

Quantification of protein quality in a fermented mixture of orange bagasse, yeast and nitrogen additives to enhance nutritional quality

Hernández-Silvestre, Sara P.¹; Serna-Lagunes, Ricardo²; Salazar-Ortiz, Juan¹; Salinas-Ruiz, Josafhat¹; Ortiz-Laurel, Hipólito^{1*}

¹ Colegio de Postgraduados, km 348 Carr Fed Córdoba-Veracruz, Congregación Manuel León, Amatlán de los Reyes, Veracruz, México. C. P. 94946.

² Universidad Veracruzana, Josefa Ortiz de Domínguez s/n, Peñuela, Amatlán de los Reyes, Veracruz, México. C. P. 94945.

* Correspondence: hlaurel@colpos.mx

ABSTRACT

Objective: To gauge the protein quality of a mixture of orange bagasse fermented with yeast and nitrogen compounds in order to determine its potential as an ingredient for animal diets.

Design/methodology/approach: The experimental design was a randomized complete block design with repeated measures. Four bagasse-based treatments were evaluated: Treatment 1: orange bagasse without additives; Trt 2: pulp + 200 g yeast + 60 g urea + 8 g (NH₄)₂SO₄; Trt 3: pulp + 100 g yeast + 30 g urea + 4 g (NH₄)₂SO₄; Trt 4: pulp + 50 g yeast + 15 g urea + 2 g (NH₄)₂SO₄. All treatments were mixed with 2 L of water per 10 kg of orange bagasse. Thus, 200 g samples were selected to measure physicochemical, nutritional, and microbiological variables at 0, 2, 4, 6, 24, 48, and 72 h of fermentation. Statistical analysis was performed using Proc Glimmix in SAS 9.4, and a Tukey mean comparison ($\alpha=0.05$).

Results: Trt 3 showed higher protein content, achieving a value of up to 18.55% at 48 h, suggesting a positive effect of the combined addition of yeast, urea, and ammonium sulfate.

Limitations of study/implications: The evaluation was only conducted at the ingredient level (bagasse); however, *in vitro* or *in situ* tests in animals are recommended.

Findings/conclusions: Bagasse fermented with nitrogen additives showed a greater tendency toward higher protein content, although no significant differences were observed, highlighting its potential as an ingredient in animal feed.

Keywords: fermentation, food waste, bagasse, ruminants, dietary constituent.

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INTRODUCTION

Globally, continuous population growth poses significant challenges to agri-food systems, which must meet a sustained and increasing demand for food. In this context, the 2030 Agenda for Sustainable Development highlights the need to achieve food security while promoting sustainable practices that help reduce environmental impact (UN, 2015).



In this regard, animal nutrition aims to provide animals with the appropriate quantity and quality of nutrients to ensure maximum productivity while maintaining good health status (Tripathi *et al.*, 2019).

In the case of ruminant animals, although pastures and forages constitute the basis of their diet, they are supplemented with concentrated feed (Flórez *et al.*, 2018). However, the cost of these feed inputs increases total production costs by 50% to 70% (Tripathi *et al.*, 2019), which has led to the exploration of alternative raw materials to reduce their dependence. Among these inputs, protein ingredients stand out, with soybean meal being one of the main representatives; however, its cost remains inaccessible. Therefore, crop residues and some agro-industrial by-products have been increasingly used as alternative protein sources to improve livestock productivity (Guerra *et al.*, 2021; Hernández *et al.*, 2019).

On the other hand, in Mexico there is still no Official Mexican Standard (NOM) that specifically regulates the use of orange bagasse in animal feed. This regulatory framework is essential to establish the scope for the use of this co-product, including conditions for its collection, transport, and storage, as well as process-related conditions such as prior analysis, washing, disinfection, among others, including the distribution cycle of the final product. The objective should be to avoid violating guidelines that could pose risks to animal health through the consumption of feed partially based on this new input. In its support, it can be mentioned that the General Law of Circular Economy was approved by the Senate in 2021, which promotes the use and valorization of waste, prioritizing the development of sustainable schemes (INECC, 2021).

Veracruz is the leading orange-producing state in Mexico, with a production of 2,271,524 tons, representing 50% of the national total (SIAP, 2023). As a result, the citrus sector in Veracruz generates approximately 630,000 tons of waste annually (Galindo-Segura *et al.*, 2023).

Although this by-product is abundant and relatively low-cost, its use in animal feed remains limited, mainly due to its low crude protein content (Panwar *et al.*, 2021). Therefore, it is advisable to subject it to biotechnological processes to increase its nutritive potential.

Orange bagasse can be directly transformed into a viable feed resource through bioconservation and biotransformation processes, among which aerobic fermentation and ensiling (anaerobic, with dry matter content control) stand out. Through these processes, undesirable flavors and odors from essential oils are eliminated and, by conferring a pasty consistency, the product becomes more acceptable to ruminants and stimulates their intake (Fernández, 2021), although both operate under different processing conditions.

Fermentation is a process in which fresh agricultural by-products can be transformed into improved feed through modifications in their nutritional composition, achieved by the consumption of carbohydrates by microorganisms, usually added to the substrate (Acosta *et al.*, 2015). Thus, the process can be carried out with minimal prior preparation and even accelerated with the integration of appropriate inputs, allowing the resulting ingredient to be incorporated into the animal diet. In addition, adjustments can be made during the process if necessary.

Therefore, the purpose of this study was to evaluate the degree of protein enrichment in orange bagasse fermented with yeast, urea, and ammonium sulfate, in order to obtain the best product under an appropriate dosage of inputs and the optimal processing time.

MATERIALS AND METHODS

Study location and experimental characterization

The orange waste fermentation process was carried out in the livestock area, and the microbiological analysis was conducted in the dairy laboratory of Colegio de Postgraduados, Córdoba Campus (COLPOS), located in the municipality of Amatlán de los Reyes. The average daily maximum and minimum temperatures range between 29-36 °C and 14-18 °C, respectively (SIEGVER, 2023).

The proximate chemical analysis was conducted in the Microalgae Biotechnology and Bioenergy Laboratory at the Instituto Tecnológico de Boca del Río, located at km 12, Veracruz-Córdoba Highway.

Biological material: Orange bagasse was obtained through donation from the company Jugos y Aceites del Golfo (JAG), located at Prolongación Josefa Ortiz de Domínguez No. 110, Peñuela, Veracruz.

Preparation of fermented orange bagasse: After collection and without prior storage, the bagasse was ground to obtain a particle size of approximately 10-40 mm in order to promote a stable fermentation process. Subsequently, the bagasse and materials were weighed, labeled, and identified for each treatment (Figure 1).

Treatments and experimental design

The experimental design was a randomized complete block design with repeated measures. The experimental unit consisted of 200 g samples in triplicate for each treatment, while physicochemical, proximate chemical, and microbiological variables were evaluated



Figure 1. Process flow for the fermentation of orange bagasse.

at time intervals of 0, 2, 4, 6, 24, 48, and 72 h. Prior to fermentation, the base inputs were mixed to obtain a homogeneous mass (Table 1).

Physicochemical analysis: Measurements were taken at the indicated time intervals (0, 2, 4, 6, 24, 48, and 72 h). pH was measured using a potentiometer (model PH10), previously calibrated with buffer solutions at pH 4 and 7 (HYCEL, Mexico) and equipped with a Conductronic electrode (Mexico). Soluble solids ($^{\circ}$ Brix) were measured using a refractometer (model HI96801; Hanna Instruments, USA). Both pH and $^{\circ}$ Brix were evaluated according to AOAC (1997) methods. Finally, sample temperature was recorded using an industrial digital infrared thermometer (BENETECH, model GM320), and ambient temperature was measured with a thermohygrometer (model HTC-2; HTC).

Proximate analysis: All samples were dried at 60 $^{\circ}$ C and, once a constant weight was reached, they were stored in Ziploc[®] bags for subsequent analysis. Dry matter (DM) and moisture content (M) were determined using AOAC method 925.09 (AOAC, 1997), while ash content (Ash) was determined using AOAC method 923.03 (AOAC, 1997).

The previously dried samples were sieved through a 680 μ m mesh. Subsequently, 0.05 g of each sample was weighed and mixed with 50 mL of water in a beaker, followed by ultrasonication using a YUCHENGTECH ultrasonic processor set at a frequency of 24.20 Hz for 10 min at 60 $^{\circ}$ C in continuous mode with intermittent agitation. The volume was then adjusted to 100 mL in a volumetric flask to homogenize the sample. The resulting solution was used to determine total carbohydrates (TC) using the phenol-sulfuric acid method described by DuBois *et al.* (1956) and total protein (TP) using the method described by Lowry *et al.* (1951).

Yeast quantification: Serial dilutions (10^{-1} to 10^{-8}) were prepared and plated using the pour plate method according to the Official Mexican Standard NOM-111-SSA1-1994 (Secretaría de Salud, 1994). The plates were incubated in a culture incubator (Riossa, model ECF-82) at 30 $^{\circ}$ C for 168 h. After incubation, colony-forming units (CFU) were counted using a colony counter (model CVP-CM3) (Figure 2).

Statistical analysis

The results were analyzed using PROC GLIMMIX in SAS[®] 9.4. The variables were evaluated through analysis of variance (ANOVA), and multiple comparisons of adjusted means (LS-means) similar to Tukey's test were performed using conservative T-grouping ($\alpha=0.05$).

Table 1. Treatments and their corresponding dosages.

Input	Control (Trt 1)	Treatment high dose (Trt 2)	Treatment medium dose (Trt 3)	Treatment low dose (Trt 4)
Orange bagasse	10 kg	10 kg	10 kg	10 kg
Water	2 L	2 L	2 L	2 L
Urea		60 g	30 g	15 g
(NH ₄) ₂ SO ₄		8 g	4 g	2 g
Yeast of <i>S. cerevisiae</i>		200 g	100 g	50 g

Tr1, Tr2, Tr3, Tr4, correspond to treatment 1, treatment 2, treatment 3, treatment 4, respectively.

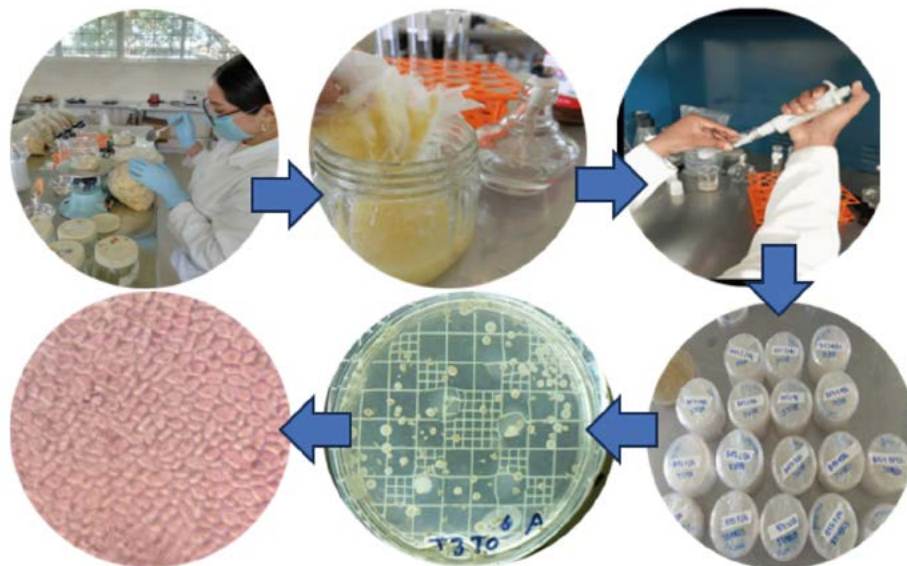


Figure 2. CFU quantification during the fermentation process.

RESULTS AND DISCUSSION

Physicochemical analysis: For all analyzed variables, a mixed model was applied using a TOEP(1) covariance structure and multiple comparisons of adjusted means (LS-means) similar to Tukey's test, using conservative T-grouping ($\alpha=0.05$).

Type III analysis of variance (ANOVA) for fixed effects on temperature showed a significant effect of time ($p<0.0001$), with no significant differences among treatments. Table 2 presents the adjusted mean comparisons of physicochemical parameters. Initial temperature values ranged from 22.36 to 22.70 °C, followed by a progressive increase until 48 h of fermentation, when the highest values were recorded: 28.05 °C (Trt 2), 27.84 °C (Trt 1), 27.67 °C (Trt 3), and 27.10 °C (Trt 4). At 72 h, temperature decreased to a minimum of 23.48 °C (Trt 4). The increase in temperature observed at the beginning of the experiment may be attributed to the metabolic activity of microorganisms, as described by Perwez and Al Asheh (2025) in a bioreactor study, where a low initial thermal incidence was recorded and temperature increased over time. Likewise, the temperature behavior observed in this study remained within the optimal range for metabolism and single-cell protein synthesis by *S. cerevisiae* (25-32 °C). This thermal increase can be attributed to metabolic heat release from yeast during the exponential growth phase, followed by a temperature decrease corresponding to the stationary phase (Walker and Stewart, 2016). The maximum temperatures recorded in this study were lower than those reported by Grizotto *et al.* (2020), who observed maximum temperatures of approximately 33 °C during the ensiling process of treatments containing 30% pelleted citrus pulp.

For the pH variable, the ANOVA showed a significant effect of time ($p<0.0001$), with numerically different but statistically non-significant differences among treatments. Initial pH values ranged from 4.00 to 4.03, followed by a progressive decrease until 48 h, reaching values of 3.76 (Trt 3), 3.74 (Trt 2), 3.68 (Trt 4), and 3.57 (Trt 1), and a slight

increase was observed at 72 h. The acidity trends observed in the bagasse are associated with the production of organic acids as a result of metabolic activity, a common process in fermentations (González-Guerra *et al.*, 2025). Therefore, a comparison among treatments indicates that those supplemented with yeast, ammonium sulfate, and urea showed the highest pH values. According to Yang *et al.* (2021), who compared urea and ammonium sulfate on the growth and fermentative metabolism of *S. cerevisiae*, urea promoted higher pH values. Likewise, Zhang *et al.* (2022) reported that urea hydrolysis contributes to pH increase in fermentation systems.

Regarding °Brix, significant differences were observed over time $p < 0.0001$ and in the interaction between time and treatments $p = 0.0230$. Initial values ranged from 5.78 to 6.23 at 0 h, and a progressive decrease was recorded as fermentation progressed. Trt 3 and Trt 4 showed more pronounced declines, reaching final values of 3.78 and 4.01, respectively. Trt 1 reached 4.19 at 72 h, while Trt 2 recorded the highest final value (4.91), which is associated with more efficient sugar utilization due to metabolic activity (Li *et al.*, 2021). The decrease in °Brix can be explained by yeast metabolism of simple sugars (sucrose, glucose, and fructose) during fermentation, as reported by Yafetto *et al.* (2023). Additionally, during orange juice extraction, soluble sugars are often inadvertently lost, reducing the sugar content of the bagasse (Rossit *et al.*, 2020). Therefore, soluble solids in citrus by-products typically range from 2 to 5 °Brix (Zhao and Yu, 2008), which may affect the reliability of recorded values.

Table 2. Least squares mean of physicochemical parameters during orange bagasse fermentation.

Temperature (°C)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	22.41 ^e	24.20 ^{edc}	25.15 ^{ebdac}	25.16 ^{ebdac}	25.04 ^{ebdac}	27.84 ^a	26.07 ^{bdac}
Trt 2	22.70 ^{ed}	25.52 ^{ebdac}	26.44 ^{bac}	25.75 ^{ebdac}	25.46 ^{ebdac}	28.05 ^a	26.83 ^{bac}
Trt 3	22.68 ^{ed}	25.18 ^{ebdac}	25.98 ^{ebdac}	25.62 ^{ebdac}	26.50 ^{bac}	27.67 ^{ba}	27.04 ^{bac}
Trt 4	22.36 ^e	24.89 ^{ebdc}	25.43 ^{ebdac}	25.16 ^{ebdac}	26.30 ^{bdac}	27.10 ^{ba}	23.48 ^{edc}
pH							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72h
Trt 1	4.00 ^{ba}	3.98 ^{bac}	3.94 ^{bac}	3.92 ^{bac}	3.72 ^{bac}	3.57 ^d	3.76 ^{bdac}
Trt 2	4.03 ^{ba}	4.05 ^a	3.97 ^{bac}	4.00 ^{ba}	3.90 ^{bac}	3.74 ^{bdc}	3.90 ^{bac}
Trt 3	4.03 ^{ba}	3.97 ^{bac}	4.00 ^{ba}	3.90 ^{bac}	3.76 ^{bdac}	3.76 ^{bdac}	3.97 ^{bac}
Trt 4	4.02 ^{ba}	3.97 ^{bac}	3.95 ^{bac}	3.92 ^{bac}	3.80 ^{bdac}	3.68 ^{dc}	3.85 ^{bdac}
°Brix							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72h
Trt 1	6.23 ^a	5.70 ^{ebdac}	5.63 ^{ebdac}	5.17 ^{ebdgcf}	4.95 ^{ebdhgcf}	5.40 ^{ebdacf}	4.19 ^{hgf}
Trt 2	5.93 ^{bac}	5.18 ^{ebdagcf}	5.18 ^{ebdgcf}	4.31 ^{hgf}	4.73 ^{edhgcf}	4.88 ^{ebdhgcf}	4.91 ^{ebdhgcf}
Trt 3	6.00 ^{ba}	5.63 ^{ebdac}	5.41 ^{ebdgcf}	4.86 ^{ebdhgcf}	4.73 ^{edhgcf}	4.58 ^{ehgf}	3.78 ^h
Trt 4	5.78 ^{bdac}	5.63 ^{ebdac}	5.63 ^{ebdac}	5.32 ^{ebdacf}	4.80 ^{ebdhgcf}	4.58 ^{ehgf}	4.01 ^h

Data were analyzed using a factorial ANOVA (treatment × time). Subsequently, Tukey’s test ($p < 0.05$) was applied for mean comparison. Means with the same letter in the same column indicate no significant differences. Trat=group of treatments for each variable. Trt=treatments. Fermentation time: 0, 2, 4, 6, 24, 48, and 72 h.

Proximal analysis: For MS and H variables, the ANOVA showed a significant effect of time in both $p < 0.0001$ and a significant time \times treatment interaction MS $p = 0.0408$; $H_p = 0.0026$, while the trend among treatments was not significant for either variable. Table 3 shows the behavior of MS and H, where initial values were close to 16-18% and final values were around 15-16% at 72 h, demonstrating that orange bagasse continues to exhibit a high moisture content. This agrees with López *et al.* (2023), who reported that at the end of the process orange bagasse registered a moisture content ranging between 75 and 80%. The reason for this is explained by Morovat *et al.*

Table 3. Least squares mean of chemical-proximal parameters during orange bagasse fermentation.

Dry matter (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	16.30 ^{ebdc}	16.40 ^{ebdac}	15.61 ^{edc}	14.94 ^e	15.40 ^{ed}	15.58 ^{ed}	15.87 ^{edc}
Trt 2	17.65 ^{bac}	16.41 ^{ebdac}	16.51 ^{ebdac}	16.45 ^{ebdac}	17.29 ^{bdac}	16.12 ^{ebdc}	16.00 ^{ebdc}
Trt 3	17.71 ^{ba}	17.10 ^{bdac}	16.52 ^{ebdac}	16.39 ^{ebdac}	16.58 ^{ebdac}	16.95 ^{ebdac}	16.58 ^{ebdac}
Trt 4	18.46 ^a	17.45 ^{bdac}	15.58 ^{edc}	16.66 ^{edac}	16.36 ^{ebdc}	16.19 ^{ebdc}	16.06 ^{ebdc}
Moisture content (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	83.72 ^{bac}	83.60 ^{bdac}	84.06 ^{bac}	84.59 ^{ba}	84.60 ^{ba}	84.42 ^{bac}	84.05 ^{bac}
Trt 2	82.35 ^{dc}	83.68 ^{bdac}	84.99 ^a	83.55 ^{bdac}	82.80 ^{bdc}	83.88 ^{bac}	83.61 ^{bdac}
Trt 3	82.29 ^d	82.90 ^{bdc}	83.48 ^{bdac}	83.61 ^{bdac}	83.42 ^{bdac}	83.06 ^{bdac}	83.42 ^{bdac}
Trt 4	81.53 ^d	82.56 ^{dc}	84.51 ^{ba}	83.26 ^{bdac}	83.64 ^{bdac}	83.81 ^{bac}	83.95 ^{bac}
Ashes (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	5.30 ^a	5.17 ^a	5.43 ^a	5.61 ^a	5.72 ^a	5.32 ^a	8.68 ^{ed}
Trt 2	5.38 ^a	5.48 ^a	5.53 ^a	5.30 ^a	5.09 ^a	5.26 ^a	11.33 ^{ebdc}
Trt 3	5.43 ^a	5.41 ^a	5.53 ^a	6.00 ^a	5.78 ^a	5.61 ^a	16.34 ^{bac}
Trt 4	5.42 ^a	5.78 ^a	5.20 ^a	6.07 ^a	6.07 ^a	5.71 ^a	15.80 ^{bac}
Total protein (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	8.21 ^e	10.17 ^{ebdc}	10.17 ^{ebdc}	10.51 ^{ebdc}	9.76 ^{ed}	8.68 ^{ed}	8.68 ^{ed}
Trt 2	10.80 ^{ebdc}	12.10 ^{ebdac}	12.10 ^{ebdac}	15.14 ^{ebdac}	16.01 ^{bac}	15.43 ^{bdac}	11.33 ^{ebdc}
Trt 3	15.24 ^{bdac}	15.76 ^{bac}	15.76 ^{bac}	16.03 ^{bac}	17.11 ^{ba}	18.55 ^a	16.34 ^{bac}
Trt 4	10.69 ^{ebdc}	11.90 ^{ebdc}	11.90 ^{ebdc}	12.31 ^{ebdac}	12.66 ^{ebdac}	13.07 ^{ebdac}	15.80 ^{bac}
Total carbohydrates (%)							
Trat	0 h	2 h	4 h	6 h	24 h	48 h	72 h
Trt 1	14.13 ^{hgf}	18.47 ^{ebdac}	20.07 ^a	19.56 ^{ba}	19.33 ^{bac}	19.15 ^{bdac}	20.04 ^a
Trt 2	10.83 ^h	13.87 ^{hg}	14.76 ^{ehgf}	15.44 ^{ebdgcf}	16.05 ^{ebdagcf}	16.75 ^{ebdagcf}	14.62 ^{ehgf}
Trt 3	14.30 ^{hgf}	13.45 ^h	16.04 ^{ebdgcf}	15.52 ^{ebdgcf}	14.45 ^{ehgf}	17.43 ^{ebdacf}	14.71 ^{ehgf}
Trt 4	16.75 ^{ebdagcf}	15.03 ^{edhgcf}	14.55 ^{ehgf}	15.22 ^{edhgcf}	17.00 ^{ebdagcf}	15.32 ^{ebdgcf}	14.68 ^{ehgf}

Data were analyzed using a factorial ANOVA (treatment \times time). Subsequently, Tukey's test ($p < 0.05$) was applied for mean comparison. Means with the same letter in the same column indicate no significant differences. Trat=group of treatments for each variable. Trt=treatments. Fermentation time: 0, 2, 4, 6, 24, 48, and 72 h.

(2014), who stated that during the fermentation of citrus by-products microorganisms metabolize fermentable sugars (glucose, fructose, sucrose, and maltose), which causes a partial reduction in dry matter.

Regarding the ash variable, ANOVA showed a significant effect of time ($p=0.0016$) and a significant treatment \times time interaction ($p=0.0023$). Mean comparison indicated that the initial ash content was 5.30% (Trt 1), 5.38% (Trt 2), 5.43% (Trt 3), and 5.42% (Trt 4), and after 72 h of fermentation the values were 8.68% (Trt 1), 11.33% (Trt 2), 16.36% (Trt 3), and 15.30% (Trt 4). Thus, Trt 3 recorded the highest value at the end of fermentation. Ash content decreased predominantly in the control treatment compared to the others (Trt 2, Trt 3, and Trt 4), indicating lower metabolic activity, a result similar to that reported by Castillo *et al.* (2011) in a study on solid-state fermentation of agro-industrial by-products. Furthermore, Erismann *et al.* (2025) reported that ash content commonly fluctuates during fermentation due to the consumption and release of soluble mineral components and inorganic compounds.

Regarding protein, according to the ANOVA, the time factor significantly affected the variable $p=0.0021$ in the treatment \times time interaction, while no significant differences were observed among treatments ($p>0.05$). Figure 3 shows the least squares means, where a relatively high standard error was observed, particularly at the beginning of the experiment, with higher values recorded in Trt 3. This dispersion may be due to environmental conditions during the process, the composition of the bagasse itself, and possibly the sensitivity of the Lowry method (Lowry *et al.*, 1951). This method was used because it quantifies true protein as soluble fractions and nitrogenous compounds, and the presence of yeast and nitrogen sources (urea and ammonium sulfate) increases the detectable fraction at the beginning of fermentation. Under this premise, protein content increased over time, with Trt 3 showing the highest values compared to Trt 1, which recorded the lowest values. The maximum protein value was observed at 48 h of fermentation, followed by a slight decrease at 72 h.

This finding corresponds to that reported by Morovat *et al.* (2014), who observed that lemon pulp supplemented with *Saccharomyces cerevisiae* and urea showed an increase in

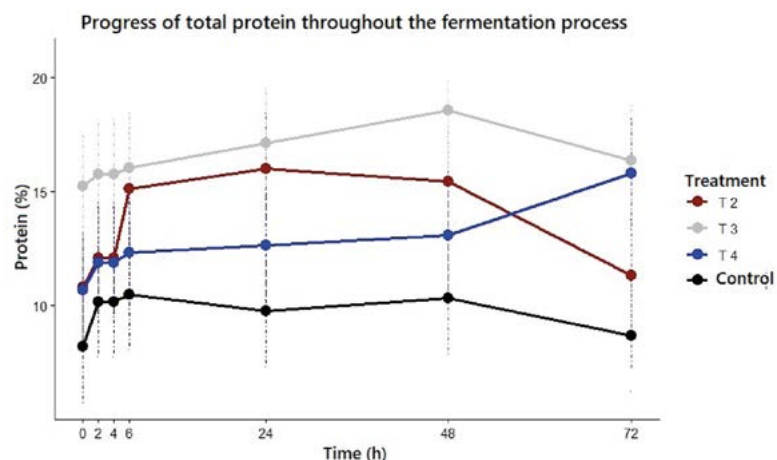


Figure 3. Evolution of the protein variable over the fermentation time in different treatments.

protein at the beginning of fermentation compared to the control. Likewise, the highest PT content throughout fermentation was observed in treatments that combined yeast with nitrogen sources. This can be explained by the premise that microorganisms utilize nitrogen sources (urea and ammonium sulfate) and transform non-protein nitrogen (NPN) into protein nitrogen (PN), which is partially incorporated into the cellular protoplasm (Rodríguez *et al.*, 2006).

Regarding total carbohydrate content, ANOVA showed a significant effect of time $p < 0.0001$ and a significant time \times treatment interaction $p = 0.0024$, while no significant differences were observed among treatments ($p = 0.1801$). In Figure 4, total carbohydrate content exhibited a significant increase during the first hours of the process, reaching its highest value at 4 h (20.07 ± 1.59). Likewise, Trt 1 showed the greatest increase compared to the other treatments (Trt 2, Trt 3, and Trt 4). These latter treatments showed similar contents, although with irregular variations, and most of them reached their highest increase within the 24-48 h range.

Similarly, a contrasting effect was observed in carbohydrate content relative to protein. On the one hand, the increase in carbohydrates was lower in the treatments supplemented with nitrogen sources (Trt 2, Trt 3, and Trt 4); however, these registered a higher protein content. This behavior can be explained by the fact that these sugars are consumed by microorganisms, which use them as an energy source during fermentation (Peters, 2006). This assertion agrees with the findings of Rossit *et al.* (2020) and Zhao & Yu (2008), who report that added nitrogen sources, such as urea and ammonium sulfate, contribute to an efficient and rapid consumption of sugars.

Yeast count: Given the nature of yeast colonies—usually dispersed and forming large aggregates during colony development—the negative binomial model adequately fits cell count data (Lindén and Mäntyniemi, 2011). In addition, this model appropriately handles processes involving repeated analyses, as in this study, where the model fitting was performed using Microsoft Excel[®]. On the other hand, ANOVA results indicated significant differences among treatments ($p = 0.004$) and over time ($p < 0.0001$), with no significant time \times treatment interaction ($p = 0.1092$). An exponential growth was observed

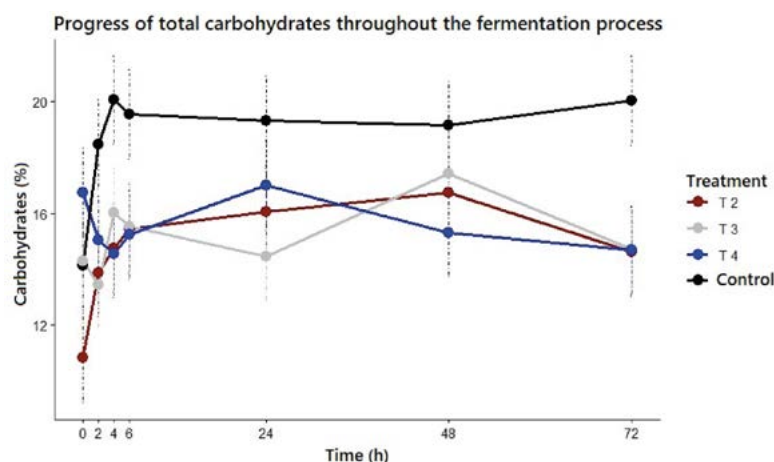


Figure 4. Evolution of the carbohydrate variable over fermentation time in different treatments.

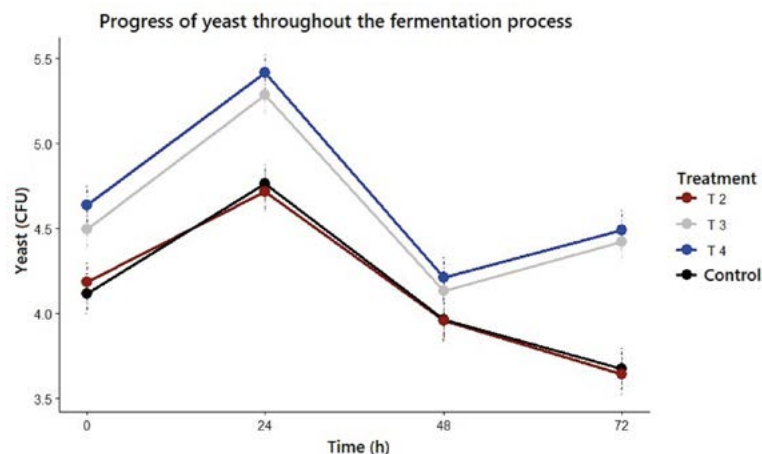


Figure 5. Evolution of yeast (CFU) over fermentation time across different treatments.

up to 24 h, followed by stabilization and a slight decrease at 72 h (Figure 5). Trt 4 showed the greatest increase (4.21×10^6 CFU/g), while Trt 1 and Trt 2 exhibited a similar and generally lower behavior compared to the other treatments. The highest yeast colony counts were recorded in treatments supplemented with nitrogen sources. This statement agrees with the findings reported by Schön *et al.* (2024), who indicated that the addition of nitrogen sources promotes the growth of *S. cerevisiae* yeast. Likewise, Li *et al.* (2024) reported that the use of nitrogen compounds contributes to the bioconversion of citrus residues, resulting in greater microbial biomass production, which in turn contributes to increasing the protein content of the substrate. In the same context, Grizzotto *et al.* (2020) reported yeast counts close to 10^6 CFU/g in orange peel silage, confirming the reliability of the values reported in this study.

CONCLUSIONS

The fermentation of orange bagasse enriched with *S. cerevisiae*, urea, and ammonium sulfate did not show statistically significant increases in the physicochemical parameters (pH and temperature) or in the proximate chemical parameters (DM, M, Ash, CP, and TC) evaluated among treatments ($p > 0.05$). Fermentation time was the most determining factor in the numerical changes observed in the variables ($p < 0.05$). The mixture and dosage of inputs in Trt 3, in the physicochemical analyses of the studied parameters, recorded significant values, allowing the optimization and enhancement of fermented orange bagasse.

An 18.55% increase in protein was observed, higher than the 10.51% recorded in Trt 1 (control), requiring 48 h of processing. This value corresponds to a descriptive value across the different sampling times. In this regard, Trt 3 and Trt 4 exhibited the highest yeast counts. A reduction in TC, °Brix, and pH was also observed throughout fermentation, along with an increase in temperature as the process progressed.

These results provide preliminary information on the potential value of orange bagasse and support future research on its utilization.

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