary distilled water to reach the 600 µL. Six repetition were made for each extract. Measurements were in equivalent µg of GA (GAE) μL^{-1} as suggested by Makkar[28]; for that, the value µg GAE mL^{-1} was estimated in base to absorbance averages by volume analyzed. This was made by means of linear regression[29] estimators, with intercept and slope of 0.359 ($p < 0.001$) and 20.081 ($p < 0.001$) respectively, which meant that for each unit of absorbance (nm), concentration increased in 20.081 µg GAE. The obtained values were expressed as mg GAE 100 g^{-1} of dry weight (DW).

Bovine raw milk microbiological analysis

From recently milked RM, stored in a cold 500 lts tank, 3.500 mL were transported at 4 °C in a sterile hermetic container, according to the Mexican official norm[30]. From the total RM obtained seven treatments were performed, each one with five samples (100 mL of RM per sample). Treatments were as follows: 1) control: RM without extract; 2) RM with cladode extracts of OFI in PCP; 3) RM with cladodes extracts of OFI in PWCP; 4) RM with cladodes extracts of OA in PWCP; 5) RM with cladode epidermis extracts of OFI in PCP; 6) RM with cladodes epidermis extracts of OFI in PWCP and 7) RM with cladode epidermis extracts of OA in PWCP.

Methanolic extract addition to RM was equal to PT dose: 1390 µg GAE mL^{-1} per sample. In order to remove methanol, a sufficient volume extract was concentrated based on PT values found for each treatment (Table 2), then concentrated extracts were dissolved in 4 mL of sterile distilled water. To each 100 mL RM sample, the water-diluted extract (1390 µg per 4 mL) was added. All samples were stored at room temperature (25 °C) for 2 h.

Sample microbiological analysis was carried out 2 h after nopal cactus extracts were added to RM, according to Mexican official norms criteria: NOM-110-SSA1[31], NOM-113-SSA1[32] and NOM-092-SSA1[33]. Colony forming unit counts (CFU) mL^{-1}, for both TC and AMB, were carried out at 24 and 48 h.

The data set built had microbiological response variables transformed into to log_{10}. Statistical analysis was done under general linear models methodology (GLM)[34] and statistical differences between treatments were taken by least square means (Lsmeans)[34].

RESULTS

OFI and OA cladodes bromatological analysis was performed as well as statistical comparison between these species. They showed that DM was greater ($p < 0.05$) in OA as compared to OFI in both levels (Table 1). EE was greater ($p < 0.05$) in the third cladode level of both species compared with fourth cladode level within the same species (Table 1). However, OFI showed greater EE concentration ($p< 0.05$) than OA. In relation to CF, OA cladodes showed less CF (9.68 ± 1.08 and 6.51 ± 2.28% for third and fourth level, respectively) than OFI ($p < 0.05$). FNE content was higher in OA ($p < 0.0\ 5$) than OFI. With regard to dehydrated mucilage production, OA had between 2.77 and 3.96 g of (DM) from 300 g of fresh weight (FW). Finally, for CP there were not differences ($p > 0.05$) according to species or cladode levels, whose means were in the range from 4.10 to 5.61%.

Table 1. *Opuntia ficus-indica* and *O. atropes* cladode bromatological characteristics for plot without agronomic practices.

Species	Variable	□ Level 3	SD	□ Level 4	SD
O. ficus-indica	Moisture$^{g\%}$	93.28^{a1}	0.38	93.08^{a1}	0.48
	Dry matter$^{g\%}$	6.72^{a1}	0.38	6.92^{a1}	0.48
	Ether extract $^{g\%}$	2.25^{a1}	1.65	0.72^{a2}	0.29
	Crude fibre$^{g\%}$	22.62^{a1}	9.11	8.04^{a2}	3.92
	Crude protein$^{g\%}$	4.76^{a1}	1.55	5.61^{a1}	0.97
	Ash$^{g\%}$	22.22^{a1}	4.74	21.07^{a1}	2.43
	F.N.E$^{g\%}$	47.51^{a1}	13.84	64.17^{a2}	2.36
	Mucilage^{g}	1.75^{a1}	0.82	1.41^{a1}	0.20
O. atropes	Moisture$^{g\%}$	88.26^{b1}	1.96	88.72^{b1}	1.22
	Dry matter$^{g\%}$	11.74^{b1}	1.96	11.28^{b1}	1.22
	Ether extract $^{g\%}$	1.23^{b1}	0.59	0.90^{a1}	0.66
	Crude fibre$^{g\%}$	9.68^{b1}	1.08	6.51^{b1}	2.28
	Crude protein$^{g\%}$	4.10^{a1}	1.26	4.33^{a1}	1.00
	Ash$^{g\%}$	20.05^{a1}	4.82	19.96^{a1}	5.14
	F.N.E$^{g\%}$	64.95^{b1}	4.50	68.29^{a1}	5.69
	Mucilage^{g}	3.24^{b1}	1.12	2.77^{b1}	0.77

$^{g\%}$= percentage of sample in grams, ^{g}= grams,
^{a,b}= statistical differences ($p<0.05$) within column.
^{1,2}= statistical differences ($p<0.05$) within row.

In relation to total phenols in methanolic extracts there were significant interactions between extract sources and species and for plot types by specie ($p < 0.01$) (Table 2). Extracts obtained from cladode epidermis showed greater amounts of total phenols ($p < 0.05$) (348 - 569 mg GAE 100 g^{-1}) ($p < 0.05$), compared to extracts from complete cladodes (174 - 268 mg GAE 100 g^{-1}). On the other hand, extracts from OFI in PCP and PWCP showed greater ($p < 0.05$) total phenols amounts (268 - 569 mg GAE 100 g^{-1}) compared to OA in PWCP: 174 - 348 mg GAE 100 g^{-1}, for complete cladode and epidermis, respectively (Table 2).

Table 2. Total phenols in methanolic extracts from cladode and cladode epidermis from nopal cactus according to specie and plot.

	Species	Plot	Mean (mg GAE100g^{-1})	SD (mg GAE100g^{-1})
Cladode	*O. ficus-indica*	With practices	268^{a}	43
	O. ficus-indica	No practices	263^{a}	24
	O. atropes	No practices	174^{b}	48
Epidermis	*O. ficus-indica*	With practices	500^{c}	39
	O. ficus-indica	No practices	569^{d}	30
	O. atropes	No practices	348^{e}	33

a, b, c, d and e= statistical differences ($p < 0.05$) within column.
SD= standard deviation.

According to values shown in Table 2, epidermis extracts from OFI cultivated in PWCP showed better results ($p < 0.05$) for total phenols (569 mg EAG 100 g^{-1}). Reading epidermis extracts, OA cultivated in PWCP produced less total phenol amounts (348 mg EAG 100 g^{-1}) ($p < 0.05$).

In relation to the effect of adding methanolic extracts to RM on AMB bacterial counts (CFU mL^{-1}) it was found extract source (cladode or epidermis) and plot did not affect AMB in RM CFU mL^{-1} count. On the contrary, species of nopal cactus did affect CFU mL^{-1} count of these bacteria in RM ($p < 0.05$). It was found that adding OA, had reduced ($p < 0.05$) the UFC mL^{-1} of AMB ($5.0x10^4$ and $7.9x10^4$ UFC mL^{-1}, for extracts of cladodes and epidermis, respectively) in comparison with the bacterial counts of RM added with OFI extracts ($3.1x10^5$ and $1.9x10^5$ UFC mL^{-1} of AMB, for extracts of cladodes and epidermis, respectively). However, the UFC mL^{-1} of AMB in RM of all analyzed treatments were smaller ($p < 0.05$) to the control treatment ($1.5x10^6$ UFC mL^{-1} of AMB in RM without addition of nopal cactus extracts) (Table 3).

For total Coliformes an effect was found ($p = 0.0001$) for extract source (cladode or epidermis) and of species. The UFC mL^{-1} of TC in RM added with extracts of nopal cactus (cladode or epidermis), independently of the species, they were less ($p < 0.05$) to the control treatment ($1.2 x10^6$ UFC mL^{-1} of TC in RM). Nevertheless, the UFC mL^{-1} of TC in RM added with extracts of cladodes of OFI, independently of the type of parcel in which it was cultivated, they showed greater reduction TC in RM ($3.9x10^4$ - $7.9x10^4$ UFC mL^{-1}) in comparison with extracts of epidermis of OFI (1.0^5x10 - $1.9x10^5$ UFC mL^{-1}). For the case of the treatment with extracts of cladodes or epidermis of OA, it was found that both extracts had the same effect ($p < 0.05$) on the reduction of bacterial counts of TC in RM ($5.0x10^4$ and $3.9x10^4$ UFC mL^{-1}, respectively). These results were similar ($p > 0.05$) to the ones observed in RM added with OFI cladode extracts (Table 3).

Table 3. Raw milk added with methanol extracts for nopal cactus cladode and epidermis microbiological analysis according to specie and plot type.

Section	Specie	Plot	Aerobic mesophilic		Total coliforms	
			Mean (UFCmL^{-1})	SE	Mean (UFCmL^{-1})	SE
Witness*	---	---	$1.5x10^{6a}$	$0.14x10^{1}$	$1.2x10^{6a}$	$0.13x10^{1}$
Cladode	*O. ficus-indica*	WP	$1.9x10^{5b}$	$0.14x10^{1}$	$7.9x10^{4b}$	$0.13x10^{1}$
	O. ficus-indica	NP	$3.1x10^{5b}$	$0.14x10^{1}$	$3.9x10^{4b}$	$0.13x10^{1}$
	O. atropes	NP	$5.0x10^{4c}$	$0.14x10^{1}$	$5.0x10^{4b}$	$0.13x10^{1}$
Epidermis	*O. ficus-indica*	WP	$1.9x10^{5b}$	$0.14x10^{1}$	$1.9x10^{5c}$	$0.13x10^{1}$
	O. ficus-indica	NP	$1.5x10^{5b}$	$0.14x10^{1}$	$1.0x10^{5c}$	$0.13x10^{1}$
	O. atropes	NP	$7.9x10^{4c}$	$0.14x10^{1}$	$3.9x10^{4b}$	$0.13x10^{1}$

a, b, c= differences estadísticas ($p < 0.05$) within column; SE= standard error.
*= raw milk without extracts; WP= with practices; NP= no practices.
UFC= colony forming units.

DISCUSSION

It has been stablished that bromatological composition can affect synthesis and amounts of total phenols on plant extracts. Giletto et al.[17] observed that for greater nitrogen concentration, (CP) DM decreased and phenol amounts increased. With respect to the results of DM obtained from both species, these values were within the range of nopal cactus species: 76 - 165 g of DM for each 1000 g of fresh matter (FM), Andrade et al.[35]. In the aspect of CP of analyzed species cladodes (Table 1), values were consistent with those established for *Opuntia spp*. Torres[36] found 5.0 and 5.3% of CP for OFI round and giant varieties respectively, which are similar to the obtained results. Hernandez et al.[37] reported 8.48% of CP for tender cladodes (450 g WF); however, these researchers suggested that CP in nopal cactus increased in response soil acidity or salinity. As previously stated, until certain point, the factor that can determine, total phenol amounts in nopal cactus extracts analyzed in this research could be DM.

Total phenols in the range between 900 and 1100 mg GAE 100 g^{-1} were reported for OFI and OA[38]; previously Guevara et al.[39] found higher values in the range from 1780 and 1990 mg GAE 100 g^{-1} for OFI complete cladodes. Which are higher than those obtained in this research for cladodes from OFI (263 ± 28 - 268 ± 43 mg GAE 100 g^{-1}) and OA (174 ± 48 mg GAE 100 g^{-1}). These differences could possibly be related to factors, such as: region agro ecological conditions where the cladodes were collected; species; cladode maturity, extraction methods and phenol analysis[16]. For example, in this research the correlation between cladode DM and total phenols was (r = -0.54; p = 0.003). linear regression estimates suggested that for each DM cladode gram a reduction of -0.012 mg GAE 100 g^{-1} ((β_0 = 0.345; (p < 0.01) and β_1 = -0.012; (p < 0.01)). The cladode DM average (90 days of age) used to obtain extracts were 6.7 ± 1.65 and 11.7 ± 1.96 g (for each 100 g of fresh matter) for OFI and OA, respectively. Whereas Guevara et al.[39] used younger cladodes, which must have between 3.0 and 3.7 g DM, according to age classification for OFI[40].

Cai et al.[41] found 555.0 mg GAE 100 g^{-1} for total phenols of cladodes epidermis, which is similar to the value found for OFI epidermis in PCP, however this specie under PWCP had less amounts, on the other hand, greater values were found for OA (Table 2). Some investigations suggested that for this extract types the total phenols quantity is greater in epidermis or fruits as compared to pulp[42,43,44]. In this sense, Moussa et al.[38], reported greater total phenols amounts in OFI fruit skin (Tuna), compared to values on the pulp. These findings agree to results of this investigation, in which epidermis extracts had greater amounts (p < 0.05) compared to those from complete cladodes (Table 2).

The importance of the extracts analyzed in this investigation was in proving its effect on aerobic mesophilic bacteria (AMB) and total coliformes (TC) in bovine raw milk (RM). At this respect, RM added with cladode extracts or epidermis showed lower AMB and TC load (P < 0.05) as compared to the control (Table 3). In addition, the values of AMB in RM, added with these extracts of nopal cactus, were within the quality standards according to the Mexican official norm (NMX-F-700-COFOCALEC[45]), as to be considered suitable for human consumption or product processing ($1.2x10^6$ UFC mL^{-1} of AMB). This was not the case for TC, even when the counts of this bacteria type in RM were reduced in comparison to the control, values found for this variable did not fulfill the standards established by the official Mexican norm ($1x10^2$ UFC mL^{-1} of CT) in order for milk to be considered suitable for human consumption (NMX-F-700-COFOCALEC[45]).

In relation to the bacteriostatic effect of the nopal cactus extracts analyzed (OFI and OA) on the bacterial load (AMB and TC) of the RM, Sanchez el at.[46], found effect of ethanolic and methanolic OFI cladode extracts on Gram-negative bacteria, such as *Vibrio cholera*. On the other hand, in related research, were results similar to those found in this research. Adding directly of fresh and dehydrated mucilage, OFI epidermis and cladodes fragments in fresh base, UFC mL^{-1} of AMB were reduced and the values established by the official mexican norm were achieved (p < 0.05), however, for the case of TC counts in RM, even when they were reduced in comparison to the control (p < 0.05), the official mexican norm was not met[21,47].

The smaller effect of the methanólic extracts on TC can be due to the fact that Gram-negative bacteria offer greater resistance than Gram-positive to the entrance of antimicrobial agents to the cell[48]. Perhaps because they have an external cellular membrane which, has an exclusion limit greater than 600 kDa molecules; whereas, the limit of exclusion in Gram-positive that lack an external cellular membrane is only 100 kDa, much greater than the majority of compounds that act as antimicrobial[49]. On this matter, total coliforms bacteria are Gram-negative from the family *Enterobacteriacea*[50]. However, the aerobic mesófilas bacteria include Gram-positive and Gram-negative bacteria that can grow at temperatures between 35 - 48°C [33]. Thus, the TC of RM added with extracts of cladode and epidermis *Opuntia spp.* offer greater resistance than the AMB.

The effects of cladode extracts or epidermis on the bacterial counts in RM can be due to phenols found in polar extracts (methanol, ethanol and water) of cladodes of OFI like phenolic acids, flavonoids and tannins[51,52,38]. With respect to phenolic acids, Gutiérrez et al.[53], evaluated the minimum inhibiting concentration of phenolic acid like the hydroxybenzoic, protocatechuic and galic, on pathogenic Gram-negative and Gram-positive bacteria on food and they found inhibition of the bacteria by effect of all evaluated phenolic acids. In this respect, Otshudi et al.[54], found that plants that have greater phenol amounts, also have greater antimicrobial activity.

Phenols action on bacteria, according to Sanchez et al.[46] can be attributable on the basis that these compounds damage the cellular membrane; also, they can cause reduction of cytoplasmic pH and ATP production. Ultee et al.[55], suggested that phenols antimicrobial activity must be on its aromatic ring and mainly associated to the hydroxyl (-OH) group, which they have (sometimes with more than one). With the –OH group, phenols can act like carriers of monovalent cations of the cytoplasm to the extracellular space; when a phenol arrives at the cytoplasm it interchanges its proton hydrogenate (H+) for a potassium ion (K+) or another cation[55]. This interchange causes cellular membrane depolarization[56] and, the depolarization or polarization of the cellular membrane is considered a cellular damage[57,58], affecting the homeostasis and finally cellular death[55].

The greater effect of OA cladode or epidermis extracts in comparison to OFI on AMB (Table 3), could be due more to phenol types in each extract rather than in total phenol quantity. Nevertheless, in this research total phenol composition were not determined according to the extract type (epidermis or cladode complete) and species (OFI or OA). However, because the reference compound was galic acid, the type of phenols can vary and this variation affects its antimicrobial capacity[59]. Ultee et al.[55], stated that although two phenols can be very similar these do not reflect the same antimicrobial and antioxidant activity. This could suggest that phenols present in the OA extracts of could display more antimicrobial activity independently, even when that specie had lower phenol amounts.

CONCLUSION

Addition of methanolic extracts from epidermis and complete cladodes of OFI and OA to raw milk, from production systems with deficient udder health systems and milking control, improved microbiological milk quality, due to the fact that these compounds reduced UFL mL^{-1} count number of aerobic mesophilic bacteria and total coliforms. Even though, methanolic extracts from OA showed lower values of total phenols than those from OFI both had greater effect on bacterial counts when added directly to raw milk.

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