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RESEARCH PAPER

Protein enrichment of *Opuntia ficus-indica* using *Kluyveromyces marxianus* in solid-state fermentation

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Abstract

E. Herrera, M. Murillo, L. Berumen, N.O. Soto-Cruz, and J.B. Páez-Lerma. 2017. Protein enrichment of *Opuntia ficus-indica* using *Kluyveromyces marxianus* in solid-state fermentation Cien. Inv. Agr. 44(2): 113-120. *Opuntia ficus-indica* is used in animal feed but has a low protein content (above 4%) in comparison to high-quality forage (above 16%). It is necessary to develop technologies that improve the nutrimental value of *Opuntia ficus-indica* through the process of solid-state fermentation (SSF). Therefore, the objective of this study was to evaluate the changes in the chemical composition of *Opuntia ficus-indica* during the SSF with the yeast *K*luyveromyces *marxianus* ITD00262. *K*luyveromyces *marxianus* ITD00262 was grown in glucose, peptone, yeast (GPY) broth, at 28 ºC and pH 4.8 for 12 h. *Opuntia ficus-indica* was incubated in SSF at 28 °C without inoculation (T1) or inoculated with *K. marxianus ITD00262 (T2)*. Fermented samples of *Opuntia ficus-indica* were dried, and the *dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIGN) and in vitro dry matter digestibility (IVDMD)* contents were ascertained. Data were analyzed with a completely randomized design with a factorial arrangement of 2×6. A decrease of 11% in DM was observed for T1 compared with T2 at 48 h of fermentation. A 300% increase in PC content was observed for T2 at 120 h of fermentation. The DMIVD was increased by 3%, whereas the values of NDF and ADF decreased by 18% in T2. According to our results, it is confirmed that the use of *K. marxianus* in the SSF of *Opuntia ficus-indica* has a positive effect and improves the nutrimental quality of this forage.

Key words: Fermentation, nutritional quality, protein, *Opuntia*, yeast.

Introduction

Irregular annual rainfall distribution in the northern region of Mexico limits natural forage availability, especially during the dry season (Murillo *et al.*, 2000). Consequently, commercial concentrates must be used during this period to provide the protein and energy required for cattle. However, a 40% increment in the cost of supplements has generated interest in using alternative food sources to reduce costs (Herrera, 2011). *Opuntia ficus-indica* represents one such alternative that has been used in cattle feed. *O. ficus-indica* is advantageous because its substrate is easily converted to biomass and it can potentially be used as a substrate on which to

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grow microorganisms in complex environments (Gutierrez *et al*., 2009). The main disadvantage of using *O. ficus-indica* is that it has low crude protein content (4%). However, solid-state fermentation (SSF) biotechnology may enhance the nutritional quality of *O. ficus-indica* because this technology is able to produce biomass from the carbohydrates present in *O. ficus-indica* (Peláez *et al*., 2011). Solid-state fermentation allows the growth and cultivation of microorganisms under controlled conditions in the absence of free water, yielding products of interest (Pandey *et al*., 1999). Examples of products obtained by SSF include industrial enzymes, fuels, and nutrient-enriched animals. Yeasts, such as *Saccharomyces cerevisiae* and some species of *Kluyveromyces*, are among the microorganisms that are frequently used in this process (Van Markis *et al*., 2006). These yeasts generate microbial biomass with a high protein content in the form of unicellular proteins (Grba *et al*., 2002). *K*luyveromyces *marxianus* is a versatile yeast that may be economically exploited for a wide range of applications, including the production of various enzymes (such as inulase, pectinase, and lactase) and for unicellular protein production (Schultz *et al*., 2006). Moreover, *K. marxianus* has a clear advantage over other yeasts because of its thermo-tolerance, high growth rates, absence of fermentative metabolism in excess sugar and broad substrate spectrum (Van Dijken *et al*., 2000). However, information about the overall nutritional quality of *O. ficus-indica* fermented with *K. marxianus* is lacking. Therefore, this study aimed to investigate the effect of *K. marxianus* on the nutritive variables of *O. ficus-indica* during different times of solid state fermentation.

Materials and methods

Study area

The present study was performed in the Postgraduate Laboratory of the Faculty of Veterinary and Husbandry of Juarez University at the Durango and Microbial Biotechnology Laboratory of the Graduate and Research Unit of the Technological Institute of Durango.

Yeast strain

The yeast strain *K. marxianu*s ITD00262 was obtained from the collection of the Microbial Biotechnology Laboratory (Technological Institute of Durango). This strain was previously isolated by traditional agave fermentation and identified using restriction fragment length polymorphism and the Yeast-id database (Páez *et al*., 2013).

Physiological characterization

Cultures were obtained after inoculation on agar plates and overnight incubation at 28 °C in 10 ml of yeast, nitrogen, and base (YNB) medium (containing 1.7% YNB w/w nitrogen, 2% lactose and 2% inulin) with 0.5% ammonium as the sole nitrogen source.

Cultivation

*K*luyveromyces *marxianus* ITD00262 was grown in peptone, 2% dextrose and 1% yeast extract broth at pH 4.8 and 28 °C for 12 h with agitation (120 rpm) to obtain an initial count of 10^8 cells ml^{-1}.

Solid-state fermentation

Fermentation was carried out in flasks with 250 g of chopped *Opuntia ficus-indica* (T1) without the yeast strain and chopped *Opuntia ficus-indica* inoculated with $2.6x10^6$ cells g^{-1} DM *K. marxianus* ITD00262 (T2). The flasks were prepared in triplicate for each treatment and each time and were incubated at 28 °C for 0, 24, 48, 72, 96 and 120 h in an incubator. Triplicate samples at each fermentation time were used to assess pH and chemical analyses.

Chemical analysis

The *Opuntia ficus-indica* samples and fermented *O. ficus-indica* were dried at 55 °C for 24 h in a forced air oven. All samples from all fermentation times were subjected to several nutritional analyses. The dry matter (DM; #934.01) and crude protein (CP; #954.0) concentrations were determined according to the AOAC (1997). The neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIGN; #973.18) percentages were determined by procedures proposed by Van Soest *et al*. (1991). *In vitro* dry matter digestibility (IVDMD) was determined using methods suggested by ANKOM Technology, Macedon, New York, USA.

Statistical analysis

Data were analyzed according to a completely randomized design with a factorial arrangement of 2×6 with measures from each 24-hour period using GLM procedures in SAS. The factors evaluated were with and without yeast and different fermentation times (0, 24, 48, 72, 96 and 120 h). The model included the effects of the yeast, fermentation time and interactions between the two variables.

Results

Fermentation and chemical characteristics

Treatment × time interactions were observed for DM and CP ($P < 0.05$, Table 1). The dry matter content decreased with increasing fermentation time, whereas the CP content increased. The dry matter content was 11% lower for T1 compared with T2 at 48 h of fermentation. The crude protein concentration increased by 300% in T2 at 120 h of fermentation with respect to T1. Increased fermentation time increased the CP concentration in T2 by 119%.

Table 1. Least square means of the chemical composition of *Opuntia ficus-indica*.

	Time				
	T1	T2	Mean	SEM	P<
Dry matter (%)					
0 h	11.30	12.37	11.83	0.045	*
24 h	11.98	11.80	11.89	0.045	*
48 h	10.19	11.46	10.82	0.045	*
72 h	10.46	10.79	10.62	0.045	*
96 h	10.00	10.19	10.09	0.045	*
120 h	9.48	10.11	9.79	0.045	*
Mean	10.57	11.12			
Crude protein (%)					
0 h	3.85	7.82	5.83	0.054	*
24 h	7.35	9.51	8.67	0.054	*
48 h	5.95	9.89	7.92	0.054	*
72 h	4.72	11.85	8.28	0.054	*
96 h	4.65	14.65	9.65	0.054	*
120 h	4.25	17.14	10.69	0.054	*
Mean	5.12	16.31			
Dry matter digestibility (%)					
0 h	90.02	87.39	88.70	0.246	NS
24 h	85.80	88.19	86.99	0.246	NS
48 h	90.57	90.19	90.38	0.246	NS
72 h	86.58	88.13	87.35	0.246	NS
96 h	81.10	80.31	80.70	0.246	NS
120 h	80.23	78.79	79.51	0.246	NS
Mean	85.71	85.5			

SEM: Standard error of the mean, *(P<0.05), NS: not significant , T1=without yeast and T2=with yeast

No treatment × time interaction was detected for IVDMD (P>0.05, Table 1). The IVDMD of *O. ficus-indica* with *K. marxianus* increased by 3% after 48 h fermentation.

Treatment × time interactions were recorded for NDF, ADF and LIGN concentrations (P<0.05, Table 2). The neutro detergent fiber and ADF concentrations of *O. ficus-indica* decreased by 137 and 18%, respectively, after 120 h SSF with *K. marxianus*. Moreover, NDF and ADF contents were lower in *O. ficus-indica* fermented with *K. marxianus* at all fermentation times. However, the LIG concentration increased with longer fermentation times.

A treatment × time interaction was observed for pH (P < 0.05, Figure 1). The pH registered in *O. ficus-indica* fermented with *K. marxianus* was lower than that of *O. ficus-indica* fermented within yeast at all fermentation times.

Discussion

Physiological characterization

Isolates of strains from agave fermentation are able to grow in inulin, which is the energy storage form for this type of plants. The principal structure in agave is agavina, which is a type of

Table 2. Least square means of the fiber content in fermented *Opuntia ficus-indica*.

	Time		Mean	SEM	P<
	T1	T2			
Neutral detergent fibre (%)					
0 h	27.17	34.96	31.06	0.311	*
24 h	37.06	36.36	36.71	0.311	*
48 h	24.22	21.22	22.72	0.311	*
72 h	24.10	19.69	21.89	0.311	*
96 h	29.10	17.23	23.16	0.311	*
120 h	28.32	15.04	21.68	0.311	*
Mean	28.32	24.08			
Acid detergent fibre (%)					
0 h	15.53	13.12	14.32	0.415	*
24 h	21.23	15.09	18.16	0.415	*
48 h	17.99	13.69	15.84	0.415	*
72 h	15.81	11.38	13.59	0.415	*
96 h	19.23	11.06	15.14	0.415	*
120 h	20.13	11.16	15.64	0.415	*
Mean	18.32	12.58			
Lignin (%)					
0 h	2.83	1.74	2.28	0.042	*
24 h	3.02	2.18	2.6	0.042	*
48 h	2.54	3.27	2.9	0.042	*
72 h	2.69	4.00	3.34	0.042	*
96 h	2.50	5.11	3.80	0.042	*
120 h	2.32	6.24	4.28	0.042	*
Mean	2.65	3.75			

SEM: Standard error of the mean, *(P<0.05), NS: not significant, T1=without yeast and T2=with yeast

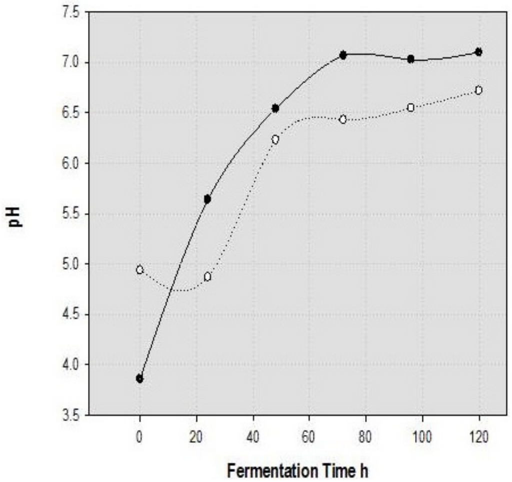

Figure 1. Changes in pH during fermentation of *Opuntia ficus-indica* in both treatments without *K. marxianus* (●) T1, and inoculating with *K. marxianus* (○) T2

inulin. The carbohydrate content in Agavaceae and Nolinaceae plants is one of the most important attributes influencing their commercial use as fibers, sweeteners and supplement ingredients (Mancilla and López, 2006). Fructans extracted from Agave, Dasylirion and *O. ficus-indica* may be fermented by this type of yeast during the early stage of SSF. These carbohydrates may be used as an energy source to produce unicellular protein.

Fermentation and chemical characteristics

The reduction observed in DM content may be attributed to yeast growth on the substrate, resulting in metabolism of the carbohydrates present in the substrate to produce CO_2, H_2O and energy (Sato and Sudo, 1999). Moreover, the reduction of DM consumption is revealed by the intake of fibrous components (Darwish *et al*., 2012). Our results supported those of Chiteva and Wairagu (2013) and Melo *et al*. (2003), who reported 13% and 10.70% DM in *O. ficus-indica* without fermentation, respectively. In this study, the DM content decreased with increased fermentation time in *O. ficus-indica* fermented with *K. marxianus,* but was higher than T1.

To our knowledge, this study is the first to publish the CP values of *O. ficus-indica* fermented with *K. marxianus*. Further, protein enrichment was a result of increased microbial biomass, considering the absence of nitrogen sources. Hu *et al*. (2012) stated that supplementation with 2.5% nitrogen source is necessary to increase the protein content from 5–6% to 16–20%. In another study, Diaz (2011) reported an increase of 24.52% CP for *O. ficus-indica* fermented with *K. lactis,* while Diaz *et al*. (2012) reported an increase of 19.36% CP for cactus fermented with *K. lactis*, urea, ammonium sulfate and minerals. The CP values obtained in both studies are lower than those recorded in this study. The general increase in CP may be explained by the growth of native microorganisms in *O. ficus-indica* without inoculum. Moreover, the high increase documented for *O. ficus-indica* fermented with *K. marxianus* in this study may be due to growth of yeasts during fermentation. In this work, an increase of 30% (from 3.85 to 5.12%) is observed for CP in T1 (Table 1). In T2, CP initial was 3.97% but increased more than three times to 12.34%, without regard to *K marxianus* from CP. Overall, the final CP was 16.31%, similar to high-quality forages (Table 1).

The high digestibility values obtained in this study (approximately 90%) may be attributed to the soluble carbohydrates present in the cactus, which are related to the nitrogen-free extract and low NDF content (Northon, 1982). According to (Do Santos *et al*., 2015) an increase in degradability of biomass is due to higher cell fiber degradation of cactus pear by microorganisms present in the rumen, resulting in greater nutrient absorption. Similarly, Murillo *et al*. (2000) recorded 90% IVDMD in cactus AN-TV6, and Mcitekah (2008) reported 75.21% in silage cactus.

Previous studies have not reported the concentration of NDF, ADF and lignin in fermented *O. ficus-indica* with *K. marxianus*. However, in our research, we observed a decrease in FDN content (34 to 15%) in T2 with increasing fermentation time. These results are similar to those reported by Pinos *et al*. (2010) in *O. ficus-indica* without fermentation (28.8%). Moreover, de Souza *et* *al*. (2016) registered reductions of cellulose and hemicelluloses content (FDN components) in cactus pear fermented with *Aspergillus niger,* at 240 h of fermentation. While the FDA content was lower in *O. ficus-indica* fermented with *K. marxianus*, according to Van Soest (1982), the *O. ficus-indica* fermented with *K. marxianus* is considered to be high-quality forage because it has less than 40% FDN.

pH is a critical factor during the SSF process (Pastrana, 1995). The optimal pH for *K. marxianus* growth is 4.5 to 5.0, although it may occur at pH 7.5 (Costa, 2000). In this study, the highest PC was recorded at pH 6.75. In contrast, Díaz (2011) reported lower optimal pH values for cactus fermented after 48 h with *K. lactis* (5.05), while Mcitekah (2008) recorded an optimal pH of 5.67 for fermented cactus under silage conditions.

The fermented *O. ficus-indica* obtained in this study represents a promising non-conventional alternative feed source for cattle. However, further studies are required to examine certain factors, such as the use of certain nutrient conditions, which could improve *K. marxianus* performance during SSF. Moreover, scale-up studies are required to develop a viable commercial process that could generate a low-cost technology for using fermented *O. ficus-indica* as high-quality forage. This study confirmed that solid-state fermentation with *K. marxianus* successfully enriches the PC of *O. ficus-indica* and removes NDF, which significantly increases the nutritional quality of this forage and may even promote animal performance.

Resumen

E. Herrera, M. Murillo, L. Berumen, N.O. Soto-Cruz, y J.B. Páez-Lerma. 2017. Protein enrichment of *Opuntia ficus-indica* using *Kluyveromyces marxianus* in solid-state fermentation Cien. Inv. Agr. 44(2): 113-120. *Opuntia ficus-indica* es utilizado en la alimentación animal, a pesar de su bajo contenido de proteínas (4%) comparado con forrajes de alta calidad (16%). Es necesario desarrollar tecnologías que permitan incrementar el valor nutrimental de *O. ficus-indica*, mediante procesos como la fermentación en estado sólido (FES). Por lo que el objetivo de este trabajo fue evaluar los cambios en la composición química de *O. ficus-indica* durante la FES al ser inoculado con *K. marxianus* ITD00262, para esto la cepa se activó en caldo de Glucosa peptona, extracto de levadura (GPY), a 28°C y pH de 4.8, el proceso de fermentación consistió de dos tratamientos, el primero sin inoculación de *K. marxianus* (T1), el segundo inoculando *K marxianus* (T2). Las muestras de *O. ficus-indica* fermentadas fueron secadas, y se les determinó los contenidos de materia seca (MS), proteína cruda (PC), fibra detergente neutra (FDN), fibra acido detergente (FDA), lignina (LIGN), digestibilidad in vitro de materia seca (IVDMD). Los datos fueron analizados con un arreglo factorial de 2×6. Se observó una disminución en la MS, el cual fue 11% menor para T1 comparado con T2 a las 48 h de fermentación. El contenido de PC, se incrementó 300% en T2 a las 120 h de fermentación. La DIVMS aumentó 3% mientras que los valores de FDN y FDA disminuyeron 18% en T2. De acuerdo con nuestros resultados, se confirma que el uso de *K. marxianus* en la FES de *O. ficus-indica*, tiene un efecto positivo porque incrementa la calidad nutritiva de este forraje.

Palabras clave: Calidad nutricional, fermentación, levadura, *Opuntia*, proteína.

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