JOURNAL OF INTERNATIONAL CRISIS AND RISK COMMUNICATION RESEARCH
ISSN: 2576-0017
2024, VOL 7, NO S6

USE OF TWO NATURAL ANTIMICROBIAL ADDITIVES IN THE SOLID FERMENTATION OF APPLE BAGASSE

José Luis Guevara Valdez[1], Daniel Díaz Plascencia*[2], Perla Lucía Ordoñez Baquera[3], Pablo Fidel Mancillas Flores[4], José Roberto Espinoza Prieto[5], Díana González López[6]

1,2,3,4,5,6. Universidad Autónoma de Chihuahua, Facultad de Zootecnia y Ecología. Periférico Francisco R. Almada Km 1. CP. 33820. Chihuahua, Chihuahua México.

∗Correspondence Author: dplascencia@uach.mx

SUMMARY

The antimicrobial effect of oregano essential oil and zeolite on solid-state fermentation (FES) of apple bagasse was evaluated. Zeolite 5% clinoptilolite (ZEO), 0.1% oregano essential oil (AEO) and both (ZXA) were used, against the traditional method of molasses broth added with urea, ammonium sulfate and minerals as control (CTL). Three repetitions per treatment were performed in 1 L tanks, with sampling at 0, 6, 12, 24, 48, 72 and 96 h. The variables evaluated were plate microbial count, pH and yeast count with a Neubauer chamber. The data were analyzed with a model of repeated means over time and comparison of multiple means. Maximum yeast growth was obtained at 48 h in ZXA (462 x106 cel/g), while in CTL at 96 h (470.5 x106 cel/g), reducing fermentation time and optimizing the process. The aerobic bacteria count was decreased (P <0.01) at hour 48, from 0.70 x106 CFU/g in CTL to 0.25 x106 CFU/g in ZXA, with AEO having the lowest bacterial count (0.11 x106 CFU/g). pH was higher (P <0.01) in ZEO (4.80) and ZXA (4.62), against CTL (4.06) and AEO (4.03). It is concluded that the use of both additives provides microbiological advantages over the traditional FES process, which reduces the fermentation time and avoids bacterial contamination, guaranteeing a process free of microbiological contaminants.
Keywords: Oregano essential oil, zeolite, yeast, apple bagasse.

INTRODUCTION

Humanity is currently facing the great challenge of producing enough food to sustain the enormous population growth that has occurred in recent years. However, this leads to more intensive harvesting, the search for more efficient breeds of animals and, above all, more waste to be produced when producing food for the population. Apple production in Mexico reached a volume of 714,203.20 tons in 2020, with the state of Chihuahua being the first national apple producer, with 594,711 tons just over 83% of total production (SIAP, 2020), so it can be estimated that a quarter of this is waste apple that does not meet quality standards. This waste, together with the by-products generated by the apple processing industry, generates waste, mainly apple bagasse, being 25 to 30% of the weight of fresh fruit, which represents a serious pollution problem, since being highly biodegradable significantly alters the environment in which it is discarded thanks to its high carbohydrate content. acids, fibers, vitamin C, and minerals (Joshi and Sandhu, 1996).

Thanks to the low nitrogen content of apple bagasse, it has been difficult to include it in animal diets, a situation that makes it imperative to develop techniques that allow consumption and reduce the environmental impact it generates. Therefore, one of the most successful alternatives is the solid-state fermentation (FES) of bagasse, added with urea nitrogen and mixtures of vitamins and minerals that allow the development of yeasts, which in addition to degrading fiber, can provide protein of microbial origin, that is, supplementing high-quality protein at low cost (Dhillon *et al.,* 2013; Mora de Alba *et al.,* 2018).

In solid-state fermentation, physicochemical parameters must be controlled to avoid contamination, since apple bagasse provides a medium with high humidity and a large amount of available carbohydrates (Castillo *et al.,* 2011). This raises the option of using by-products through the FES to maintain an adequate microbiological environment, reduce microbiological contamination, but without altering the development of fermentative yeasts. Oregano essential oil (OEA) has been used as a natural alternative to artificial preservatives, given its antioxidant activity, safety, antioxidant capacity and, above all, its antimicrobial capacity (Teixeira *et al.,* 2013; Ibarra-Cantún *et al.,* 2022). Thanks to these properties, it is considered that the addition of AEO to the FES of apple bagasse will provide a defense against contamination by bacteria that would consume the substrates and decrease the fermentative efficiency of the yeasts.

Zeolites are clayey materials obtained from sedimentary deposits. Of the more than 40 types of zeolites that occur naturally, clinoptilolite is especially functional when adsorbing nitrogenous compounds, especially NH4+. Clinoptilolite is a type of zeolite that has an open molecular structure, with a porous volume close to 35% and is readily found in mineral deposits worldwide. This characteristic is obtained thanks to the three-dimensional structure of aluminosilicates with rings of four and five members, which forms micropores capable of hosting exchangeable cations such as NH4+, Na+, K+ and Ca2+ (Leung *et al.,* 2007).

Therefore, the objective of this work was to evaluate the microbiological effect of the addition of micronized zeolite and oregano essential oil to the solid-state fermentation of apple bagasse, in addition to determining the adsorptive effect of zeolite on the nitrogenous compounds of the apple.

MATERIALS AND METHODS

Localization

The experiment was carried out in a greenhouse of the Faculty of Zootechnics and Ecology (FZyE) of the Autonomous University of Chihuahua (UACH), located near the city of Chihuahua, Mexico. The unit is located between parallels 28° 38' LN and 106° 04' LO, at an altitude of 1,440 meters above sea level. The average annual temperature is 18.6 °C with an average annual rainfall between 200 and 600 mm (INEGI 2017).

Raw material: The apple bagasse was kept in plastic containers of 1 $^{L-1}$ capacity, to which urea and ammonium sulfate were added as a source of nitrogen and a mineral supplement to provide the necessary nutrients for the development of yeasts (Control Treatment). The raw material used was apple bagasse from CONFRUTTA, a company dedicated to the production of apple juice, from Ciudad Cuauhtémoc, Chihuahua, being apples of the *Golden Delicious variety.*

Treatments: The control treatment consisted of apple bagasse without additives, while the AEO treatment was added with oregano essential oil (AEO) at 0.1%, the ZEO treatment was added with 5% of micronized zeolite and, finally, the ZXA treatment included both oregano essential oil and zeolite, with three replications per treatment. as it appears in (Table 1).

The zeolite used was obtained commercially from a mine in San Luis Potosí, Mexico, with a porosity of 45 to 52%, density of 700 to 850 kg/m3, hardness of 2 to 3 Mohs, water absorption capacity of 42 to 50%, pores of 4 to 7 Å and a melting point of 1300 °C. The composition guaranteed by the zeolite supplier is found in the (Table 2).

Once the mixture was made, it was homogenized and four manual agitations were made per day, completely stirring the product to generate adequate oxygenation for four days. Samples were taken at h 0, 6, 12, 24, 48, 72 and 96 for yeast counting, and at h 0, 48 and 96 for bacterial count.

Variables Evaluated

Bacteria count. The microbiological count was carried out in accordance with the Official Mexican Standard NOM-092-SSA1-1994. Method for counting aerobic bacteria in plaque. 5 samples were taken from different places in the FES, and homogenized, of this one gram is taken, diluted 6 times in the diluent medium, and inoculated in each Petri dish, running all the samples in triplicate. After inoculating the sample dilutions into the petri dishes, add 12 to 15 mL of the prepared standard bead medium, mix it using 6 right-to-left, 6 clockwise, 6 counterclockwise and 6 back-to-front, on a smooth, horizontal surface until the inoculum is completely incorporated into the medium and allowed to solidify. A non-inoculum box is included for each medium and diluent prepared as a sterility control. The boxes are incubated in an inverted position at 35 ± 2 ºC for 48 ± 2 h.

In the reading, those plates where between 25 and 250 CFU appear are selected and all the colonies developed in the selected plates are counted. After incubation, plates in the range of 25 to 250 colonies are counted, using the colony counter and recorder. The average count per gram of this dilution is calculated.

Yeasts. The yeast count was carried out by a simple count in an improved Neubauer chamber, by the method described by Díaz-Plascencia (Díaz-Plascencia, 2011), where a sample of 1 g of the product was taken, which was taken to serial dilutions in phosphate regulating solution, and then each of them was placed in a Neubauer chamber. for the yeast count (individual or budding) in the four quadrants of the striping. From these data, the amount of yeast per gram of apple bagasse ferment is calculated.

pH. The pH was measured throughout the experiment with a Hanna digital potentiometer, which was sterilized by immersion in alcohol, dried and immersed in the broth to take the measurement according to the manufacturer's instructions.

Antioxidant activity. To determine the antioxidant activity of fermented apple bagasse, the DPPH method (1,1-diphenyl-2-picrylhydrazalin) was used according to Ajila, (Ajila *et al.,* 2011), for which the extract of fermented apple bagasse (200 ∟l) was mixed with 1 mL of the DPPH solution. After being homogenized in vortex, it was left to react for 20 minutes in total darkness. The absorbance was read at 517 nm, with which the free radical scavenging activity was calculated as a percentage with the equation (% of capture activity) = 1 – (*As/A0*) x 100, where A0 is the absorbance of the control while *As* is the absorbance of the sample.

Protein. The protein variable was determined by the methodology of Fagbenro, who used the method for precipitation with trichloroacetic acid to determine true protein and discern it from non-protein nitrogen (Fagbenro and Jauncey, 1998).

Statistical analysis

The data obtained were analyzed by means of a model of means repeated over time, with the help of the Mixed procedure of SAS 9.1.6 (SAS, 2006) and the comparison of means was carried out by means of Least Square Means, from the same computer package.

RESULTS AND DISCUSSION

A significant effect ($P < 0.01$) of the inclusion of AEO and Zeolite clinoptilolite for the variable of mesophilic aerobic bacteria in the FES of apple bagasse was found, decreasing the bacterial count compared to the control at hour 48 and creating a significant difference between treatments that contained AEO and those that did not ($P < 0.01$) at hour 96. These results can be explained by the antimicrobial effect of AEO, observing the decrease in the total number of bacteria thanks to the antibacterial activity of Thymol and Carvacrol contained in AEO, this being consistent with the work of Lambert *et al.,* (2001), who inhibited bacterial growth with thymol concentrations in AEO of 5%. There was also an interaction between treatments and time ($P < 0.01$), so it can be said that as the bacteria develop in the FES of apple bagasse, they are affected by the presence of AEO or ZEO, so that their number is reduced compared to the control treatment.

At 48 h after fermentation, the difference between the Control and ZEO treatments is clearly observed, with respect to the other two containing AEO (Graph 1), which indicates that, at the moment of maximum growth of bacteria, they are significantly inhibited by AEO ($P < 0.01$). This effect of AEO is observed throughout fermentation, making clear the inhibitory effect of AEO on mesophilic aerobic bacteria.

The quantified yeasts showed the characteristic multiplication of these microorganisms, in accordance with the growths presented by other authors (Díaz-Plascencia, *et al.,* 2012) and it is observed at time 0 of the FES that there is a significant effect ($P < 0.01$) due to the addition of the treatments, observing a significant decrease in the amount of yeasts (Table 3). so it is clear that both Zeolite and AEO have an effect on yeast counting.

In the first 24 h, poor growth can be observed in the yeasts of the ZXA treatment ($P < 0.01$) compared to the other treatments, recovering in h 48, where this treatment presents the highest yeast count, comparable only to the final count of the control treatment. This may indicate an adequate use of the fast substrates present in apple bagasse and a subsequent depletion of them, since from this inflection point onwards, the yeast count of the ZXA treatment is in sharp decline. The three treatments have their maximum peak of yeast growth at 48 h, and from then on there is a decrease in the count, while the control presents the highest count at 96 h.

At the end of the experiment, a significant difference can be found between the control treatment and the others, with a higher count in the control, followed by the ZEO treatment, which from h 48 stabilized its growth and kept its count relatively stable. However, the two treatments containing AEO had a significant decrease in their yeast population, evidencing the antimicrobial power of AEO (Lambert *et al.,* 2001; Torres & Zeledón, 2018).

The measurement of pH during the experiment shows that there is an effect of zeolite to maintain a higher pH, since the two treatments that include zeolite have a significantly higher pH than the other treatments ($P < 0.01$).

The effect generated by zeolite can be clearly appreciated, as it maintains the stable pH from the moment it is added to the FES, since from the h 0 of fermentation its pH regulatory activity is present, statistically separating the ZEO and ZXA treatments from those that do not contain zeolite in their formulation ($P < 0.01$). Graph 2 shows the pH fluctuation that occurred in the AEO and Control treatments during the first 24 h, so that the effect of ammonium adsorption on the pH of the zeolite is evident. These results are similar to those presented by other authors, as they explain the ability of zeolite to adsorb ammonium molecules and other ions, thus regulating pH for the benefit of the microbiological system (Forte & Maugeri, 2007; Kardaya *et al.,* 2012; Espinoza & Montiel, 2016; Mejía-Tinoco, 2017).

The antioxidant activity of fermented apple bagasse increased as the fermentation time elapsed (Graph 3), doubling its activity from 6.44 to 12.43%, which is consistent with what was presented by Ajila *et al.* (2011); (Ibarra-Cantún *et al.,* 2022), as the increase in antioxidant capacity can be demonstrated by two main effects: as fermentation is carried out in the solid state, the substrate is stirred to aerate, which causes the stems, seeds and cells of the apple to break, releasing antioxidant compounds; On the other hand, the growth of yeasts itself causes their enzymes to digest cell walls, membranes and release compounds with antioxidant activity from apple tissues.

The results of true protein show that there was an increase in it during the course of fermentation, almost doubling its content in the final product. Despite having a similar behavior, in the last stage of fermentation it was possible to differentiate perfectly between treatments ($P < 0.05$) at hours 72 and 96 (Table 4), perfectly differentiating between the control and the other treatments. The maximum concentration of protein in fermentation occurred at hour 48 for all treatments, except for the control, where it was present until hour 96. This work is consistent with the work of Bhalla and Joshi (1994); (Rodríguez-Muela *et al.,* 2010), who using controlled strains of *Saccharomyces* achieved proteins of up to 30%, but presented results lower than those of Pirmohammadi *et al.,* (2006); Aguirre *et al.,* (2018), who managed to obtain protein concentrations of up to 40.1%, although no additives from other carbon sources were used in this work, as in the aforementioned work.

From the growth curves that were generated from the count of bacteria and yeasts, it can be concluded that after 48 h the substrates of rapid metabolization such as simple carbohydrates are exhausted, since a marked decrease in the growth speed is observed, as well as in the number of yeasts and bacteria. so it is understood that microorganisms are forced to use other substrates as an energy source for their development.

In the control treatment, this turning point was not observed, so when observing the slope of the control treatment, it is seen that growth was slower, so the turning point, which is when they exhausted the rapidly metabolized substrates, occurs until 72 h, so it is established that it is less efficient. because at 96 h it has a yeast count of 470.5 x106/g, equivalent to that presented by the ZXA treatment at 48 h (462 x106/g), so with the addition of Zeolite and Oregano Essential Oil the result is obtained in less time, improving the process.

It was also observed that the appearance of the treatments that included zeolite was much drier and earthier than the others, thanks to the chemical characteristics of the zeolite, since it had a drying effect, adsorbing water and limiting the growth of microorganisms by decreasing the activity of water in the FES, a situation that easily affects bacteria. but yeasts are better able to resist. This effect can be observed by having a lower development in the first h, since zeolite treatments had a slower microbiological development compared to the other treatments.

CONCLUSIONS AND RECOMMENDATIONS

It is concluded that adding the solid state fermentation of apple bagasse with zeolite and oregano essential oil provides productive advantages over the control treatment, since a maximum development of yeasts is achieved in a shorter time by adding both AEO and zeolite clinoptilolite, compared to the control treatment, which achieves a faster process, safe and stable.

It is concluded that the addition of oregano essential oil causes a decrease in the count of aerobic mesophilic bacteria in solid state fermentation, avoiding bacterial contamination.

The addition of oregano essential oil and zeolite does not affect the production of true protein, so the efficiency of fermentation in the solid state is not diminished. It is also concluded that the pH values remained constant in the treatments that contained zeolite, so this has a pH regulating effect that served as a buffer.

Table 1. Formulations of the FES treatments of apple bagasse

Treatment1	Urea %	Ammonium Sulfate %	Mineral supplement %	Zeolite %	AEO %
1.- Control	2.0	0.4	1.0	-	-
2.- ZEO	2.0	0.4	1.0	5.0	-
3.- AEO	2.0	0.4	1.0	-	0.1
4.- ZXA	2.0	0.4	1.0	5.0	0.1

[1] ZEO indicates treatment with 5% Zeolite, AEO treatment with 0.1% Oregano Essential Oil and ZXA treatment with both (5 and 0.1% respectively).

Table 2.- Chemical composition of the zeolite clinoptilolite1

Compound	Concentration %
Silicon oxide (SiO2)	67.9
Aluminum oxide (Al2O3)	13.7
Potassium oxide (K2O)	4.7
Sodium oxide (Na2O)	2.2
Calcium oxide (CaO)	1.7
Ferric oxide (Fe2O3)	1.8
Magnesium oxide (MgO)	1.8

[1] Analysis provided by the chemical analysis laboratory of the Metallurgy Institute of the U.A.S.L.P.

Table 3.- Least squares means (±EE) of the total yeast count (Yeasts x106/g) in the FES of apple bagasse added with AEO and ZEO [1,2]

Fermentation time, h	Treatment3				±EE
	Control	Zeo	AEO	ZXA	
0	40.00th	18.50b	25.50b	27.00b	3.04
6	94.00th	37.50b	37.00b	34.00b	4.26
12	111.50th	96.00b	106.50ab	45.00c	2.76
24	152.00th	143.50th	159.50th	74.50b	7.53
48	362.00b	339.5.00b	384.50b	462.00th	14.83
72	447.00th	337.00b	247.50c	273.00c	14.22
96	470.50th	326.50b	281.00bc	247.50c	17.25

[1] Different literals between columns indicate significant difference ($P < 0.01$).
[2] Literals differ between rows.
[3] ZEO indicates treatment with 5% Zeolite, AEO treatment with 0.1% Oregano Essential Oil and ZXA treatment with both (5 and 0.1% respectively).

Table 4.- True protein over time of the FES of apple bagasse added with AEO and ZEO [1,2]

Fermentation Time (h)	Treatment3				±EE
	AEO	AXZ	Control	Zeo	
0	18.51ab	18.74a	18.10b	18.60ab	0.1333
6	18.73a	19.10a	18.72a	19.09a	0.1219
12	20.15a	19.32b	19.20b	20.15a	0.1778
24	22.65a	19.94b	23.13a	22.57a	0.1851
48	30.49a	30.81a	30.47a	30.25a	0.2822
72	29.21c	28.66c	33.83a	30.26b	0.1466
96	29.23c	28.78c	34.42a	30.74b	0.2445

[1] Different literals between columns indicate significant difference ($P < 0.01$).
[2] Literals differ between rows.
[3] ZEO indicates treatment with 5% Zeolite, AEO treatment with 0.1% Oregano Essential Oil and ZXA treatment with both (5 and 0.1% respectively).

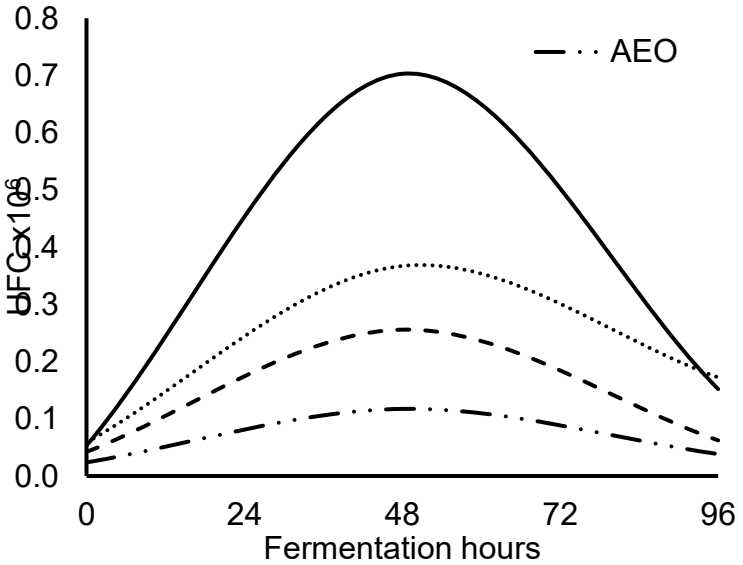
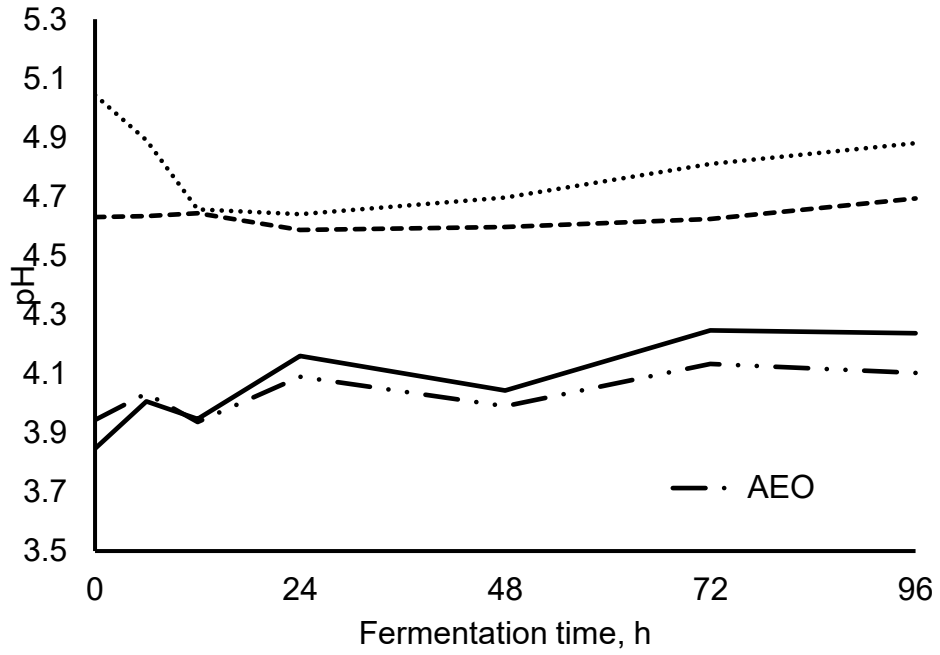
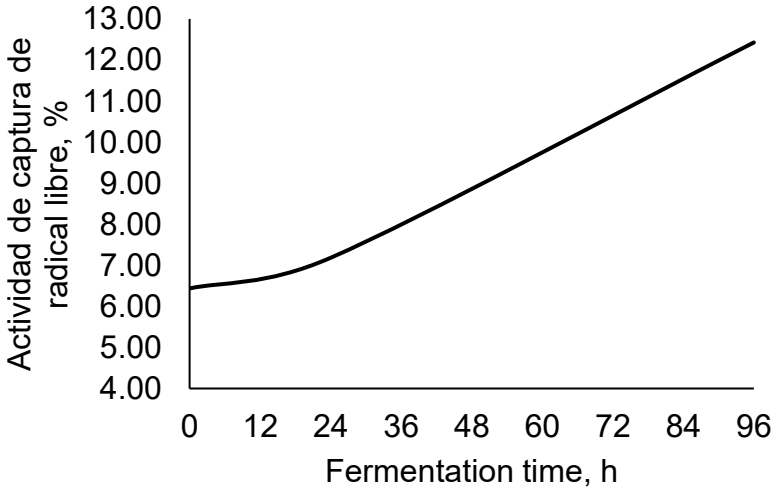


Figure 1. Least squares means of the total mesophilic aerobic bacteria count (CFU x106) in the FES of apple bagasse added with AEO and ZEO.



Graph 2.- Means of least squares of pH of the FES of apple bagasse added with AEO and ZEO.



Graph 3.- Free radical scavenging activity in percentage of fermented apple bagasse.

REFERENCES

Aguirre, L. A., Rodríguez, Z., Boucourt, R., Saca, V., Salazar, R., & Jiménez, M. (2018). Effect of whey on solid-state fermentation of coffee pulp (Coffea arabica L.) for use in ruminant feed. Cuban Journal of Agricultural Science, 52(3), 303-312.

Ajila, C. M., F. Gassara, S. K. Brar, M. Verma, R. D. Tyagi and J. R. Valéro. 2011. Polyphenolic antioxidant mobilization in apple pomace by different methods of solid-State fermentation and evaluation of Its antioxidant activity. Food Bioprocess Technol. 5:2697–2707.

Bhalla, T. C. and M. Joshi. 1994. Protein enrichment of apple pomace by co-culture of cellulolytic moulds and yeasts. World J. Microbiol. Biotechnol. 10:116–7.
Castillo, Y., O. Ruiz, C. Angulo, C. Rodríguez, A. Elías and O. La O. 2011. Inclusion of bakery residues in some metabolites and bromatological indicators of solid-state fermentation of apple bagasse. Cuban Journal of Agricultural Science. 45 141–144.
Dhillon, G. S., S. Kaur and S. K. Brar. 2013. Perspective of apple processing wastes as low-cost substrates for bioproduction of high value products: A review. Renew. Sustain. Energy Rev. 27:789–805.
Díaz-Plascencia, C. Rodríguez-Muela and P. Mancillas-Flores. 2012. In vitro fermentation of forage nopal with *Kluyveromyces lactis* yeast inoculum obtained from waste apple. Rev. Electron. Vet. 13:1–11.
Díaz-Plascencia, D. 2011. Development of a yeast-based inoculum and its effect on in vitro fermentation kinetics in rations for high-yielding Holstein cows. Doctoral Thesis. Faculty of Zootechnics and Ecology. Autonomous University of Chihuahua. Chihuahua, Chih. Mexico.
Espinoza Gonzales, J. J., & Montiel Fernández, J. A. (2016). Solid-state fermentation of Saccharum officinarum with different levels of inclusion of fresh foliage of Moringa oleifera Managua, 2016 (Doctoral dissertation, National Agrarian University, UNA).
Fagbenro, O. and K. Jauncey. 1998. Physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia (*Oreochromis niloticus*). Anim. Feed Sci. Technol. 71:11–18.
Forte, M. and F. Maugeri. 2007. Purification of clavulanic acid from fermentation broth using zeolites. J. Biotechnol. 131: S191.
Ibarra-Cantún, D., Ramos-Cassellis, M. E., Sánchez-Arzubide, M. G., Castelán-Vega, R. D. C., & Marín-Castro, M. A. (2022). Total phenolic compounds and antioxidant activity of solid-state fermentation of apple bagasse (Malus domestica Borkh., var. panochera). Agricultural Science and Technology, 23(2).
INEGI (2017) Mexico in figures: National Information by Federal Entity and Municipalities. Retrieved from: Accessed on: October 20, 2019.
Joshi, V. K. and D. K. Sandhu. 1996. Preparation and evaluation of an animal feed byproduct produced by solid-state fermentation of apple pomace. Bioresour. Technol. 56:251–255.
Kardaya, D., D. Sudrajat and E. Dihansih. 2012. Efficacy of dietary urea-impregnated zeolite in Improving rumen fermentation characteristics of local lamb. Indonesians. J. Anim. Sci. Technol. 35:207–213.
Lambert, R. J. W., P. N. Skandamis, P. J. Coote and G. J. E. Nychas. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91:453–462.
Leung, S., S. Barrington, Y. Wan, X. Zhao and B. El-Husseini. 2007. Zeolite (clinoptilolite) as feed additive to reduce manure mineral content. Bioresour. Technol. 98:3309–3316.
Mejía Tinoco, W. A. (2017). Solid-state fermentation of Saccharum officinarum with foliage of Moringa oleifera for pig feed. (Master's Thesis). National Agrarian University. Managua-Nicaragua.
Mora de Alba, M. E., Tirado-González, D. N., Quezada-Tristán, T., Guevara-Lara, F., Jáuregui-Rincón, J., Larios-González, R., & Tirado-Estrada, G. (2018). Nutritional quality of apple bagasse ensiled with organic and inorganic nitrogenous sources. Mexican Journal of Agricultural Sciences, 9(1), 229-235.
Pirmohammadi, R., Y. Rouzbehan, K. Rezayazdi and M. Zahedifar. 2006. Chemical composition, digestibility and in situ degradability of dried and ensiled apple pomace and maize silage. Small Rumin. Res. 66:150–155.
Rodríguez-Muela, C., Díaz, D., Salvador, F., Ruiz, O., Arzola, C., Flores, A., ... & Elías, A. (2010). Effect of urea and soybean paste level on protein concentration during solid-state fermentation of waste apple (Malus domestica). Cuban Journal of Agricultural Science, 44(1), 23-26.
SAS Institute, Inc (2006) SAS/STAT users guide: Statics Version 9 Cary, North Carolina. U.S.A.
Ministry of Health. 1995. Official Mexican Standard NOM-092-SSA1-1994 Goods and Services. Method for counting aerobic bacteria in plaque.
SIAP-Ministry of Agriculture and Rural Development. 2020. https://www.gob.mx/agricultura/prensa/estima-agricultura-crecimiento-de-dos-digitos-en-produccion-nacional-de-manzanas-al-cierre-del-ciclo-agricola-2021. Accessed November 13, 2023.
Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N. R., ... & Nunes, M. L. (2013). Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oil. Journal of the Science of Food and Agriculture, 93(11), 2707-2714.
Torrez, A., Martin, J., & Zeledón Ballesteros, A. A. (2018). Effect of inclusion of different levels of zeolite on the solid-state fermentation of sugarcane (Saccharum officinarum) and Maringa (Moringa oleifera) (Doctoral dissertation, National Agrarian University).