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The Effect of Fermentation with *Saccharomyces cerevisiae* on the Release of Bound Phenolic Compounds from Wheat Bran and Its Effect on Antioxidant Capacity

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Abstract

Wheat bran (WB) is a rich source of phenolic compounds (PCs) with antioxidant capacity (AOX). Approximately 90% of these PCs are bound to the cell wall matrix, which limits their bioavailability. Fermentation with *Saccharomyces cerevisiae* is an effective strategy to release these bound phenolics. This study aimed to evaluate the effect of fermentation on the release of bound PCs in WB and their AOX during an in vitro digestion system. WB was fermented with *Saccharomyces cerevisiae* for 2, 4, and 6 days and subsequently subjected to simulated digestion. Free PCs were extracted with methanol, while bound PCs were obtained through alkaline hydrolysis. Total PCs were quantified using the Folin–Ciocalteu method, and AOX was assessed through DPPH, TEAC, and FRAP assays. The content of bound PCs significantly increased after fermentation ($p < 0.05$): 30.24 ± 0.06 mg GAE/g (day 2), 27.18 ± 0.40 mg GAE/g (day 4), and 28.41 ± 0.40 mg GAE/g (day 6), compared with unfermented WB (7.7 ± 0.21 mg GAE/g) ($p < 0.05$). AOX was notably enhanced; DPPH reached its peak on day 4 (47.38 ± 0.07 µmol TE/g) ($p < 0.05$). TEAC was highest on day 2 (26.20 ± 0.43 µmol TE/g) compared with the control (20.14 ± 0.22 µmol TE/g) ($p < 0.05$) and FRAP showed a slight improvement on day 6 (57.38 ± 0.10 µmol TE/g) relative to the control (56.22 ± 0.13 µmol TE/g) ($p < 0.05$). Fermentation with *Saccharomyces cerevisiae* promotes the release of bound PCs in WB and enhances its AOX, highlighting its potential as a functional food ingredient.

Keywords: antioxidant capacity; fermentation; phenolic compounds; *Saccharomyces cerevisiae*; wheat bran

1. Introduction

Cereals are among the most important sources of energy, protein, vitamins, and minerals for the global population [1,2]. Within this group, wheat (*Triticum aestivum* L.) stands out as the most cultivated and consumed grain worldwide [3]. Its nutritional value lies not only in carbohydrates, proteins, lipids, and vitamins, but also in bioactive compounds that confer health-promoting properties. Among these, phenolic compounds (PCs) are particularly relevant due to their diverse biological activities [4]. Beyond its nutritional role, wheat bran represents a major agro-industrial byproduct generated during flour milling. Globally, millions of tons are produced annually, yet a large fraction is used as low value animal feed or discarded. In recent years, the valorization of wheat bran as a source of bioactive compounds has gained attention due to its potential application in functional foods, nutraceuticals, and sustainable ingredients aligned with circular bioeconomy principles [5]. In wheat, most PCs are concentrated in the bran fraction, where approximately 75% occur bound to cell wall constituents such as cellulose, hemicellulose or arabinoxylans, lignin, and proteins [6–9]. As a result, these bound phenolics are poorly bioaccessible and bioavailable. Hydroxycinnamic acids, especially ferulic acid, are the predominant phenolics in wheat bran, with nearly 90% ester-linked to arabinoxylans and only about 10% present in free or soluble forms [10].

Phenolic compounds are associated with antioxidant, antimicrobial, anticancer, and other bioactivities, with their antioxidant role being the most recognized [11–14]. By neutralizing free radicals and reactive oxygen species, polyphenols reduce oxidative stress, which is implicated in the development of cardiovascular diseases and other chronic conditions [15,16]. Given their potential health benefits, strategies to release and improve the bioaccessibility of bound phenolics in wheat bran have been widely studied. Various approaches such as alkaline and acid hydrolysis, enzymatic hydrolysis, germination, extrusion, fermentation, and baking have been reported [7–9]. Among these, fermentation has emerged as a low-cost, sustainable, and controllable process capable of enhancing phenolic content while preserving antioxidant functionality [17,18]. *Saccharomyces cerevisiae*, a Generally Recognized as Safe (GRAS) yeast, is widely used in food fermentation and known for producing hydrolytic enzymes (xylanases, esterases, and feruloyl esterases) that facilitate the release of bound phenolics from the cell wall matrix [19].

Previous studies have demonstrated that fermentation enhances the phenolic content and antioxidant activity of cereal matrices; however, most available reports focus on specific microbial strains, short fermentation periods, or omit the integration of in vitro gastrointestinal digestion models to evaluate phenolic bioaccessibility [20–22]. Despite these advances, limited information is available on how controlled solid-state fermentation with *Saccharomyces cerevisiae* affects both the release of free and bound phenolics and their subsequent behavior during digestion. This gap still constrains the full valorization of wheat bran as a functional food ingredient.

Moreover, there is still limited information regarding how controlled solid-state fermentation with *Saccharomyces cerevisiae* impacts both the release of free and bound phenolics and their subsequent behavior during digestion. In this study, two days of fermentation were identified as the optimal treatment time, providing the highest phenolic content and antioxidant capacity. Although product formulation was beyond the present scope, the fermented wheat bran can be stabilized by drying and stored under vacuum-sealed condi-

tions for later incorporation into functional food prototypes. This finding contributes to bridging the gap between the biochemical understanding of fermentation and its potential technological applications.

Previous research has demonstrated that fermentation can modify the food matrix and enhance the release of phenolic compounds during simulated gastrointestinal digestion. For example, in vitro models have shown that fermentation increases the bioaccessibility and antioxidant activity of cereal-derived phenolics by promoting enzymatic hydrolysis and matrix disintegration [23,24]. These findings support the rationale for combining *Saccharomyces cerevisiae* fermentation with digestive simulation to assess the functional potential of wheat bran.

Antioxidant activity is one of the main indicators used to evaluate the functional potential of phenolic compounds released during food processing [25]. Their antioxidant potential is commonly assessed through complementary assays based on different reaction mechanisms. The ABTS assay measures the ability of antioxidants to neutralize radical cations via electron and hydrogen atom transfer, while DPPH mainly reflects hydrogen-donating capacity and FRAP evaluates electron-donating power through the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions [26]. Each radical shows distinct affinities toward antioxidant molecules, which explains the variability observed among assays. ABTS reacts with both hydrophilic and lipophilic antioxidants through a single electron transfer (SET) mechanism, whereas DPPH preferentially interacts with compounds containing hydroxyl groups near conjugated double bonds, favoring hydrogen atom transfer (HAT) [27]. Together, these methods provide a comprehensive view of redox behavior in complex food matrices [28]. Therefore, evaluating ABTS, DPPH, and FRAP activities in fermented wheat bran helps to identify the specific antioxidant mechanisms enhanced by *Saccharomyces cerevisiae* fermentation and clarifies how bioprocessing can improve phenolic release, bioaccessibility, and the overall functional value of wheat bran.

In this context, the present study aimed to evaluate the effect of *Saccharomyces cerevisiae* fermentation on the release of bound phenolic compounds in wheat bran and to determine its impact on antioxidant capacity during an in vitro gastrointestinal digestion system. The novelty of this research lies in demonstrating how controlled fermentation not only enhances the concentration of free and bound phenolics but also improves their bioaccessibility throughout digestion, thereby offering new insights into the design of antioxidant-enriched functional ingredients from wheat bran.

2. Materials and Methods

2.1. Preparation of the Raw Wheat Bran

Wheat bran (variety *C.V Kronstad*), donated by Molino "La Fama" in Hermosillo, Sonora, Mexico, was used as raw material. The wheat sample was subjected to cleaning following the Reyes-Pérez [29] methodology. Wheat milling was carried out in a Brabender Quadrumant Senior (Brabender Instruments, Duisburg, Alemania) obtaining wheat flour and bran. The wheat bran was subjected to a second grinding in a Laboratory Mill Model 1100 (Thomas Scientific, Swedesboro, NJ, USA), obtaining a particle size of less than 0.05 mm.

2.2. Microbial Fermentation of Wheat Bran

Fermentation was conducted following the methodology of Călinoiu et al. [30] with minor modifications, using *Saccharomyces cerevisiae*. The yeast employed in this study was a commercial baker's yeast (Tradi-Pan®, Mexico City, Mexico), corresponding to a domesticated industrial strain of *Saccharomyces cerevisiae* commonly used in food fermentation. The solid-state fermentation (SSF) was carried out in 500 mL Erlenmeyer flasks

containing 25 g of sterile, ground wheat bran (particle size < 1 mm) with a fixed moisture content of 70% (*w*/*w*). The substrate was aseptically inoculated with 4.5 mL of yeast suspension (1×10^8 CFU/mL) and 50 mL of sterile distilled water, thoroughly mixed, and incubated under static conditions at 30 °C for up to 6 days. Non-inoculated flasks (54.5 mL of sterile distilled water only) served as controls.

Although pH and microbial viability were not monitored directly, several measures were implemented to ensure that the observed effects were due to active fermentation rather than spontaneous chemical changes. Controlled inoculation and standardized SSF conditions (70% moisture, 30 °C, 6 days) promoted the typical metabolic behavior of *Saccharomyces cerevisiae*, including CO_2 release, ethanol odor development, and time-dependent increases in phenolic and antioxidant contents. These changes are consistent with previously reported yeast-driven SSF in cereal substrates [23,24,30].

For analytical determinations, aliquots of approximately 17 g of culture were withdrawn from the Erlenmeyer flasks every 48 h. The aliquots were inactivated in a convection oven at 60 °C for 24 h and then stored in a desiccator under dark at room-temperature conditions. Dried samples were ground using an electric coffee grinder (Hamilton Beach, 80350R, Glen Allen, VA, USA). Approximately 3.0 g of each ground sample was defatted three times with hexane (1:5 *w*/*v*, 5 min per extraction) and air-dried for 48 h under an extraction hood at room temperature.

For the activation, 0.1 g of yeast was weighed and inoculated into 10 mL of potato dextrose broth, previously sterilized (15 min at 121 °C and 15 lb pressure), and then was incubated for 12 h at 30 °C. A cell suspension of 1×10^8 CFU/mL 0.5 McFarland standard solution was prepared. The solid-state fermentation culture was carried out in 500 mL Erlenmeyer flasks containing 25.0 g of the sterile grinded wheat bran, with a fixed humidity of 70% (*w*/*w*). The wheat bran samples were aseptically inoculated with 4.5 mL of yeast suspension (1×10^8 CFU/mL) and 50 mL of sterile distilled water, were mixed, and then incubated up to 6 days at 30 °C under static conditions. Samples of the same sterile grinded wheat bran that were treated with this procedure but inoculated with 54.5 mL of only sterile distilled water were used as the control. For the different analyses, aliquots of approximately 17.0 g of culture were withdrawn from the Erlenmeyer flasks at 48 h intervals. The aliquots were inactivated by heat in a convection oven at 60 °C for 24 h, and later were placed in a desiccator under dark conditions at room temperature. The dried samples were ground using an electric coffee grinder (Hamilton Beach, 80350R, Glen Allen, VA, USA). Approximately 3.0 g of each ground sample were transferred to test tubes and defatted three times with hexane (1:5 *w*/*v*) for 5 min per extraction. The samples were then air-dried for 48 h under an extraction hood at room temperature.

The extraction of phenolic compounds was carried out under standardized conditions following procedures previously validated for cereal-based matrices. Although no internal standard was used, the method has demonstrated reproducible recoveries (90–95%) in similar wheat bran systems. All extractions were performed in triplicate using identical solvent ratios, agitation speeds, and extraction times to ensure consistency and analytical reliability.

2.3. Free Phenolic Compound Extraction

Free phenolic compounds were extracted according to the method described by Chiremba et al. [31]. For this purpose, 1.0 g of sample (control or fermented wheat bran) was mixed with 15 mL of 80% methanol, sonicated (Branson 1800 Digital Bath, Emerson, St. Louis, MO, USA) for 1 h, and centrifuged (OHAUS-FC5718R 120V, Frontier™ Series 5000 Multi Pro, Parsippany, NJ, USA) for 15 min at 100 rpm. The supernatant was collected in a glass container, and the extraction was repeated three times. The combined supernatants were concentrated under vacuum in a rotary evaporator at 50 °C (DLAB

RE 100-Pro, Beijing, China), and the phenolic compounds were re-dissolved in 5 mL of HPLC-grade methanol. The resulting extract was stored in amber vials at −20 °C until analysis. The precipitate obtained during this procedure was reserved for the subsequent extraction of bound phenolic compounds.

2.4. Bound Phenolic Compound Extraction

Bound phenolic compounds were extracted following the method described by Guo and Beta [32]. The precipitate obtained from the previous extraction was dried at 45 °C for 24 h. Subsequently, 100 mg of the dried sample (control and fermented) were mixed with 5 mL of 2 M NaOH for alkaline hydrolysis and sonicated (Branson 1800 Digital Bath, Emerson, St. Louis, MO, USA) for 3 h. The mixture was then acidified with 6 M HCl to a pH of approximately 1.5–2.0. A liquid–liquid extraction was performed with ethyl acetate at twice the volume of the acidified preparation, followed by centrifugation (OHAUS-FC5718R 120V, Frontier™ Series 5000 Multi Pro, Parsippany, NJ, USA) for 15 min at 100 rpm. The supernatant was collected and concentrated under vacuum using a rotary evaporator at 40 °C (DLAB RE 100-Pro, Beijing, China). The residue was re-dissolved in 5 mL of HPLC-grade methanol, and the resulting extract was stored in amber vials at −20 °C until analysis.

2.5. Determination of Total Phenolic Compounds

Total phenolic content was determined using the Folin–Ciocalteu spectrophotometric method described by Singleton and Rossi [33]. This method is based on the reaction of phenolic groups with oxidizing agents. The Folin–Ciocalteu reagent, which contains sodium molybdate and sodium tungstate, reacts with phenolic compounds to form a phosphomolybdic–phosphotungstic complex. In an alkaline medium, this complex undergoes electronic reduction to tungsten oxide (W_8O_3) and molybdenum oxide (Mo_8O_{23}), producing an intense blue color proportional to the number of hydroxyl groups in the phenolic compounds. For analysis, 10 µL of each extract (free and bound phenolics from control and fermented samples) were mixed with 25 µL of 1 M Folin–Ciocalteu reagent and incubated for 5 min at room temperature. Subsequently, 25 µL of 20% Na_2CO_3 and 140 µL of distilled water were added, and the absorbance was measured at 760 nm using a microplate reader (Thermo Fisher Scientific Inc. Multiskan GO, New York, NY, USA). Results were expressed as mg gallic acid equivalents per gram of sample (mg GAE/g) based on a calibration curve prepared with gallic acid standard solutions ranging from 0.01 to 1.0 mg/mL. This phenolic acid is commonly used as the standard polyphenol because it is a representative natural phenolic found in many plants [34]. All measurements were performed in triplicate.

2.6. Trolox Equivalent Antioxidant Capacity (TEAC) Quantification of Phenolic Compounds

The antioxidant capacity was measured using three methods based on the comparation with a Trolox standard. Trolox, a synthetic water soluble analogue of α-tocopherol, known as the most active form of vitamin E, serves as the reference compound for expressing results as Trolox equivalent antioxidant capacity (TEAC), which allows the reliable comparison of antiradical activity among samples [35]. These methods are the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) decolorization, the DPPH (1,1-difenil-2-picrihidrazil), and the ferric-reducing ability of plasma (FRAP) assays [36–38].

2.6.1. ABTS Decolorization Assay

Antioxidant activity was determined using the $ABTS^+$ radical cation decolorization assay described by Re et al. [36]. This method is based on the quantification of $ABTS^+$ discoloration resulting from its reduction to ABTS by antioxidant compounds. The $ABTS^+$