

Nutritional properties of defatted and hydrothermally pretreated meal of *Jatropha curcas* fermented in solid-state with *Saccharomyces cerevisiae*

Propiedades nutrimentales de harina desgrasada y pretratada hidrotérmicamente de *Jatropha curcas* fermentada en estado sólido con *Saccharomyces cerevisiae*

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ABSTRACT

In this research, the effect of solid-state fermentation on the nutritional properties of defatted *Jatropha curcas* meal was evaluated, with and without hydrothermal treatment application, using *Saccharomyces cerevisiae* (6×10^9 CFU/g), and incubating for 24, 72, and 144 h at 36 °C. The antinutrient content was determined by spectrophotometric methods; the phorbol esters content was evaluated by high-resolution thin layer chromatography; and the nutritional composition was evaluated by proximal analysis. A two-factor (type of treatment and fermentation time) design was used. The response variables were the content of phytates, saponins, phorbol esters, and nutritional content. The meal that had the characteristics of our interest was the one fermented for 144 h without hydrothermal treatment because it presented a significant reduction of 74 % of phytates, and 51 % of saponins, in addition to the fact that no phorbol esters were detected. The protein content (44.50 %) did not have significant changes. Additionally, decreases of 8-15 %, 28-44 %, and 1.6-7 % in fat, fiber, and carbohydrate content, respectively, were observed. Therefore, this fermented meal is recommended to be implemented in balanced diets for *Oreochromis niloticus*.

KEY WORDS: *Jatropha*, Fermentation, Hydrothermal, Yeast, Fish food.

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RESUMEN

En este estudio se evaluó el efecto de la fermentación en estado sólido sobre las propiedades nutrimentales en harina desgrasada de *Jatropha curcas*, con y sin aplicación de tratamiento hidrotérmico, utilizando *Saccharomyces cerevisiae* (6×10^9 CFU/g) e incubación durante 24, 72 y 144 h a 36 °C. El contenido de antinutrientes se determinó por métodos espectrofotométricos, por cromatografía de capa fina de alta resolución se evaluó el contenido de ésteres de forbol y la composición nutrimental por análisis proximal. Se empleó un diseño bifactorial (tipo de tratamiento y tiempo de fermentación) con variables de respuesta contenido de fitatos, saponinas, ésteres de forbol y contenido nutrimental. La harina que tuvo las características de nuestro interés fue la que solamente se fermentó por 144 h sin tratamiento hidrotérmico debido a que presentó una reducción significativa del 74 % de fitatos, 51 % de saponinas, además de que no se detectaron ésteres de forbol. El contenido de proteínas (44.50 %) no tuvo cambios significativos. Además, se observaron disminuciones entre el 8 – 15 %, 28 – 44 % y 1.6 – 7 % en el contenido de grasa, fibra y carbohidratos, respectivamente. Por lo tanto, se recomienda esta harina fermentada para implementarse en dietas balanceadas para *Oreochromis niloticus*.

PALABRAS CLAVE: *Jatropha*, Fermentación, Hidrotérmico, Levadura, Alimento para peces

Introduction

Plant-based ingredients are suitable for fish feed formulation, with oilseed meals standing out. The use of plant proteins has been investigated for various commercial fish species, as these food sources offer a higher content of proteins, amino acids, and fatty acids than those of animal origin (Mondal & Payra, 2015). However, the use of oilseed meal in aquaculture feed is limited by the low content of essential amino acids and the presence of antinutritional factors (Ghosh & Mandal, 2015). Due to the expansion of aquaculture activity, there is a need to seek alternative plant-based protein sources to replace fishmeal in aquaculture diets (Moss *et al.*, 2019). Fishmeal in diets enhances quality and accelerates growth due to its palatability, better absorption, digestion, and nutrient uptake (Hodar *et al.*, 2020). Fish consumption has increased from 5.2 kg per capita in 1961 to 19.4 kg in 2017, with an average annual growth rate of 2.4 % (FAO, 2020). Hence, it is important to have an alternative to partially replace fishmeal with plant-based meals, such as *Jatropha curcas*.

Jatropha curcas is a plant native to Mexico and continental America (Achten *et al.*, 2010), found in tropical and subtropical climates, and is resistant to high temperatures (da Schio, 2010; Makkar & Becker, 2009). This plant is considered an oilseed due to its high oil content, with at least 50 % oil, making it highly promising for biodiesel production (Gomes *et al.*, 2018). The meal obtained after oil extraction is considered nutrient-rich due to its high protein content. However, toxic and antinutritional compounds in raw *Jatropha* limit its employment in diets for feeding several animal species (Abasubong *et al.*, 2023). Therefore, it is necessary to apply a detoxification process, such as solid-state fermentation (SSF). Due to its low production cost and high nutritional value, agro-industrial waste from oilseed crops can be considered suitable substrates for fermentation (Dharmakar *et al.*, 2022).

SSF is a microbial process that involves the breakdown and transformation of toxic compounds into products that promote the growth of microorganisms (Krishna 2005; Lizardi-Jiménez & Hernández-Martínez 2017). Additionally, it has been reported that the use of plant protein fermented with *Saccharomyces cerevisiae* does not have negative effects on growth, immunity, and stress resistance in various fish species (Hassaan *et al.*, 2015; Plaipetch & Yakupitiyage, 2014; Soltan *et al.*, 2015). Moreover, *S. cerevisiae* is a yeast with a mechanism similar to probiotics as it increases the secretion of extracellular enzymes, proteases, and amylases, as well as improving nutrient digestibility, which leads to increased growth and feed efficiency in Nile tilapia (Ahmadifar *et al.*, 2020; Van-Doan *et al.*, 2020).

This study aims to characterize defatted *J. curcas* meal fermented in solid-state with *S. cerevisiae*, with and without the application of a hydrothermal pretreatment. The results obtained from this work can be used for the implementation of fermented defatted *J. curcas* meal in the production of balanced aquaculture feed, specifically for Nile tilapia.

Materials and Methods

Sample preparation

J. curcas fruits were collected in Ejido de la Campana (24° 53'52.3" N; 107° 27' 18.3" W and 94 masl) in Culiacán, Sinaloa, Mexico, during September 2021 to January 2022. Fruits were transported to the Bioresources Laboratory at the Research Center for Food and Development (CIAD) in Culiacán subsede, Sinaloa, Mexico. The fruits were then dried at ambient temperature (25 – 33 °C) to facilitate the removal of the shell and testa. A sheller (REINMAC) and a testa remover (REINMAC) were used for this process. The remaining testa was manually separated to obtain the kernel, which was then subjected to a cold press (KOMET DD 85G) for partial oil extraction, resulting in a partially defatted meal with a fat content of 58.96 %. Finally, the meal underwent a maceration process for 13 days with hexane at a 1:3 ratio to remove the remaining oil. The resulting defatted *J. curcas* meal (MJc), with a fat content of 15.84 %, was placed in an oven (TERLAB) for 5 hours at 70 °C to evaporate the remaining solvent and stored at 4 °C until its respective use.

Solid-state fermentation (SSF)

SSF was carried out following the methodology of Medina-Rodelo *et al.* (2023). Before fermentation, one MJc batch was hydrothermally treated and compared to a non-treated batch. A total of 120 g of MJc was placed in 500 mL Erlenmeyer flasks, inoculated with 19.5 mL of *S. cerevisiae* (6×10^9 CFU/g), and 72 mL of distilled water (60 % humidity) was added. The mixture was manually homogenized, and the flask openings were sealed with an airlock. The samples were incubated (Incubator IC603CW, Yamato) at 36 °C for 24, 72, and 144 hours. After the incubation period, the samples were placed in an oven (TERLAB) at 65 °C for 24 hours to stop microbial growth. The experiment was performed in duplicate (**Figure 1**).

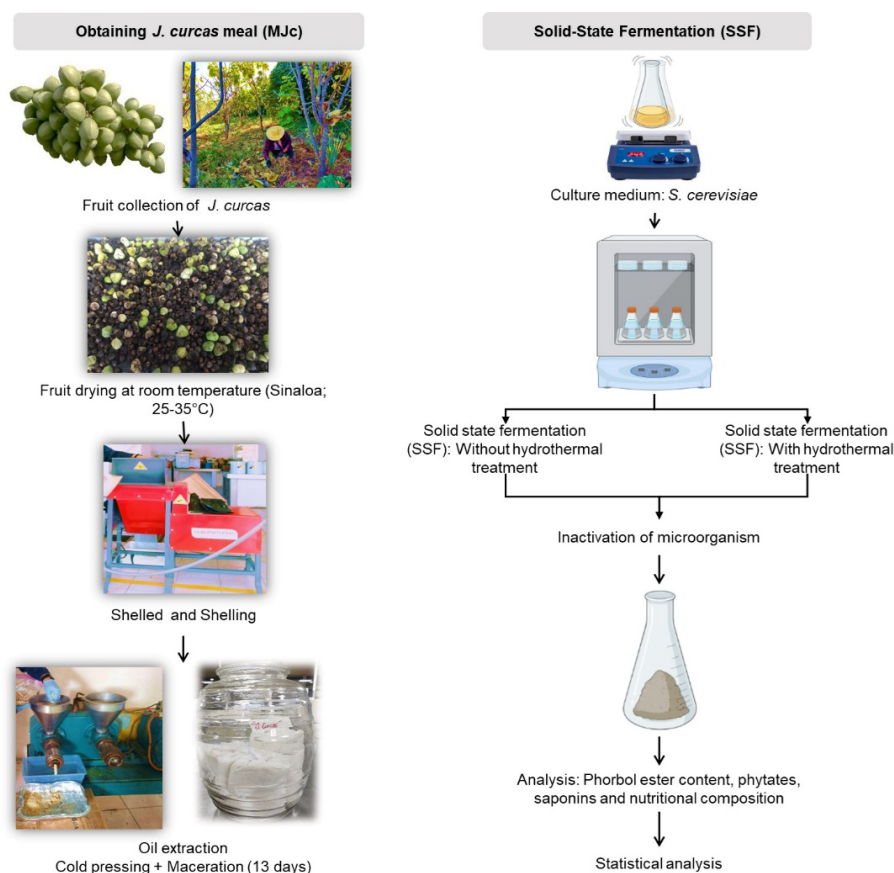


Figure 1. General outline of the research work: obtention of defatted *J. curcas* meal (MJc) and solid-state fermentation process and corresponding analyses.

Quantification of phorbol esters

Phorbol esters quantification was carried out according to the methodology described by Makkar *et al.* (2007) and Devappa *et al.* (2013). One gram of each sample was weighed, and 10 mL of HPLC-grade methanol (SIGMA Aldrich) was added. Then, the mixture was placed in an ultrasonic bath (Model 2800 Branson) at 240 W for 25 minutes at 15 °C. The supernatant was then collected and filtered using filter paper (Whatman No. 40) into a 250 mL round-bottom flask and evaporated to dryness (Mod R-215 Buchi). Afterward, 1 mL of methanol was added, and the sample was sonicated for 2 minutes. Finally, the sample was filtered with a 0.45 µm acrodisc (Millex) and collected in an amber vial. To carry out the High-Performance Thin-Layer Chromatography (HPTLC) analysis, a semi-automatic applicator [Limonat 5, CAMAG (chromatographic chamber)] was used, and 6 µL of the sample was injected into 20 x 10 cm TLC Silica Gel 60 F254 aluminum plates in 85 mm long bands. The plates were placed in a chromatographic chamber (CAMAG), previously saturated for 20 minutes, with a mobile phase of acetone-petroleum ether 4:6 (v/v). The plates were visualized in a UV light chamber (Cabinet 4, CAMAG) at 254 and 366 nm. A densitometric scan was then performed with a TLC scanner (TLC Scanner 4, CAMAG) with an absorbance wavelength of 280 nm using deuterium and tungsten lamps. The concentration of phorbol esters (PE) was calculated using a calibration curve of phorbol-12-myristate-13-acetate (PMA), which is considered a standard (Medina-Rodelo *et al.*, 2023). Finally, derivatization was performed using Liebermann's reagent (vanillin-sulfuric acid solution).

Determination of phytates

Following the spectrophotometric method described by Vaintraub & Lapteva (1988), 0.5 g of each sample was weighed, 10 mL of 3.5 % HCl was added, and the mixture was stirred for 1 hour on a stirring plate (DLAB, Model SK-D1807-S). The mixture was centrifuged (Hermle Labortechnik, Thermo Scientific, Germany) at 3400 rpm for 10 minutes and the pellet was discarded. A 200 µL aliquot of the supernatant was mixed with 2.8 mL of distilled water and 1 mL of Wade reagent. The phytate concentration was determined by spectrophotometry at 500 nm, using a calibration curve from a sodium phytate standard solution (SIGMA Aldrich) (160 µg/mL). Results were reported as equivalents of µg of phytic acid per gram of dry sample.

Saponins determination

Saponins were determined following the methodology described by Hiai *et al.* (1976). A 0.5 g sample was mixed with 10 mL of 80 % aqueous methanol (99.8 % purity, SIGMA Aldrich). The mixture was stirred for 12 hours on a stirring plate (DLAB, Model SK-D1807-S), and centrifuged (Hermle Labortechnik, Thermo Scientific, Germany) for 10 minutes at 10,000 rpm. The supernatant was collected, and the precipitate was resuspended in 80 % aqueous methanol. This process was repeated three times, resulting in a total volume of 25 mL. A 200 µL sample reacted with 50 µL of 80 % methanol and 125 µL of 8 % vanillin reagent (SIGMA Aldrich). While on an ice bath, 2.5 mL of 72 % H₂SO₄ (SIGMA Aldrich) was slowly added, and the samples were vortexed (VORTEX-GENIE 2, Scientific Industries) for 3 minutes. Finally, the samples were heated in a water bath at 60 °C for 10 minutes. The saponin concentration was determined by

spectrophotometry at 544 nm using a diosgenin calibration curve. Results were reported as µg of diosgenin equivalents per gram of dry sample.

Nutritional composition

Following the AOAC (2001) methodology, the sample nutritional content (ash, crude fiber, fat, and crude protein) was determined. The carbohydrate content was estimated by difference ($100 - \sum$ data obtained from proximate analysis).

Statistical analysis

A two-factor design (fermentation time and type of treatment) was used with three levels of fermentation (24, 72, and 144 h) and two levels of treatment type (no treatment and hydrothermal treatment). This resulted in six treatments derived from the combination of the factor levels. The response variables were the content of phytates, saponins, phorbol esters, and nutritional composition. A two-factor ANOVA was performed, followed by a means comparison using Tukey's test with $\alpha = 0.05$ and Dunnett's test with $p \leq 0.05$ to compare with the control sample, which was raw *Jatropha curcas* meal (unprocessed). The experiment was conducted in duplicate, and the statistical analysis was performed using MINITAB 18.0 software.

Results and Discussion

Effect of fermentation on phorbol esters in MJc

Tables 1 and **2** show the effect of solid-state fermentation (SSF) on the phorbol ester (PE) content in treated (hydrothermal treatment) and non-treated fermented MJc. These tables show the sample retention factors (Rf), which ranged from 0.234 to 0.826 and 0.108 to 0.786. Makkar *et al.* (1997) reported that PEs are present in *J. curcas* within an Rf range of 0.42 to 0.55. Additionally, Medina-Rodelo *et al.* (2023) mention that phorbol-12-myristate-13-acetate (PMA) appears with an Rf of 0.54. The sample chromatograms are illustrated in **Figures 2** and **3**, showing the absence of PEs. Therefore, since the Rf values of our samples do not fall within the reported ranges for PEs, we can conclude that no detection of these PEs was found under the chromatographic conditions used.

Table 1. Chromatographic profile of defatted *J. curcas* meal (MJc) samples without hydrothermal treatment.

Figure	Peak	Rf	Area	Substance
Figure 2a)	1	0.549	0.00551	PMA
Figure 2b)	1	0.234	0.00082	ND
	2	0.826	0.00572	ND
Figure 2c)	1	0.210	0.00050	ND
Figure 2d)	1	0.204	0.00122	ND

Rf: Retention factor, PMA: Phorbol-12-Myristate-13-acetate, ND: Not detected

Table 2. Chromatographic profile of defatted *J. curcas* meal (MJc) samples with hydrothermal treatment.

Figure	Peak	Rf	Area	Substance
Figure 3a)	1	0.549	0.00551	PMA
Figure 3b)	1	0.108	0.00551	ND
	2	0.328	0.00232	ND
	3	0.786	0.00136	ND
Figure 3c)	1	0.116	0.00466	ND
	2	0.269	0.00243	ND
Figure 3d)	1	0.111	0.00388	ND
	2	0.261	0.00219	ND

Rf: Retention factor, PMA: Phorbol-12-Myristate-13-acetate, ND: Not detected

Effect of fermentation on phytates in MJc

The phytate content in raw MJc (unprocessed) was 374.96 µg phytic acid per gram of flour. As shown in **Figure 4**, the phytate content decreased between 16 % and 74 % after fermentation. In contrast, the MJc batch subjected to hydrothermal treatment and SSF showed a reduction in phytates between 55 % and 59 %. Therefore, SSF is considered an effective process for the degradation of this compound. Tanasković *et al.* (2021) used SSF on wheat bran meal and found that as fermentation time increased, there was a reduction in phytates, with a final degradation of 34 %. Similarly, Olukomaiya *et al.* (2020) fermented canola meal with *Aspergillus* species, resulting in a significant reduction in phytate content, which is attributed to the increased activity of enzymes such as phytase due to SSF. Terefe *et al.* (2021) used corn meal for SSF with *Lactobacillus plantarum* and *Saccharomyces cerevisiae*. Unlike our study, they performed SSF over shorter periods (12, 24, 36, and 48 hours), resulting in approximately 67 % phytate degradation. In our study, we used a temperature of 37 °C during SSF, which favored the production of phytases, as phytases can be produced naturally (endogenous phytase) or by microorganisms (exogenous phytase) within a temperature range of 35 °C to 45 °C (Sindhu & Khetarpaul, 2001). Therefore, SSF with *S. cerevisiae* can produce phytase enzymes, which participate in the action of repressible acid phosphatases secreted by yeast cells, enabling the hydrolysis of phytates (Haraldsson *et al.*, 2005). Furthermore, the presence of phytases increases the bioavailability of minerals (Vásquez-Villalobos *et al.*, 2019).

Effect of fermentation on saponins content

The saponin content in raw MJc (unprocessed) was 1594.04 µg diosgenin equivalents per gram of meal. **Figure 5** shows a reduction in saponins in MJc, regardless of the hydrothermal treatment. A 49-51 % decrease was observed in the non-treated fermented MJc, while in the MJc subjected to hydrothermal treatment, the reduction ranged from 82 to 96 %. Sharath *et*

al. (2014) fermented defatted *J. curcas* meal and observed a degradation of saponins over time, achieving a 96 % reduction after six days of fermentation using *Rhizopus oryzae* and *Cunninghamella echinulata*. Similarly, Adedayo & Sani (2019) conducted SSF on *Adansonia digitata* seed flour, finding a significant reduction in saponin content. It is also important to note that saponins have a bitter taste, which can lead to a lower preference for foods that contain them (Ocampo *et al.*, 2015).

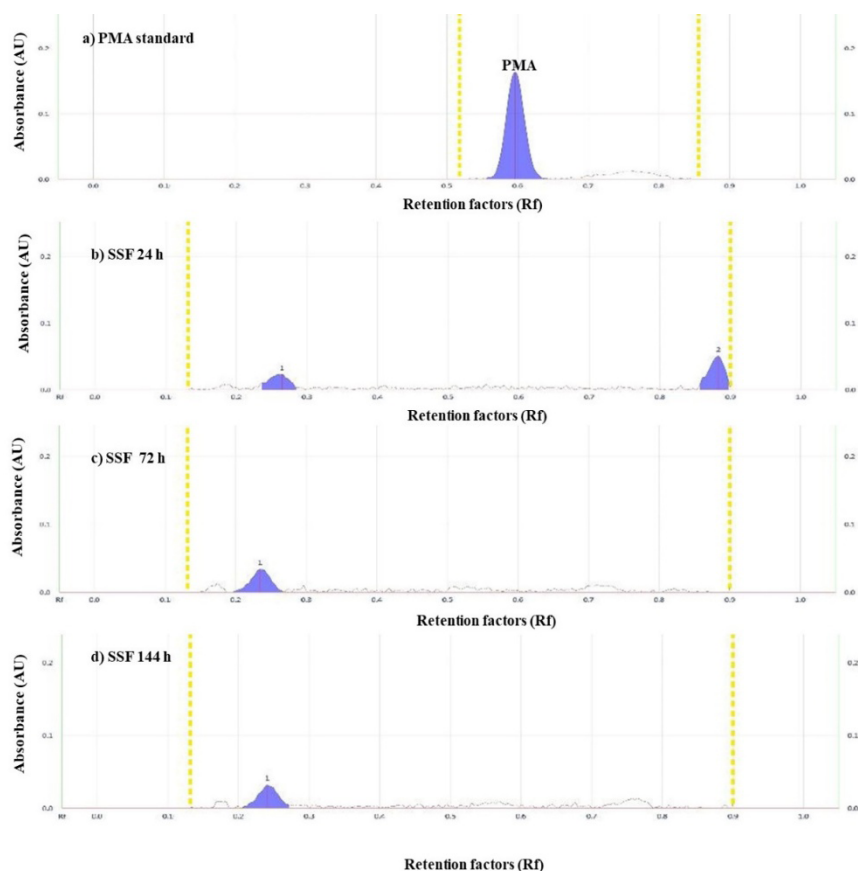


Figure 2. HPTLC chromatograms:

a) Phorbol-12-myristate-13-acetate (PMA); b) defatted *J. curcas* meal (MJc) without hydrothermal treatment fermented for 24 h; c) defatted *J. curcas* meal (MJc) without hydrothermal treatment fermented for 72 h; d) defatted *J. curcas* meal (MJc) without hydrothermal treatment fermented for 144 h.

Effect of fermentation on the MJc nutritional content

Raw MJc showed a content of protein, fat, ash, crude fiber, and carbohydrates of 40.43 ± 0.22 %, 15.84 ± 0.99 %, 10.07 ± 0.01 %, 9.29 ± 0.21 %, and 24.37 ± 0.86 %, respectively. The protein content was lower than that reported by Martinez-Herrera *et al.* (2006), which ranged from 62 % to 65 %, while the crude fiber and ash content were higher than their reported values (3.9 % to 4.5% and 4.9 % to 8.8 %, respectively).

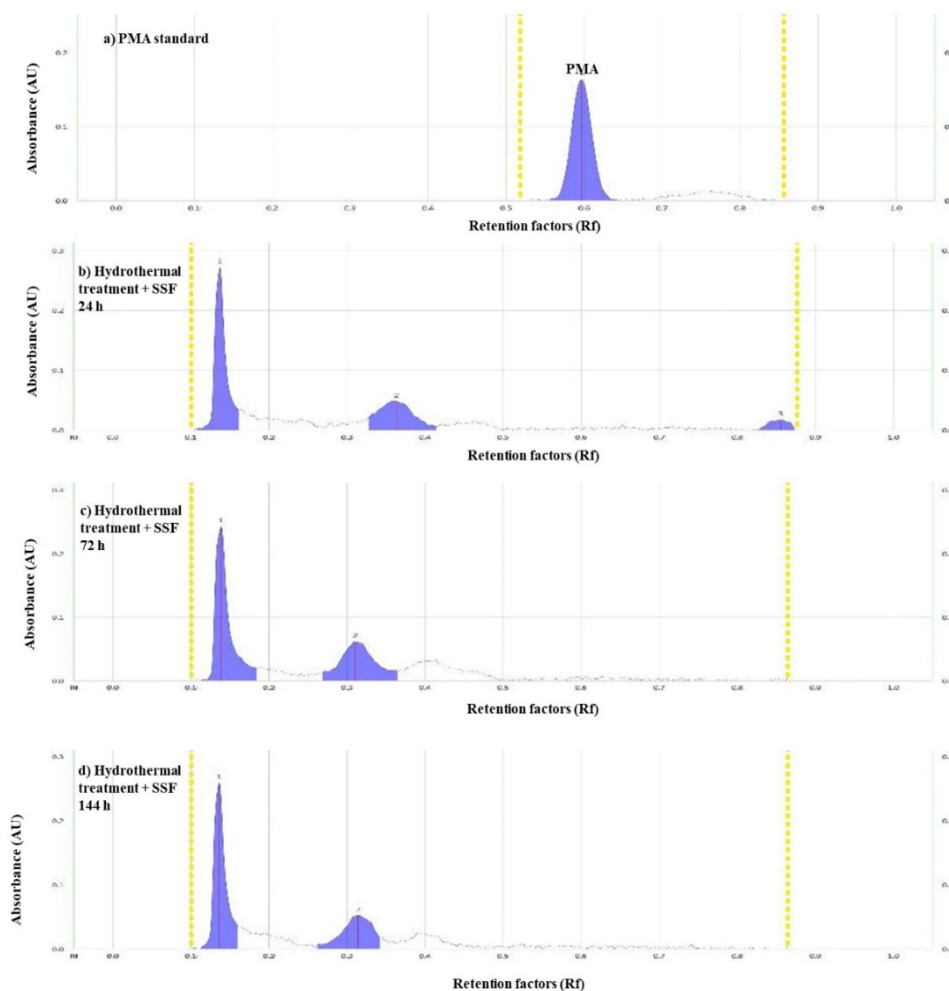


Figure 3. HPTLC chromatograms:

a) Phorbol-12-myristate-13-acetate (PMA); b) defatted *J. curcas* meal (MJc) with hydrothermal treatment fermented for 24 h; c) defatted *J. curcas* meal (MJc) with hydrothermal treatment fermented for 72 h; d) defatted *J. curcas* meal (MJc) with hydrothermal treatment fermented for 144 h.

The effect of fermentation with and without hydrothermal treatment on the nutritional content of MJc can be observed in **Table 3**. No significant difference in protein content was found in MJc due to SSF, hydrothermal treatment, or the combination of both treatments. This contrasts with the findings of Darwish *et al.* (2012), who applied SSF with *S. cerevisiae* to corn stalks and observed a 7.5 % increase in protein over a 14-day fermentation period. Additionally, the final protein content in our study ranged from 41 to 44 %, which falls within the recommended range (18-50 %) to use in balanced fish feed.

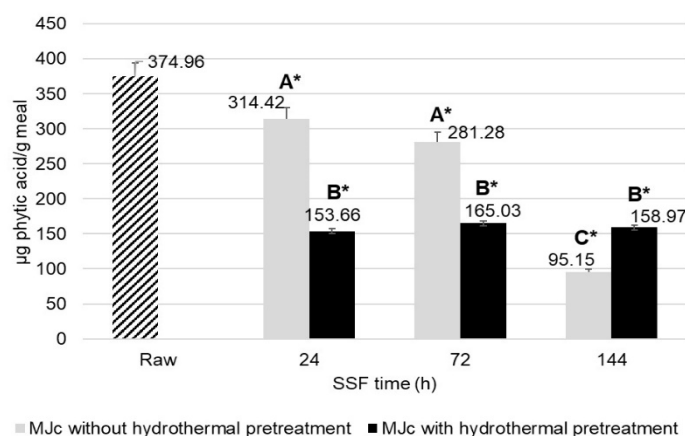


Figure 4. Phytate concentration (µg phytic acid/g meal) in defatted *J. curcas* meal (MJc), with and without hydrothermal treatment, at different fermentation times.

Values are means $\pm$ standard deviation (n=2). ^{A, B, C} Means with different capital superscripts in the corresponding bars are significantly different between samples with and without hydrothermal treatment (Tukey, $p \leq 0.05$). *Means that show significant differences with raw (unprocessed) MJc (Dunnet, $p \leq 0.05$). MJc: defatted *J. curcas* meal.

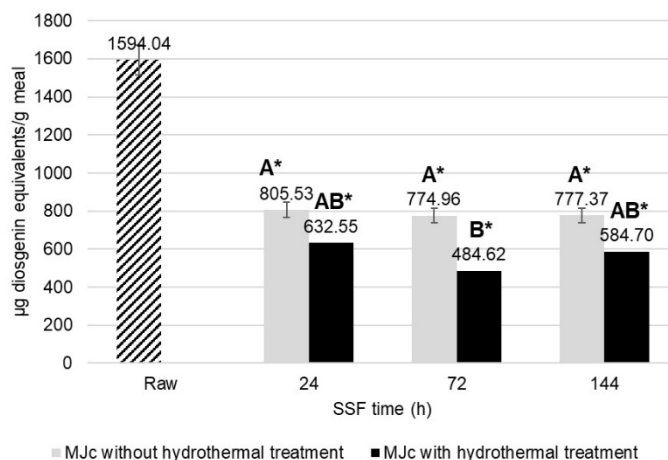


Figure 5. Saponin concentration (µg diosgenin equivalents/g meal) in defatted *J. curcas* meal (MJc), with and without hydrothermal treatment, at different fermentation times.

Values are means $\pm$ standard deviation (n=2). ^{A, B} Means with different capital superscripts on the corresponding bars are significantly different between samples with and without hydrothermal treatment (Tukey, $p \leq 0.05$). *Means that show significant differences with raw (unprocessed) MJc (Dunnett, $p \leq 0.05$). MJc: defatted *J. curcas* meal.

A significant decrease in fat content was observed in non-treated fermented MJc. The fermented MJc showed lower crude fat values (13.45 % to 14.59 %) compared to the raw sample (15.84 %). In contrast, when hydrothermal treatment was applied, there was an increase in fat content (18.19 % to 20.19 % in fermented MJc vs. 15.84 % in the raw sample). The fermentation time did not have a significant effect on fat content, regardless of the applied hydrothermal treatment.

In contrast, the crude fiber content showed a significant decrease due to SSF application (approximately 4 %). Hydrothermal treatment did not have a significant effect on this variable. This decrease in crude fiber during SSF can be attributed to the secretion of extracellular enzymes that are capable of degrading fibers, including cellulose and hemicellulose (Darwish *et al.*, 2012; Rashad *et al.*, 2011). Similar to crude fat content, the fermentation time and hydrothermal treatment had no significant effect on the crude fiber content.

The ash content showed an inverse pattern compared to the crude fat content. A significant increase was observed due to SSF application when the sample was not treated with a hydrothermal treatment. The ash content ranged from 12.33 to 12.66 %, and the raw sample had 10.07 %. However, when the hydrothermal treatment was applied, there was a slight decrease in ash content (9.22 to 9.37 % in fermented MJc) during the fermentation times of 24 and 144 hours. Fermentation time did not have a significant effect on the ash content when the hydrothermal treatment was not applied. However, on heat-treated samples, a reduction in ash content was observed at the previously mentioned fermentation times. The reason for these changes in ash

content is that hydrothermal treatment allowed the release of minerals through leaching, making them available to microorganisms, which causes a decrease in this nutrient. In contrast, in the non-heated samples, mineral release before SSF did not occur.

Lastly, significant differences were observed in the carbohydrate content across the six treatments without showing any apparent pattern or trend due to SSF, hydrothermal treatment, or a combination of both. This behavior in carbohydrates may be because this nutrient was calculated by subtracting the rest of the nutrients. A decrease of 6 to 8 % in carbohydrate content was observed due to SSF, regardless of the hydrothermal treatment application. This decrease in carbohydrates is attributed to the ability of *Saccharomyces* to utilize hydrocarbon fractions of 12 to 16 carbons as an energy source (Csutak *et al.*, 2010).

Table 3. Nutritional content (% ds) of defatted *J. curcas* meal (MJc), with and without hydrothermal treatment at different fermentation times

Nutritional composition	Without hydrothermal treatment			With hydrothermal treatment		
	SSF time (h)			SSF time (h)		
	24	72	144	24	72	144
Crude protein	41.82 ± 0.19 ^a	43.50 ± 0.12 ^a	44.50 ± 0.21 ^a	41.94 ± 0.20 ^a	41.80 ± 0.31 ^a	41.85 ± 0.20 ^a
Crude fat	14.59 ± 0.04 ^{ab}	14.45 ± 0.07 ^b	13.45 ± 0.30 ^a	19.79 ± 0.24 ^{ab}	20.19 ± 0.05 ^a	18.19 ± 0.07 ^{ab}
Ash	12.33 ± 0.07 ^a	12.66 ± 0.11 ^a	12.52 ± 0.01 ^a	9.22 ± 0.007 ^c	9.95 ± 0.02 ^b	9.37 ± 0.01 ^c
Crude fiber	5.64 ± 0.94 ^a	6.71 ± 1.11 ^a	5.54 ± 0.92 ^a	5.23 ± 0.06 ^a	6.08 ± 0.25 ^a	6.30 ± 0.13 ^a
Carbohydrates	25.62 ± 0.21 ^{ab}	22.68 ± 0.78 ^a	23.98 ± 0.30 ^{bc}	23.83 ± 0.28 ^c	21.97 ± 0.35 ^c	24.29 ± 0.24 ^{abc}

Note: All values are means ± standard deviation (n=2).

^{a, b, c} Means with different superscript lowercase letters on the same line are significantly different (Tukey, $p \leq 0.05$). MJc: defatted *J. curcas* meal.

Conclusions

In this study, the effects of solid-state fermentation (SSF) with *S. cerevisiae* and the application of a hydrothermal treatment before fermentation on defatted *Jatropha curcas* meal (MJc) were observed. After reviewing the response variables, MJc fermented for 144 hours without hydrothermal treatment is of particular interest, as it showed an improvement in nutritional composition, with a reduction in fat, crude fiber, and carbohydrate content. On the other hand, no

significant changes were observed in protein content that could affect the nutritional composition. Additionally, any presence of phorbol esters was detected, and a low concentration of antinutritional compounds (phytates and saponins) was obtained compared to raw (unprocessed) MJc. Therefore, this study broadens the understanding of the benefits of applying biological processes to plant materials, such as MJc, which has significant potential for use in feed production for Nile tilapia.

Author Contributions

“Work conceptualization: Medina-Rodelo, D.P.; Gutiérrez-Dorado, R.; Angulo-Escalante, M.A.; methodology development: Medina-Rodelo, D.P.; Quintana-Obregón, E.A.; Gutiérrez-Dorado, R.; software management: Medina-Rodelo, D.P.; Gutiérrez-Dorado, R.; experimental validation: Medina-Rodelo, D.P.; Gutiérrez-Dorado, R.; results analysis: Medina-Rodelo, D.P.; Gutiérrez-Dorado, R.; data management: Medina-Rodelo, D.P.; Gutiérrez-Dorado, R.; manuscript writing and preparation: Medina-Rodelo, D.P.; writing, review, and editing: Medina-Rodelo, D.P.; Quintana-Obregón, E.A.; Gutiérrez-Dorado, R.; Angulo-Escalante, M.A.; Heredia, J.B.; Puello-Cruz, A.C.; project administration: Medina-Rodelo, D.P.; Quintana-Obregón, E.A.; Gutiérrez-Dorado, R.; Angulo-Escalante, M.A.; Heredia, J.B.; Puello-Cruz, A.C.; funding acquisition: Gutiérrez-Dorado, R.; Angulo-Escalante, M.A.”.

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Ethical declarations

Not applicable.

Informed consent declaration

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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