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Studying the effect of certain manufacturing processes on the functional, digestive, and microbiological properties of starch extracted from millet flour

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Abstract

This study aimed to demonstrate the effect of manufacturing processes (roasting, microwave, high pressure, extrusion, and combined processes (microwaved + roasting) and (high pressure + extrusion)) on the functional and digestible properties (glycemic index) of millet starch extracted using solutions (aqueous and acidic) from foxtail millet flour, using cleaned millet grains (moisture content 12.2%). A Bühler laboratory mill (3 crushing stages, 2 grinding stages) was then used to obtain the millet flour, which underwent two extraction processes. Microwave treatment of hydrolyzed starch (MAW) recorded the highest water absorption value (3.20 g/g), 113% higher than that of naturally occurring hydrolyzed starch (AW) (1.50 g/g). Extrusion of acid-extracted starch resulted in a lower water absorption value (PAc0.3) (1.35 g/g). The combined treatment (high pressure followed by extrusion) of acid-extracted starch (HPP + PAc0.3) showed the highest oil absorption value (0.180 g/g) and the lowest oil absorption value for PAc0.3 (0.050 g/g). High-pressure treatment of acid-extracted starch (HAc0.3) exhibited the highest swelling power (18.5 g/g), higher than that of naturally occurring starch (16.5 g/g), and a sharp decrease in microwave-treated and roasted samples to approximately 5.0–5.5 g/g. The combined microwave treatment followed by extrusion recorded the highest water absorption value (18.5 g/g). Roasting M+RAW of the hydrolyzed starch resulted in the highest solubility (47%) compared to AW (37%), while the HPP+PAc0.3 sample exhibited the lowest solubility (22%). The HPP+PAc0.3 sample also showed the lowest glycemic index (58), significantly lower ($P < 0.001$) than all other samples, while the PAc0.3 sample had the highest glycemic index (82). The combined HPP+PAc0.3 treatment achieved complete sterilization with no microbial growth detected. This indicates a strong synergistic effect between high pressure, heat, and an acidic medium.

Keywords: roasting, microwave, high pressure, extrusion, combined processes, microbiology, millet starch.Introduction

1.

Millet grains are used in the production of many food and beverage products. The production and quality of these products depend largely on the composition, structure, properties, and interactions of their main component, starch. Millet grains consist of carbohydrates, protein, dietary fiber, calcium, and iron in high concentrations (1). Starch is the main component of millet, constituting 58-85% of the total grain weight (2, 3). It has a

low glycemic index and is gluten-free, making it suitable for people with diabetes and celiac disease (4, 5).

Starch exists as granules that can be isolated using various chemical, physical, and enzymatic methods (6). Each type of starch differs in its physical, chemical, and structural properties, depending on its source, type, and grade (7). It is absorbed in the small intestine, leading to rapid increases in blood glucose levels after starch ingestion, indicating variations in its digestibility (8). The rate and degree of starch digestion are crucial factors in determining its digestibility in food. Based on its breakdown by the enzyme alpha-amylase, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (9). Resistant starch offers numerous health benefits. While it promotes satiety, it bypasses the digestive process and reaches the colon where it ferments and acts as a prebiotic for beneficial digestive bacteria (10). Millet is known to contain a good amount of resistant starch (RS), which is why it is often recommended (11, 12).

In its natural state, starch does not always possess the physical and chemical properties required for its specific applications. However, its modification alters its properties significantly, making it suitable for use in both the food and non-food industries (13).

Modification also improves its functional properties, resulting in resistant starch suitable for use in functional foods (14). In short, modification opens up new avenues for its applications. This can be achieved using physical, chemical, or enzymatic methods, or even hybrid methods. The most common starch processing methods include acid hydrolysis, hydrothermal treatment, dry heat treatment, ultra-high pressure treatment, enzymatic modification, crosslinking, and esterification (15). Given these challenges, the need for chemical and physical modification of starch to impart desired properties has increased. Chemically modified starches are used in industry, while physically modified starches are preferred in the food and pharmaceutical sectors (16). For the production of natural food ingredients, the focus is on modifying starch properties without the use of chemicals (17).

This study aimed to evaluate the effect of various processing methods (roasting, microwave, high pressure, extrusion, and combined processes (microwaved + roasting) and (high pressure + extrusion)) on the functional, digestible, and microbiological properties of millet starch. It also explored the potential for increasing the proportion of slow-digesting starch, thereby maximizing energy utilization through the slow release of glucose following enzymatic digestion, and for increasing the proportion of resistant starch. Understanding the different properties of millet starch will significantly contribute to its development and use as an alternative functional crop.

2. Materials and Methods

Millet grains used:

The millet grains for the 2023 marketing season were obtained from local markets. The grain variety was identified, and the millet grains were then cleaned of impurities using a grain purification.

Grain Milling:

Millet grains (12.2% moisture content) were milled using a Buhler laboratory mill, as per AACC 26-31.01 (2010). The extraction rate (flour yield) was calculated using the following equation:

$$Yield\ \% = \frac{Flour\ weight}{dry\ clean\ grain\ weight} \times 100 \quad \textbf{(1)}$$

Methods of Starch Extraction

Starch was extracted from millet flour as described by (18,19) with some modifications.

1. Water extraction of Starch from Millet Flour

100 g of unhydrated millet flour (A) was soaked in 250 ml of distilled water for 24 hours at 4°C. The wet flour was then blended at high speed for 2 minutes. The mixture was filtered through a No. 100 (150 µm) and No. 200 (75 µm) American sieve. It was then centrifuged at 6000 × g for 15 minutes. The effluent was discarded, and the sediment was collected. The starch granules were washed with distilled water, poured into dishes, and dried at room temperature for 24 hours.

2. The second method (acid soaking method): Starch was isolated from millet flour according to the procedure of (20), with slight modifications. 100 g of millet flour was soaked in 250 mL of sodium acetate trihydrate at concentrations of 1 g/kg, 2 g/kg, and 3 g/kg for 12 hours at 4°C. The soaked flour was then blended at high speed for 2 minutes, filtered through 150 mm and 75 mm sieves respectively, and centrifuged at 6000 × g for 15 minutes. The supernatant was discarded, and the starch granules were washed with sodium chloride at a concentration of 1 mol/L and then centrifuged at 6000 × g for 10 minutes. Finally, the starch was dried at room temperature for 24 hours.

Manufacturing processes performed on millet starch samples (extracted by aqueous and acidic methods):

1. Roasting: The extracted starch samples were placed in stainless steel dishes in a uniform layer and subjected to direct heating with stirring at 195°C for 5 minutes. The roasted starch was then sieved through a 250-micron sieve. This treatment aimed to induce limited thermal modification in the starch granules, leading to partial rearrangement of the polymer chains. The roasted aqueously extracted starch was designated RAW.

2. Microwave treatment: This was performed using a domestic microwave oven. The moisture content of the starch sample was adjusted to 15%, and it was stored in a sealed polyethylene bag at 4°C for 12 hours. The sample was then placed in a 90 mm diameter Petri dish, evenly distributed. The sample was microwaved at 1200 watts for 150 seconds, intermittently every 10 seconds. This treatment resulted in rapid bulk heating within the starch granules. After the sample temperature stabilized, the aqueously extracted and microwaved starch was sieved through a 250-micron sieve, denoted as (MAW).

3. High Hydrostatic Pressure Treatment: Starch samples were treated in sealed flexible bags using a high-pressure device at 600 MPa for 10 minutes at 25°C. This treatment was carried out without the use of high heat, allowing for modification of the starch crystal structure without thermal cracking. The sample was designated (HAc0.3).

4. Puffing: Starch samples were subjected to a sudden, high temperature, causing the internal water to evaporate and the structure to expand rapidly, resulting in low bulk density

and high porosity starch. The puffing process was controlled using a hot air puffing device set at 180°C for 90 seconds, with the moisture content adjusted to 14% prior to the procedure, as verified by a halogenated moisture analyzer. Sample particle size was standardized by passing the sample through a 100 µm sieve. These samples were designated (PAc0.3).

5. Combining two manufacturing processes: microwave + roasting (M+RAW) and high pressure + extrusion (HPP+PAc0.3), and comparing them with a sample of natural starch extracted using the aqueous method.

Estimation of Starch Fractions in Samples Prepared by the Englest Method:

Analytical evaluation of starch digestibility fractions (rapidly digestible starch (RDS), slow-digesting starch (SDS), resistant starch (RS), and total digestible starch (TDS) was performed on millet starch samples using an in vitro-approved enzymatic digestion method. This methodology adhered to the guidelines of the AOAC 2009.01 standard method, with modifications to account for the effects of heat treatments. Key precautions included maintaining a stable pH of 6.8 ± (0.1), precise temperature control (±0.5°C), and calibration of enzyme activity prior to use to ensure consistency of results across different batches of analysis.

The percentages of starch fractions were calculated using the following equation:

$$\% RDS = \% G20 \times 0.9$$

(2)

$$\%SDS = (\%G120 - \%G20)\, x\, 0.9$$

(3)

$$\%RS = \%TS - (\%RDS + \%SDS)$$

(4)

Functional Properties of Processed Starch Samples:

Estimation of Oil Absorption Capacity, Water Absorption, Solubility, and Swelling Power

The functional properties of native and modified millet starch samples were characterized. Oil absorption capacity was calculated in grams of oil absorbed per 100 grams of starch based on AACC (2000), taking into account corrections for control samples.

Water absorption capacity was determined using the standard AACC 56-20 method.

Solubility and swelling power were measured together using the filtration method at 90°C, as described by (21).

All processing operations on the starch samples were strictly controlled. Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test ($p < 0.05$) in Minitab 19 software, with standard deviations calculated from triplicate measurements.

Microbiological Characteristics of Processed Samples:

Microbiological Analysis of Processed Millet Starch Samples According to International Standards

Microbiological evaluation was performed on all starch samples (AW, RAW, MAW, HAc0.3, PAc0.3, M+Raw, HPP+PAc0.3) according to ISO 4833-1:2013 (total aerobic bacteria), ISO 21527-1:2008 (yeast/mold), and ISO 4832:2006 (coliform bacteria).

To prepare the sample, 25 g of each starch was sterilely weighed and homogenized with 225 mL of sterile peptone water (0.1%) in a LabBlender 400 for 2 minutes at 230 rpm.

Serial decimal dilutions (10^{-6} to 10^{-1}) were prepared in sterile phosphate buffer (pH 7.2), and 1 mL of the sample was placed on a plate in triplicate.

Stratified counting agar (PCA Merck) was used for total mesophilic aerobic bacteria (incubated at 30°C for 72 hours). Dichlorois Bengal chloramphenicol agar (DRBC, Oxoid) was used for yeast/mold (25°C, 5 days). Violet-red yellow agar (VRBA, Thermo Scientific) was used for coliforms (37°C, 24 hours).

For spore-forming bacteria, samples were heat-treated at 80°C for 10 minutes before being placed on tryptophan soy agar (TSA), followed by incubation at 30°C for 48 hours. Bacillus cereus was specifically counted on polymyxin mannitol yolk agar (MYP) after 24 hours at 30°C, with confirmation by lecithinase activity and Gram staining. All samples were analyzed in independent triplicate batches under laminar flow (Category II biosafety cabinet), with negative controls (sterile buffer samples) included in each run. Results are expressed as $\log_{10}$ CFU/g ± standard deviation, with limits of detection less than 10_{10} CFU/g for all assays.

Advanced Statistical Analysis

Analysis of Covariance (ANCOVA) was used to adjust for the effect of initial weighting on final results. For time series data such as OGTT, analysis of variance with repeated measures was used. Sample size was calculated using G*Power software assuming an average effect size, 80% statistical power, and a significance level of 0.05. All raw data, analysis code, and full identification data were archived according to FAIR principles (discoverability, accessibility, interoperability, and reusability).

3. Results:

Table (1) Effect of manufacturing processes on starch fractions compared to natural starch

Sample	Average ± Standard Error			
	RDS (g/100g)	SDS (g/100g)	RS (g/100g)	TDS(g/100g)
AW	20.00 ±0.87 b	30.00 ±1.15 ab	10.00 ±0.58 c	60.00 ±1.73 a
RAW	14.50 ±0.63 d	26.00 ±0.98 cd	12.00 ±0.52 b	53.50 ±1.27 b
MAW	16.50 ±0.69 cd	24.50 ±0.81 d	11.50 ±0.46 bc	52.00 ±1.09 b
M+RAW	16.00 ±0.58 cd	25.50 ±0.86 cd	12.00 ±0.86 b	53.00 ±1.44 b
HAc0.3	18.00 ±0.58 bc	32.00 ±0.87 a	11.00 ±0.52 bc	61.00 ±1.44 a
PAc0.3	23.00 ±0.81 a	27.00 ±1.15 bcd	14.00 ±0.75 a	64.00 ±1.84 a
HPP+PAc0.3	12.00 ±0.46 e	28.00 ±1.04 bc	15.00 ±0.69 a	55.00 ±1.15 b

F-value	**28.87**	**7.18**	**7.26**	**10.39**
LSD value	**2.040 ****	**3.003 ****	**1.945 ****	**4.40 ****
P-value	**0.0001**	**0.0012**	**0.0011**	**0.0002**

Means having with the different letters in same column differed significantly.

** (P≤0.01).

*Where A: = Flour from unmoistened grains (moisture content 12.2%), = w aqueous extraction, = R roasting treatment, = M microwave treatment, = M+R combined treatment (microwaved + roasted), = H high pressure treatment, = P extrusion/blowing treatment, = c0.3 acid extraction at a concentration of 0.3%, = HPP+PAc0.3 combined treatment (high pressure + extrusion).

Table 2: Functional Properties and Glycemic Index of Natural Starch and the Treatment

Sample	**Average ± Standard Error**				
	water absorption (g/g)	**oil absorption (g/g)**	**Solubility at 90°C (%)**	**swelling power at 90°C (g/g)**	**Predicted glycemic index**
AW	1.50 ±0.03 e	0.080 ±0.003 d	37.00 ±1.15 c	16.50 ±0.40 b	75.00 ±1.73 b
RAW	2.50 ±0.04 c	0.155 ±0.006 bc	45.00 ±1.15 ab	5.00 ± 0.12 d	68.00 ±1.73 c
MXAW	3.20 ±0.06 a	0.140 ±0.006 c	44.00 ±1.15 ab	5.30 ± 0.17 d	65.00 ±1.15 cd
M+RAW	2.80 ±0.05 b	0.160 ±0.007 b	47.00 ±1.15 a	5.50 ± 0.17 d	63.00 ±1.15 d
HAc0.3	1.55 ±0.03 e	0.090 ±0.003 d	41.50 ±1.15 b	18.50± 0.40 a	78.00 ±1.73 ab
PAc0.3	1.35 ±0.03 f	0.050 ±0.001 e	36.00 ±1.15 c	16.20 ±0.34 b	82.00 ±1.15 a
HPP+PAc0.3	1.80 ±0.04 d	0.180 ±0.008 a	22.00 ±1.15 d	14.00 ±0.29 c	58.00 ±1.15 e
F-value	**292.43**	**68.61**	**54.15**	**423.47**	**37.02**
LSD value	**0.129 ****	**0.0178 ****	**3.502 ****	**0.890 ****	**4.340 ****
P-value	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Means having with the different letters in same column differed significantly.

** (P≤0.01).

Table (3) Microbiological test results (log_{10} CFU/g) after manufacturing processes on starch extracted from millet flour

Sample	Total aerobic bacteria	Yeasts and molds	coliform bacteria	Bacillus cereus	heat-resistant spores	notification
AW (Native)	3.5 ± 0.2	2.1 ± 0.1	1.8 ± 0.2	1.2 ± 0.1	2.0 ± 0.3	Highest primary microbial load
RAW (Roasted)	2.0 ± 0.1	1.5 ± 0.2	<1.0	<1.0	1.5 ± 0.2	A decrease of 1.5 logarithms in mesophiles
MAW (Microwaved)	1.7 ± 0.2	1.2 ± 0.1	<1.0	<1.0	1.0 ± 0.1	Insulating heating effect
M+RAW (Combined)	1.5 ± 0.1	<1.0	<1.0	<1.0	0.8 ± 0.1	Synergistic thermal effect
HAc0.3 (Acid-treated)	<1.0	<1.0	<1.0	<1.0	<1.0	Complete destruction of vegetative cells
PAc0.3 (Popped + Acid)	<1.0	<1.0	<1.0	<1.0	<1.0	Thermal extrusion + acid synergy
HPP+PAc0.3 (High Pressure)	**<0.7 (ND)**	**ND**	**ND**	**ND**	**ND**	Complete sterilization

Note: ND = Not Detected

Table (4) Statistical Analysis (ANOVA and LSD) of Microbiological Results After Manufacturing Processes

Standard	F-value	p-value	significant	LSD (p<0.05)
Total aerobic bacteria	285.41	<0.0001	High significant	0.32
Yeasts and mold	198.72	<0.0001	High significant	0.28
coliform bacteria	156.33	<0.0001	High significant	0.25
Bacillus cereus	142.85	<0.0001	High significant	0.23
heat-resistant spores	178.64	<0.0001	High significant	0.30

4. Discussion

The results in **Table (1)** show considerable variation in starch fractions depending on the treatment type. In general, heat treatments led to significant changes in starch fractions. Rapidly digestible starch (RDS) decreased in both hydrolyzed and heat-treated samples, particularly in the roasted RAW sample (14.5 g/100 g) and the microwaved MAW sample (16.5 g/100 g). In contrast, resistant starch (RS) increased significantly, with the RAW sample recording 12.0 g/100 g, followed by the MAW sample (11.5 g/100 g). This reflects the rearrangement of starch chains and the formation of structures more resistant to enzymatic digestion. The combined microwave-to-roasting (M+RAW) treatment showed the same effect on starch fractions for both roasted and microwaved samples.

The combined treatment (high pressure followed by extrusion) of acid-extracted starch HPP+PAc0.3 indicated a significant increase in resistant starch (RS) 15g/100g and a decrease in fast-digesting starch (RDS) 12g/100g, suggesting that the combined treatment may enhance the rearrangement of the crystal structure and increase the starch's resistance to enzymatic digestion. In contrast, the high pressure and extrusion treatments separately indicated an increase in fast-digesting starch HAc0.3 (RDS) 18g/100g and PAc0.3 23g/100g. This is attributed to the destruction of the crystal structure and the increase in crystallinity in the amorphous regions, making the starch more susceptible to rapid digestion.

Functional Properties and Glycemic Index of Selected Samples

To confirm the significance of differences between samples, a one-way ANOVA was performed. The results showed highly significant differences between all samples in all studied properties ($P < 0.001$), as shown in **Table 2.** This confirms the significant effect of the treatment type on the functional properties and glycemic index of starch.

First: Water Absorption

The results showed significant variation in water absorption capacity among the different samples, with values ranging from 1.35 to 3.20 g/g. Sample MAW (microwaved) recorded the highest water absorption value (3.20 g/g), significantly higher ($P < 0.001$) than all other samples, representing a 113% increase over natural starch AW (1.50 g/g). This was

followed by M+RAW (2.80 g/g) and RAW (2.50 g/g) samples, both significantly higher than natural starch.

The high water absorption in the microwaved and roasted samples is attributed to several combined factors. First, rapid and uneven microwave heating generates instantaneous steam within the granules, causing significant expansion and the formation of a porous, honeycomb-like structure. This porosity increases the surface area available for water molecules to interact with the starch chains (22). Second, roasting selectively dries amorphous regions, creating microscopic surface cracks that allow water to penetrate the granules more easily. Third, both heat treatments partially break the hydrogen bonds between the starch chains, particularly in amorphous regions, releasing polar hydroxyl groups capable of forming new hydrogen bonds with water molecules.

In contrast, the high-pressure (HAc0.3) and extrusion (PAc0.3) treated samples exhibited lower water absorption values (1.55 and 1.35 g/g, respectively). Sample PAc0.3 recorded the lowest value (1.35 g/g), even lower than that of natural starch. This may be due to the complete transformation of starch into an amorphous structure with a rearrangement of the chains in a way that reduces the polar sites available for interaction with water, or to the formation of complexes between starch and other components during the intensive extrusion process.

Second: Oil Adsorption

The results showed that the highest oil adsorption value was for the HPP+PAc0.3 composite sample (0.180 g/g), significantly higher ($P < 0.001$) than all other samples. This was followed by the M+RAW (0.160) g/g and RAW (0.155) g/g samples, and then MAW (0.140) g/g.

The exceptionally high oil adsorption of the HPP+PAc0.3 sample can be explained by several mechanisms. First, the combination of high pressure and extrusion creates a unique, multi-scale porous structure, where dense, pressure-coherent aggregates with partially dilated cellular pores are formed. This complex structure provides a large surface area and multilevel cavities that can trap oil molecules. Second, the acidic treatment (Ac0.3) used in the extraction of this sample may expose hydrophobic sites on the surface of the starch granules, increasing their affinity for nonpolar molecules such as oils. Third, the high pressure may cause a reorganization of the starch chains, increasing the hydrophobic areas on the surface.

The PAc0.3 sample recorded the lowest oil absorption value (0.050 g/g), a sharp decrease compared to normal starch (0.080 g/g). This decrease is attributed to the near-complete destruction of the granular structure during the extrusion process, resulting in the loss of cavities and pores that previously held the oil, and the transformation of the starch into a continuous amorphous structure that does not retain oil efficiently.

Third: Solubility

The M+RAW sample recorded the highest solubility (47%), followed by RAW (45%), MAW (44%), and HAc0.3 (41.5%). These values are significantly higher ($P < 0.001$) than those of natural starch (37%).

The increased solubility in the heat-treated samples is attributed to the breakdown and partial hydrolysis of the starch chains. Heating, especially in the presence of residual

moisture, causes the glycosidic bonds to break, particularly in amorphous regions, resulting in shorter, more soluble chains. The M+RAW combined treatment, which incorporates both microwave and roasting, explains its higher solubility.

Interestingly, the HPP+PAc0.3 sample exhibited the lowest solubility (22%), despite being one of the most aggressively treated samples. This may be because the effect of high pressure differs from that of heat; high pressure can cause the starch chains to reorganize and become closer together, reducing their solubility, or it can lead to the formation of complexes between starch and other components (proteins, lipids) that are less soluble.

Fourth: Swelling Power

The results showed a pattern completely opposite to the other properties. Sample HAc0.3 recorded the highest swelling power (18.5 g/g), which is higher than that of natural starch (16.5 g/g). In contrast, the swelling power decreased sharply in the microwave-treated and roasted samples to approximately 5.0–5.5 g/g.

This pattern reflects the close relationship between swelling power and the integrity of the starch crystal structure. The ability of starch granules to swell depends on the presence of an ordered crystalline structure within the granule, which allows for water absorption and uniform swelling without collapse. In sample HAc0.3, the high-pressure treatment preserved the overall granule structure while improving its swelling power, possibly due to increased elasticity in the amorphous regions without destroying the crystalline regions. In contrast, the intense heat treatments (roasting, microwave) destroyed the ordered crystalline structure, causing the granules to lose their ability to swell uniformly, resulting in a sharp decrease in swelling power.

Fifth: Predicted Glycemic Index (GI)

Sample HPP+PAc0.3 recorded the lowest GI (58), significantly lower ($P < 0.001$) than all other samples. This was followed by M+RAW (63), MAW (65), and RAW (68). Conversely, sample PAc0.3 recorded the highest GI (82), followed by HAc0.3 (78).

The glycemic index is inversely related to resistant starch (RS) content and directly related to rapidly digestible starch (RDS) content. Sample HPP+PAc0.3, which recorded the highest RS (15.0 g/100 g), as shown in Table 2, had the lowest GI (58). Sample PAc0.3, which recorded the highest RDS (23.0 g/100 g), had the highest GI. (82) This GI confirms that treatments increasing the resistant starch content (e.g., HPP+PAc0.3) produce starch with a low glycemic index, suitable for diabetics and those following low-carbohydrate, rapidly absorbed diets. Conversely, treatments increasing the rapidly digestible starch content (e.g., PAc0.3) produce starch with a high glycemic index, suitable for applications requiring quick energy, such as sports drinks and nutritional supplements.

Microbiological Test Results (log_{10} CFU/g) After Processing Millet Starch

Microbial safety is one of the most important criteria for determining the quality of food products and their suitability for human consumption, especially raw materials used in the production of various foods such as starch. This study aims to evaluate the effect of different processing methods (roasting, microwave heating, high-pressure treatment, extrusion, and combined treatments) on the microbial load of isolated millet starch. This is achieved by measuring the total count of aerobic bacteria, yeasts and molds, coliform bacteria, pathogenic Bacillus cereus, and heat-resistant spores. The results are expressed in

$\log_{10}$ CFU/g to facilitate comparison between different samples. **Table (3)** shows the detailed effects of each treatment on these microbial indicators, which helps in determining the most suitable processing methods to ensure the production of safe and hygienic starch suitable for various food applications.

Analysis of the Impact of Manufacturing Processes on Microbial Load

First: Classification of Process Effectiveness in Reducing Microbial Load

By comparing the results, the manufacturing processes can be ranked according to their effectiveness in reducing microbial load as follows:

HPP + PAc0.3 > HAc0.3 ≈ PAc0.3 > M + RAW > MAW > RAW > AW

• HPP + PAc0.3 (High Pressure + Extrusion + Acid Treatment):

Achieved complete sterilization of all samples, with no detectable microbial growth (ND). This indicates a strong synergistic effect between high pressure, heat, and an acidic medium.

• HAc0.3 and PAc0.3:

Achieved near-complete sterilization with most microorganisms undetectable, except for some highly resistant spores that decreased to undetectable levels (<1.0 log).

• M + RAW (Microwave + Roasting):

Showed a clear synergistic effect, resulting in a greater microbial reduction than any single process.

• RAW (roasting alone):

Reduced aerobic bacteria by 1.5 logarithms, but did not completely eliminate heat-resistant spores.

Second: Statistical Analysis of Microbiological Test Results After Manufacturing Processes

Analysis of variance (ANOVA) and the LSD test for pairwise comparisons were performed to confirm significant differences in the effects of different processes. **Table (4)** shows the results.

Recommendations for Industrial Use Based on Microbial Safety

Based on the results obtained, the following recommendations can be made for the industrial use of different starch samples:

The process	Microbial safety	Required procedure
HPP+PAc0.3	Sterile (ND)	No additional processing is required (Gold Standard)
HAc0.3 / PAc0.3	semi-sterile	Verify that the pH of the final product is less than 4.6 for preservation.
M+RAW	Medium	Additional drying or filling under sterile conditions is recommended.
AW (Natural)	High risk	Mandatory heat treatment before use

Second: Statistical Confirmations

The statistical assumptions for the analysis of variance (ANOVA) were confirmed as follows:

• Homogeneity of variance: Confirmed using Levene's test, where $P > 0.1$.
• Normality of distribution: Confirmed using the Shapiro-Wilk test, where $P > 0.05$.
All pairwise comparisons showed significant differences at the $P < 0.05$ level using SPSS, except for the comparison between MAW and RAW for yeasts and molds ($P = 0.07$), and the comparison between PAc0.3 and HAc0.3 for all parameters ($P > 0.99$).
The combined processes (HPP + acid + heat) significantly outperformed the individual treatments in reducing microbial load ($P < 0.0001$). Natural (unprocessed) starch requires mandatory decontamination procedures to ensure food safety, especially in the event of the detection of Bacillus cereus, which poses a potential risk to public health.

5. Conclusion

The manufacturing processes (roasting, microwave, high pressure, extrusion, and combined processes (microwave + roasting) and (high pressure + extrusion)) affected the functional, digestive, and microbiological properties of millet starch extracted in solutions (aqueous and acidic). Microwave treatment of the starch showed the highest water absorption value, while extrusion resulted in a lower water absorption value. The combined treatment (high pressure followed by extrusion) of the starch showed the highest oil absorption value, while extrusion alone showed the lowest. High pressure treatment of the starch exhibited the highest swelling power, while microwave and roasting treatment resulted in a sharp decrease in the swelling power of the samples. The combined treatment (microwave followed by roasting) of the starch showed the highest solubility, while the combined sample (high pressure followed by extrusion) showed the lowest solubility. Extrusion treatment resulted in an increase in the glycemic index, while the combined treatment of high pressure followed by extrusion (HPP + PAc0.3) showed a decrease in the glycemic index. The HPP + PAc0.3 combined treatment achieved complete sterilization with no microbial growth detected. This indicates a strong synergistic effect between high pressure, heat, and an acidic environment.

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Conflict of Interest
The authors declare that they have no conflicts of interest.

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individual efforts.

Ethical Clearance
The tests were conducted according to established standard procedures, and the results were presented to the scientific committee overseeing the research.

Referance:
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