

Comparative analysis of the effects of carob and wheat flours on nutritional, health, and pre-bake rheological properties of spontaneously fermented sourdough

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Article history

Received:

21 August 2024

Received in revised form:

30 September 2025

Accepted:

2 October 2025

Keywords

carob,
 fermentation,
 health,
 nutrition,
 sourdough,
 wheat

Abstract

Natural non-wheat alternatives can improve the nutritional and health properties of baked foods during dough fermentation. However, the effects of carob on these parameters are unclear. Therefore, the present work aimed to comparatively examine the effects of carob and wheat flours on the nutritional, health, and pre-bake rheological properties of spontaneously fermented sourdough. Five sourdough compositions (SC1 with 100% carob flour; SC2 with 75% carob flour; SC3 with 50% carob flour; SC4 with 25% carob flour; and SC5 with 100% wheat flour) were prepared and fermented for 5 d at 30°C. The pH, temperature, titratable acidity (TTA), phytic acid content (PAC), levels of vitamins B₁, B₂, B₃, and B₆, phenolic (TPC) and flavonoid (TFC) contents, antioxidant capacity (AC), and identification of lactic acid bacteria (16S rRNA sequencing) were evaluated. Additionally, the effect of fermented dough on the viability of human L929 fibroblast and HCT-116 colon cancer cell lines was examined using *in vitro* cytotoxicity (MTS) assay. Besides, the pre-baking farinograph and extensograph indices of flours were evaluated. Compared with wheat and wheat-carob blends, SC1 with 100% carob flour exhibited a pH of 4.2 at 21.8°C, lower TTA (2.1-fold) and PAC (3.4-fold), and higher B-group vitamin (1.9-fold), TPC and TFC (4-fold), and AC (1.2-fold) levels. *Lactiplantibacillus plantarum* was the predominant species in sourdoughs. The cytotoxicity assay demonstrated that sourdoughs exerted cytotoxic effects on HCT-116 cells ($p < 0.05$) but not on L929 cells. The addition of carob flour (5, 10, and 15%) increased the water adsorption, dough development, stability time, resistance to extension, and ratio number, but decreased energy and extensibility. Thus, carob flour can be used as a non-wheat ingredient in spontaneously fermented sourdough for preparing baked goods due to its high nutritional and health values. However, further studies are needed to understand the biochemical function of sourdough prepared with carob flour, and to validate the final product.

DOI

<https://doi.org/10.47836/ifrj.32.4.14>

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Introduction

Sourdough preparation is a fermentation process that provides products with health, nutritional, and sensorial characteristics. This process increases free amino acid and bioactive contents, improves rheological properties, and decreases pH (Fekri *et al.*, 2024). Additionally, this process decreases the levels of phytic acid or phosphorus compounds, and myoinositol and hexaphosphoric

acid present in cereal grains and legumes (Yildirim and Arici, 2019). Sourdough can be produced traditionally or industrially (Eraslan *et al.*, 2023) by spontaneous fermentation (Type-1; yeasts and lactic acid bacteria (LAB) present in the flour) or starter-mediated fermentation (Type-2; a starter culture inoculated) mixture (Sánchez-Adriá *et al.*, 2023).

The type of raw material used in sourdough preparation determines the nutritional, sensorial, and textural properties of baked products (Cardinali *et al.*,

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2022). In the bakery industry, sourdough is used as a leavening agent to reduce the need for additives (Fernández-Peláez *et al.*, 2020). Various raw materials can be used to prepare sourdough, including cereals, pseudocereals, and legumes. Globally, wheat (*Triticum aestivum* L.) is a typical cereal used in sourdough fermentation. The global annual production of wheat was 760 million tons in 2020. Sourdough prepared from wheat increases proteolytic activity, contributing to enhanced gliadin hydrolysis (Tomić *et al.*, 2023). The demand for healthy and nutritional foods has recently increased. Thus, there is a need to develop alternative methods of mixing various non-wheat ingredients with wheat flour (Cakir *et al.*, 2021).

The carob tree (*Ceratonia siliqua* L.) has contributed to the Mediterranean basin's economy since ancient times, primarily as a food source for livestock (Martins-Loução *et al.*, 2024). Additionally, the carob tree has a long history of usage as a food source, dye, and medicine (Ikram *et al.*, 2023). Previous studies have reported the importance of the carob tree in agronomy, cultivation, and industrial processing owing to its nutritional benefits (Gioxari *et al.*, 2022). Carob, an underutilised fruit tree species, is a potential alternative non-wheat ingredient for sourdough fermentation (Lima *et al.*, 2023). For instance, the seeds of carob are used as a substitute for cocoa powder (Ikram *et al.*, 2023). Carob is rich in bioactive compounds, fibre (essential for digestive health) (Papaefstathiou *et al.*, 2018), and antioxidants (gallic acid, flavonoids, and tannins) (Ydjedd *et al.*, 2017; Christou *et al.*, 2021; Laaraj *et al.*, 2023). The low glycaemic index (antihyperglycemic) of carob is beneficial for patients with diabetes (Azab, 2022; Aydın *et al.*, 2022; Ayad

et al., 2023). Additionally, carob exerts anti-dyslipidaemic effects, decreasing the risk of cardiovascular diseases (Martínez-Villaluenga *et al.*, 2018; Rašković *et al.*, 2023; Brassesco *et al.*, 2023).

Carob is a promising new frontier in modern food technology, especially fermentation (Igreja *et al.*, 2021). For instance, carob is reported to improve the quality of yoghurt and ice cream (Kulcan *et al.*, 2021), gluten-free bread formulations (Tsatsaragkou *et al.*, 2023), lactic acid production (Azaizeh *et al.*, 2022), and inulinase synthesis (Ilgin *et al.*, 2019).

Fermenting sourdough prepared with wheat and carob can meet the demands of consumers for high-value-added products and decrease the burden on the food industry (Novotni *et al.*, 2020). The baking industry is continuously exploring other sources of flour. Thus, evaluating the effects of carob flour on sourdough fermentation can aid in its application to improve the healthy, nutritional, and techno-functional properties of baked goods. Therefore, the present work aimed to examine the effect of carob and wheat flours on the nutritional, health, and pre-baking rheological properties of spontaneously fermented sourdough.

Materials and methods

Materials

The wheat grains (Mawna variety) were obtained from the Cooperative of Cereals and Pulses in Tipaza, Algeria. The carob sample was harvested from an ancient tree in Tipaza in 2023 (Figure 1). All the samples were stored in a dark room at 20 to 25°C until further analysis. Ethical approval was not required for this study.

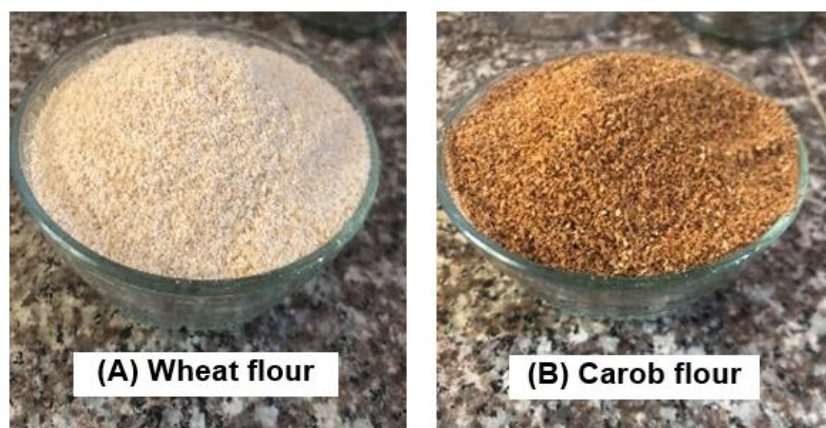


Figure 1. Wheat flour (A) and carob flour (B).

Preparation of flours and sourdoughs

The burnt and contaminated wheat and carob grains, as well as grains with physical impurities, were discarded. The samples were then ground using a cylinder tool. The wheat flour (400 g) and carob flour (400 g) were mixed with 600 mL of sterile purified water (SPW) (each 1 kg) in 1000-mL beakers. The mixture was vigorously stirred for 5 - 10 min manually. The beakers were covered with aluminium foil and incubated at 30°C for 24 h. Next, the dough was kneaded for 1 min and incubated at

30°C for 5 d. This is the standard method adopted by the industry as it simplifies the multiple-step type I sourdough preparation (Hernández-Figueroa *et al.*, 2023). Five sourdough compositions (SCs) were prepared (Table 1). They were supplemented with 50 g of wheat flour, with or without carob flour, and SPW once every 24 h. The final sourdough samples were kneaded for 1 min and stored in 1000-mL beakers at 4°C for further analyses (Lim *et al.*, 2017). The changes in sourdough samples after daily supplementation were photographed.

Table 1. Sourdough compositions (SC).

Sourdough sample		Initial composition 1 st day			Supplementation (every 24 h for 1 + 5 d)		
No.	Composition (%)	C (g)	W (g)	SPW (mL)	C (g)	W (g)	SPW (mL)
SC1	100% C	400.0	-	600.0	50.0	-	70.0
SC2	75% C + 25% W	300.0	100.0	600.0	37.5	12.5	60.0
SC3	50% C + 50% W	200.0	200.0	600.0	25.0	25.0	60.0
SC4	25% C + 75% W	100.0	300.0	600.0	12.5	37.5	70.0
SC5	100% W	-	400.0	400.0	-	50.0	50.0

SC: sourdough composition; C: carob flour; W: wheat flour; and SPW: sterile purified water.

Determination of pH, temperature, titratable acidity (TTA), and phytic acid content (PAC)

The pH and temperature were measured once every 24 h using a pH and temperature meters (Hanna Edge pH, RI, USA), respectively, which were directly inserted in the dough (Ognean, 2015).

The TTA was determined by homogenising 10 g of the dough sample with 90 mL of boiled distilled water for 20 min. The pH of the homogenate was measured using a pH meter. The suspension was titrated against 0.1 N NaOH (Merck 221465, Darmstadt, Germany) solution to a final pH value of 8.5 with phenolphthalein (1.0 g/100 mL) (Merck 319236) as the indicator. The TTA was defined as the amount of NaOH consumed (mL) (Dapčević-Hadnađev *et al.*, 2022).

The PAC was determined following the methods of Najafi *et al.* (2012). The dough sample (2 g) dried at 30°C for 16 h in an incubator (Nüve EN 120, Ankara, Türkiye) was extracted using 40 mL of HCl (Sigma Aldrich 07102.2.5L, Steinheim, Germany) solution (2.4%, w/v) at 20 to 25°C for 3 h. The suspension was vacuum filtered using a Whatman Grade 1 filter paper (Merck WHA1001090). The filtrate (1 mL) was pipetted into a 25-mL flask, mixed with 1.0 mL of EDTA (Merck

324504), and diluted up to 25 mL with distilled water. The diluted solution was injected into the ion exchange column (2 g of Purolite A-400 with a particle size of 16 - 50 mesh; Hounslow, UK). After washing with 15 mL of 0.7 M NaCl solution, the filtrate was digested using a Kjeldahl apparatus (InKJel M, Dusseldorf, Germany) with concentrated sulphuric acid (H₂SO₄) (Merck 112080.2500) and 3 mL of concentrated nitric acid (HNO₃) (Merck 309079). The digested solution was allowed to cool at room temperature and transferred to a 50-mL volumetric flask. The sample was mixed with 2 mL of ammonium molybdate [(NH₄)₂MoO₄] (Merck 277908) solution, 1 mL of sulfonic acid reagent, and diluted up to 50 mL with distilled water. A blank sample was prepared. The phosphorus level was determined by examining the absorbance of the reaction mixture at 640 nm using a BioTek 800 TS spectrometer (Santa Clara, USA). The PAC was calculated using Eq. 1:

$$\text{Phytic acid (mg/g sample)} = \frac{((\text{mean K}) \times A_{\text{sample}} \times 20) / (0.282 \times 1,000)}{\quad} \quad (\text{Eq. 1})$$

where, A = absorbance of the solution at 640 nm, K = standard phosphorus concentration (µg/mL)/A_{standard},

and mean K = mean of all K values determined for the standard solutions.

Determination of vitamins B₁, B₂, B₃, and B₆

The fermented sourdough (1 g) was weighed on an analytical balance (Sartorius Secura2131S, Göttingen, Germany) and pretreated with 26 mL of 1 N NaOH (Merck 221465) for 1 h at 50°C (Memmert UN55, Schwabach, Germany). The pH of the solution was adjusted to 5.6 using HCl (Sigma Aldrich 07102.2.5L). The solution was centrifuged at 4,000 rpm for 5 min (Mixtasel-BL, Barcelona, Spain). The supernatant was subjected to liquid-liquid extraction. The water fraction was initially double-filtered through 0.47-µm polyvinylidene difluoride (PVDF) syringe filters, followed by filtration through 0.22-µm PVDF syringe filters. The filtrate (10 µL) was injected into the high-performance liquid chromatography system (Shimadzu Nexera-I LC-2040-C, Buckinghamshire, UK) to analyse water-soluble vitamins B₁, B₂, B₃ (nicotinic acid and nicotinamide), and B₆ (pyridoxal, pyridoxine, and pyridoxamine) (Mateeva *et al.*, 2023).

Determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity (AC)

Sourdough (200 mg) was mixed with 4 mL of acidified methanol (Merck 106009) solution (HCl/methanol/distilled water; 1:80:10, v/v) for 2 h on an orbital shaker (Esco OrbiCult, Friedberg, Germany) and centrifuged (Himac CR22N, Tokyo, Japan) at 4°C and 3,000 rpm for 10 min. The supernatant was diluted up to 4 mL with distilled water and stored at -20°C for further analyses (Beta *et al.*, 2005; Banu *et al.*, 2010). After leaving the samples undisturbed at room temperature for 75 min, the absorbance at 765 nm was measured using a spectrophotometer. TPC was expressed as chlorogenic acid equivalents in µg/g of sample dry basis (db) using the Folin-Ciocalteu reagent (Merck 109001), following the methods of Singleton and Rossi (1965). TFC was determined using the aluminium chloride (AlCl₃) colorimetric method (Dewanto *et al.*, 2002). The absorbance of the reaction mixture at 510 nm was measured.

Sourdough (500 mg) was mixed with 5 mL of methanol on an orbital shaker for 2 h. The sample was centrifuged at 4°C and 3,000 rpm for 10 min. The supernatant was diluted up to 5 mL and stored at

-20°C (Beta *et al.*, 2005). AC was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent (Hara *et al.*, 2018). The absorbance at 517 nm of the samples was measured.

Isolation and identification of LAB

Homogenised sourdough (10 g) was mixed with 90 mL of physiological saline solution (PSS) (Merck Millipore 146193) to prepare serially diluted (10⁻² and 10⁻³) samples. The diluted suspension (1 mL) was transferred to *Lactobacillus* Agar De Man Rogosa (MRS Agar) (Merck 110660.0500) and incubated at 37°C for 24 - 48 h (Carl Roth IN55, Karlsruhe, Germany). The morphology of suspected LAB colonies was examined under a Leica DMi1 microscope (Wetzlar, Germany). To ensure the purity of the suspected colonies, MRS agar was inoculated into broth tubes and activated at 37°C for 24 h under aerobic/anaerobic conditions. The matte-cream-colored colonies were considered LAB strains. Pure cultures were subjected to Gram-staining. Gram-positive cocci and bacilli were observed under the light microscope and subjected to the catalase test. Catalase-negative strains were selected (Doğan and Tekiner, 2019).

The genomic DNA was extracted from single colonies using the Qiagen DNeasy blood and tissue kit (Hilden, Germany), following the manufacturer's instructions. The presumptive isolates were identified by amplifying and sequencing the 16S rRNA gene using a universal primer pair (*f*-5'-AGAGTTTGATCMTGGCTCAG-3' and *r*-5'-CTGCTGCSYCCCGTAG-3'). Eurofins Genomics (Ebersberg, Germany) sequenced the amplicons. The sequences were compared using the Basic Local Alignment Search (BLAST) tool in GenBank of the National Centre for Biotechnology Information (NCBI) database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Kuhnert *et al.*, 1996; Rehagel *et al.*, 2022).

In vitro cytotoxicity (MTS) test

The cells were cultured in Dulbecco's Modified Eagle Medium (Invitrogen 11965092, UK) supplemented with 4.5 g/L D-glucose, 10% foetal bovine serum (Invitrogen), and 1% of penicillin/streptomycin/amphotericin (Invitrogen) at 37°C and 5% CO₂ in a humidified incubator (Nüve EC160, Ankara, Türkiye). The cells at approximately 80% confluency were trypsinised using a 0.25%

trypsin/EDTA solution (Invitrogen 25200056). The cell count was determined using a haemocytometer after centrifugation (Abdik, 2022).

The sample was prepared according to UNI EN ISO 10993-5 (AOAC, 2009a) and 10993-12 (AOAC, 2009b). Homogenised sourdough (10 g) was mixed with 90 mL of PSS (Merck Millipore 146193) to prepare serially diluted (10^{-2} and 10^{-3}) samples. The diluted suspension (1 mL) was transferred to *Lactobacillus* Agar De Man Rogosa.

Human L929 fibroblast and HCT-116 colon cancer cells were seeded in 96-well plates at a density of 5×10^3 cells per well. The culture medium was supplemented with 200 μ L of prepared sourdough samples. The cells were incubated at 37°C for 24 and 48 h. The viability of cells was determined using the MTS assay (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazol)-3-(4-sulfophenyl) tetrazolium), following the manufacturer's instructions (Promega CellTiter96, Southampton, UK). The samples were incubated with 10 μ L of MTS reagent at 37°C for 1 h. The optical density at 495 nm was measured using an Agilent Biotech 800 TS (Santa Clara, USA) microplate reader (Abdik, 2022). Each assay was performed five times. The effect was considered cytotoxic when the percentage cell viability (CV) was $\leq 70\%$ and non-toxic when the percentage CV was $> 70\%$ (Cannella *et al.*, 2020).

Farinograph and extensograph indices of C-W flours Flour compositions

Five percent increasing doses of carob-supplemented flour (0% (containing only wheat flour), 5, 10, and 15% (w/w)) were prepared as described by Miś *et al.* (2012) to eliminate the effect of the dough water content on its farinograph and extensograph properties.

Farinograph indices

The farinograph indices of the wheat and wheat-carob blends during the kneading process were tested using a Brabender Farinograph, following the Cereals and Grains Association (formerly known the American Association of Cereal Chemists) AACC method no: 54-21 constant dough method (AACC, 2000). At 14% base moisture content, wheat flour with or without carob flour was transferred to the 300 g farinograph mixing bowl. Distilled water was added to the bowl using a burette (Table 1). As the blend was mixed to obtain dough, the farinograph consistency (BU) against time (min) was recorded for

30 min. The water absorption (WA) (%), development time of dough (DT) (min), and stability time (ST) (min) were determined (Novotni *et al.*, 2020).

Extensograph indices

A Brabender extensograph-E with a large mixer-S 300 H (for 300 g of flour) was utilised to determine the extensograph characteristics of the dough and its resistance following the standard AACC method (no. 54-10) (AACC, 2000). The extensibility (mm), resistance to extension (BU), ratio number, and energy (cm^2) were determined at 45, 90, and 135 min (Shongwe *et al.*, 2022). The farinograph and extensograph indices were measured in three replications.

Statistical analysis

Data were expressed as mean \pm standard deviation (SD) calculated using the MSOffice Excel Program. Means between groups were compared using One-way analysis of variance, followed by Tukey's honestly significant difference test using SPSS 19 software (IBM Corporation, USA). Differences were considered significant at $p < 0.05$.

Results

The images of sourdough samples during daily supplementation are shown in Figure 2.

pH, temperature, TTA, and PAC

The pH, temperature, TTA, and PCA of sourdough samples during fermentation are shown in Table 2.

After 5-d fermentation, the pH of SC1 and SC5 decreased by 18.8% (from 5.0 to 4.0; 4.2 ± 0.1) and 37.9% (from 5.8 to 3.6; 4.1 ± 0.5), respectively. Meanwhile, SC2, SC3, and SC4 exhibited mean pH values of 4.3 ± 0.7 , 4.0 ± 0.4 , and 4.3 ± 0.8 , respectively.

The temperature of SC1, SC2, SC3, SC4, and SC5 did not significantly vary (21.8 ± 0.5 , 22.4 ± 0.5 , 21.8 ± 0.4 , 21.9 ± 0.5 , and $21.9 \pm 0.5^\circ\text{C}$, respectively) during fermentation.

The mean TTA values (in mL NaOH consumed), which indicate the capacity of the samples to release acids during fermentation of SC1, SC2, SC3, SC4, and SC5 were 7.1 ± 1.2 (from 5.5 to 8.8 mL, 60% increase), 9.0 ± 2.7 (from 4.2 to 11.9 mL, 183% increase), 12.5 ± 4.1 (from 5.0 to 16.0 mL,

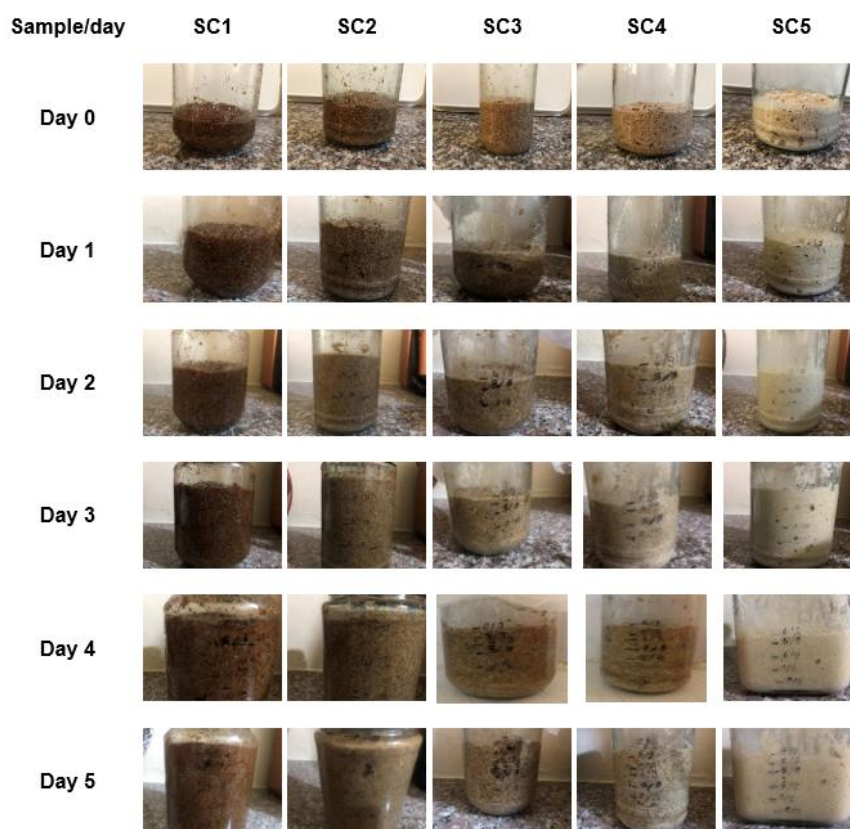


Figure 2. Evolution of sourdough samples (SC1 to SC5) during daily refreshments.

Table 2. Results of pH, T, TTA, and PAC.

C	P	US	Fermentation time (day)					Δ (\pm %)	Median \pm SD
			1 st	2 nd	3 rd	4 th	5 th		
SC1	pH	5.0	4.2	4.2	4.3	4.3	4.0	-18.8	4.2 \pm 0.1 ^c
	T	21.4	22.2	21.2	21.5	22.2	22.1	3.3	21.8 \pm 0.5 ^f
	TTA	5.5	6.0	6.2	6.6	7.8	8.8	60.0	7.1 \pm 1.2 ^g
	PAC	12.0	10.8	6.0	5.8	4.1	3.5	-70.6	6.0 \pm 2.9 ^a
SC2	pH	5.9	5.9	5.7	5.6	4.6	4.5	-23.9	4.3 \pm 0.7 ^c
	T	21.4	22.0	21.5	22.0	22.6	22.4	5.6	22.4 \pm 0.5 ^f
	TTA	4.2	6.0	6.1	10.1	10.7	11.9	183.3	9.0 \pm 2.7 ^g
	PAC	19.3	14.94	12.25	7.1	6.6	6.4	-66.8	6.7 \pm 0.3 ^h
SC3	pH	4.9	4.6	4.1	3.7	3.8	3.9	-20.6	4.0 \pm 0.4 ^d
	T	21.4	22.1	21.1	21.8	22.0	22.1	3.3	21.8 \pm 0.4 ^f
	TTA	5.0	6.0	10.9	13.9	15.6	16.0	220.0	12.5 \pm 4.1 ^g
	PAC	21.9	13.4	8.8	7.5	5.4	5.4	-75.5	8.1 \pm 3.3 ^h
SC4	pH	6.2	6.2	5.3	4.4	4.4	4.3	-30.3	4.3 \pm 0.8 ^c
	T	21.4	22.2	22.0	22.6	22.5	21.9	5.1	21.9 \pm 0.5 ^f
	TTA	2.4	6.0	13.4	14.8	15.0	15.5	545.8	12.9 \pm 4.0 ^g
	PAC	38.9	25.3	24.6	15.1	10.9	10.1	-73.9	17.2 \pm 7.3 ^h
SC5	pH	5.8	5.0	4.1	4.0	3.8	3.6	-37.9	4.1 \pm 0.5 ^c
	T	21.1	22.4	21.0	21.8	22.1	22.0	4.3	21.9 \pm 0.5 ^f
	TTA	2.4	5.3	15.0	16.8	17.5	18.2	658.3	14.6 \pm 5.3 ^g
	PAC	44.8	31.3	25.2	18.4	13.9	11.8	-73.7	20.1 \pm 8.1 ^b

C: composition; P: parameter; US: unfermented sourdough; T: temperature; TTA: titratable acidity (mL NaOH consumed); PAC: phytic acid content (mg/100 g db); and Δ : Change (\pm %). Means followed by different lowercase superscripts within similar column are significantly different ($p < 0.05$).

220% increase), 12.9 ± 4.0 (from 2.4 to 15.5 mL, 545% increase), and 14.6 ± 5.3 mL (from 2.4 to 18.2 mL, 658% increase) during fermentation, respectively.

The mean PAC values of SC1, SC2, SC3, SC4, and SC5 were 6.0 ± 2.9 mg/100 g db (from 12.0 to 3.5 mg/100 g, 70.6% decrease), 6.7 ± 0.3 mg/100 g db (from 19.3 to 6.4 mg/100 g, 66.8% decrease), 8.1 ± 3.3 mg/100 g db (from 21.9 to 5.4 mg/100 g, 75.5% decrease), 17.2 ± 7.3 mg/100 g db (from 38.9 to 10.1 mg/100 g, 73.9% decrease), and 20.1 ± 8.1 mg/100 g db (from 44.8 to 11.8 mg/100 g, 73.7% decrease), respectively.

Vitamins B₁, B₂, B₃, and B₆

The concentrations of vitamins B₁ (thiamine), B₂ (riboflavin), B₃ (niacin and niacinamide), and B₆ (pyridoxal, pyridoxine, and pyridoxamine) were quantified in the sourdough samples (Table 3 and Figure 3). The levels of vitamins B₁, B₂, B₃, and B₆ in SC1 were 314, 19, 1799, and 126 µg/100 g, respectively, while those in SC5 were 400, 86, 444,

and 245 µg/100 g, respectively.

TPC, TFC, and AC

The TPC, TFC, and AC of sourdough samples are shown in Table 4. The TPC, TFC, and AC levels in SC1 with 100% carob flour were 555.3 ± 15.0 mg FAE/100 g, 38.14 ± 4.0 mg CE/100 g, and 545.7 ± 19.1 mg TE/100 g, respectively. In contrast, the levels of TPC and AC in SC5 with 100% wheat flour were 142.3 ± 13.7 mg FAE/100 g and 450.9 ± 29.1 mg TE/100 g, respectively, whereas no TFC was measured.

Characterisation of LAB

The genomic DNA extracted from single colonies was amplified and sequenced. Analysis with the BLAST tool revealed that the predominant bacterial sequence in sourdough samples exhibited a 100% homology with *Lactiplantibacillus plantarum* strain JCM 1149 16S ribosomal RNA (NCBI Reference Sequence: NR_115605.1). Other genera of LAB were not detected.

Table 3. Vitamins B₁, B₂, B₃, and B₆ (µg/100 g) levels.

C	B ₁	B ₂	B ₃			B ₆			
			NA	NAM	∑B ₃	PXL	PXN	PXMN	∑B ₆
SC1	314 ^a	19 ^a	152	1462	1614 ^a	185	105	21	311 ^a
SC2	346 ^c	14 ^c	174	1874	2048 ^c	284	99	90	473 ^c
SC3	272 ^b	58 ^b	149	2155	2304 ^b	133	326	33	492 ^b
SC4	295 ^c	19 ^c	149	951	1100 ^c	151	75	42	268 ^c
SC5	400 ^c	86 ^c	21	336	357 ^c	87	211	34	332 ^c
Median	339.8	39.7	121.0	1270.8	1484.6	152.5	142.7	40.8	375.2
Sd. (±)	56.8	28.3	58.2	683.8	778.6	76.2	106.5	25.2	100.9

C: sourdough composition; NA: nicotinic acid, NAM: nicotinamide, PXL: pridoxal; PXN: pridoxin; and PXMN: pridoxinamin. Means followed by different lowercase superscripts within similar column are significantly different ($p < 0.05$).

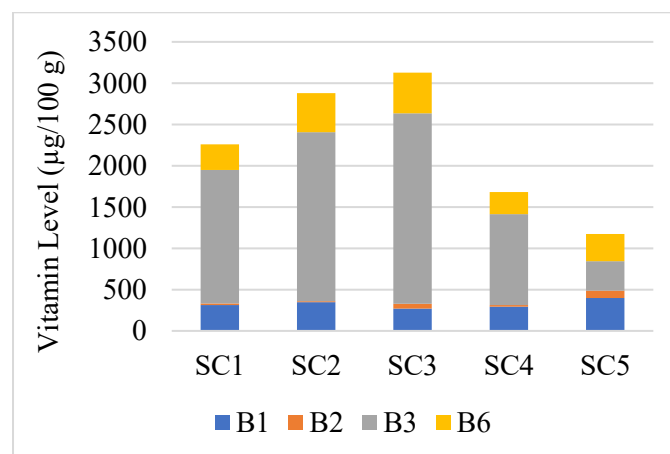


Figure 3. Comparative analysis of vitamins B₁, B₂, B₃, and B₆ concentrations (µg/100 g).

Table 4. Dynamics of TPC, TFC, and AC (DPPH) content in sourdoughs.

C	TPC (mg FAE/100 g db)	TFC (mg CE/100 g db)	DPPH (mg TE/100 g db)
SC1	555.3 ± 15.0 ^a	38.14 ± 4.0 ^a	545.7 ± 19.1 ^a
SC2	124.0 ± 11.7 ^b	n.d.	213.9 ± 14.7 ^b
SC3	959.0 ± 86.3 ^c	181.0 ± 17.0 ^c	643.3 ± 11.4 ^b
SC4	556.4 ± 53.4	172.8 ± 20.5	537.3 ± 22.7
SC5	142.3 ± 13.7	n.d.	450.9 ± 29.1

C: composition; TPC: total phenolic content; FAE: ferulic acid equivalents; TFC: total flavonoid content; CE: catechin equivalent; TE: Trolox equivalent; and n.d.: not detected. Means followed by different lowercase superscripts within similar column are significantly different ($p < 0.05$).

In vitro cytotoxicity (MTS) test

The effect of treatment with 200 μ L sourdough suspension for 24 and 48 h on the viability of L929 and HCT-116 cells was examined using the MTS assay. The treatment with SC1 with 100% carob flour, wheat-carob flour blends (SC2, SC4), and SC5 with 100% wheat flour for 24 h significantly decreased the viability of L929 cells ($p < 0.05$). However, treatment with SC3 for 24 h did not affect the viability of L929 cells ($p \geq 0.05$). Meanwhile, treatment with SC2 for 48 h significantly decreased the viability of L929 cells ($p < 0.05$). In contrast, treatment with SC1, SC3, SC4, and SC5 for 48 h did not affect the viability of L929 cells ($p \geq 0.05$) (Figure 4). These findings indicated that wheat flour, with or without carob flour, did not exert cytotoxic effects on the human L929 fibroblast cells.

Treatment with SC1 with 100% carob flour and SC5 with 100% wheat flour, as well as carob and wheat flour blends, for 24 or 48 h, significantly decreased the viability of HCT-116 colon cancer cells ($p < 0.05$) (Figure 5). These findings indicated that wheat flour, with or without carob flour, exerted cytotoxic effects on the colon cancer cells.

Effect of carob flour supplementation on farinograph and extensograph indices

Farinograph indices

The effects of carob flour on WA (%), DT (min), and ST (min) are shown in Table 5. The WA increased from $57.34 \pm 0.23\%$ (blends with 0% carob flour) to $60.20 \pm 0.02\%$ (wheat dough supplemented with 0, 5, and 15% carob flour). Compared with other formulations, the WA was higher in blends with 10% carob flour ($62.33 \pm 0.05\%$).

DT, which is the time required for the dough to reach maximum consistency, increased from 3.65 ± 0.05 min in blends with no addition to 25.50 ± 0.02

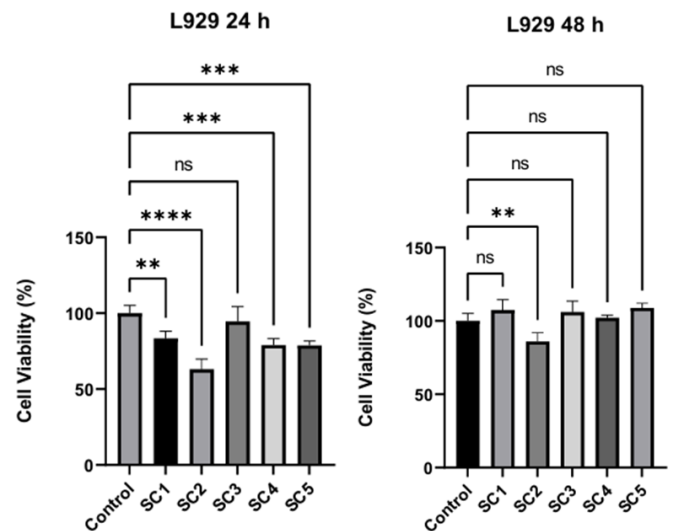


Figure 4. MTS assay, the CV results on Human L929 fibroblast cell line to sourdough samples after 24 and 48 h of incubation. One-way ANOVA with Tukey's *post hoc* test ($*p < 0.05$). ns: not significant.

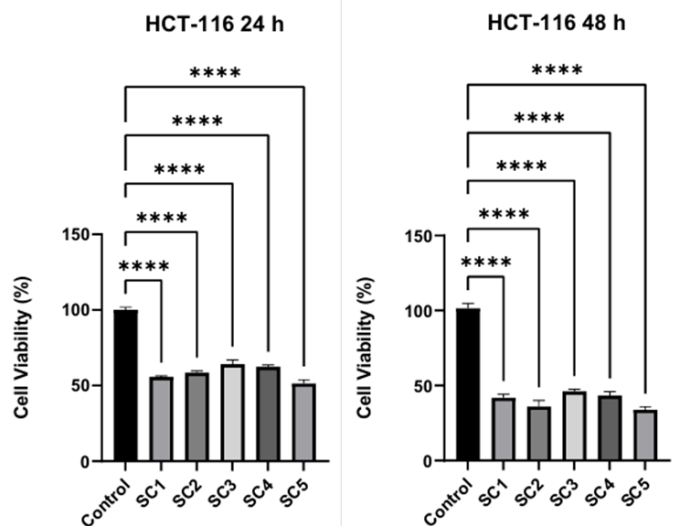


Figure 5. MTS assay, the CV results on HCT-116 colon cancer cell line to sourdough samples after 24 and 48 h of incubation. One-way ANOVA with Tukey's *post hoc* test ($*p < 0.05$). ns: not significant.

Table 5. Farinograph characteristics of W and W-C blends.

Farinograph indice	Blend proportion of C (%)			
	0	5	10	15
Water absorption (WA) (%)	57.34 ± 0.23 ^{aA}	61.32 ± 0.39 ^{bA}	62.33 ± 0.05 ^{cA}	60.20 ± 0.02 ^{dA}
Development time (DT) (min)	3.65 ± 0.05 ^{aB}	10.96 ± 0.01 ^{bB}	18.21 ± 0.14 ^{cB}	25.50 ± 0.02 ^{dB}
Stability (S) (min)	5.78 ± 0.02 ^{aC}	9.32 ± 0.03 ^{bC}	10.73 ± 0.02 ^{cC}	9.94 ± 0.05 ^{dC}

Means followed by different lowercase superscripts within similar column are significantly different ($p < 0.05$). Means followed by different uppercase superscripts within similar row are significantly different ($p < 0.05$).

min in wheat dough supplemented with 0, 10, and 15% carob flour. Compared with that in other formulations, the DT was higher in blends with 15% carob flour.

ST, which is the time at which the dough consistency is ≥ 500 BU, increased from 5.78 ± 0.02 in blends with no addition to 9.94 ± 0.05 min in wheat dough with 0, 5, and 15% carob flour. Compared with that in other formulations, the ST was higher in blends with 10% carob.

Extensograph indices

The effects of carob flour supplementation on energy (cm^2), extensibility (mm), resistance to extension (BU), and the ratio number are shown in Table 6. The blends with 0% carob flour exhibited increased energy values at 45, 90, and 135 min.

Energy and extensibility decreased with the increase in the amount of carob flour. Resistance to extension significantly increased in blends with $> 5\%$ carob flour at all time points. The ratio number was not significantly different between blends with 0% carob flour and those with 5% carob flour. However, the ratio number gradually increased when the carob flour levels increased more than 5%. The extensibility (mm) time-dependently decreased, whereas the energy value, maximum resistance to extension, and the ratio number time-dependently increased. In wheat flour, the extensibility time-dependently decreased and the maximum resistance to extension time-dependently increased. Meanwhile, the energy and ratio number values of wheat flour slightly increased.

Table 6. Extensograph characteristics of W and W-C blends.

Extensograph indice	Blend proportion of C (%)	45 min	90 min	135 min
Energy (cm^2)	0	131.48 ± 0.54 ^{abdA}	160.50 ± 0.21 ^{abdA}	152.69 ± 0.13 ^{abdA}
	5	116.69 ± 0.55 ^{abdA}	178.20 ± 0.40 ^{abdA}	139.21 ± 0.24 ^{abdA}
	10	102.31 ± 0.29 ^{abdA}	196.03 ± 0.76 ^{abdA}	125.93 ± 0.63 ^{abdA}
	15	88.26 ± 0.24 ^{abdA}	214.70 ± 0.14 ^{abdA}	113.28 ± 0.20 ^{abdA}
Extensibility (mm)	0	180.60 ± 0.42 ^{acfB}	154.73 ± 0.10 ^{acfB}	67.51 ± 0.29 ^{acfB}
	5	145.98 ± 0.67 ^{acfB}	124.70 ± 0.41 ^{acfB}	129.23 ± 0.40 ^{acfB}
	10	112.66 ± 0.25 ^{acfB}	95.19 ± 0.36 ^{acfB}	110.84 ± 0.12 ^{acfB}
	15	79.37 ± 0.09 ^{acfB}	63.88 ± 0.99 ^{acfB}	12.96 ± 0.07 ^{acfB}
Resistance to extension (BU)	0	621.35 ± 0.33 ^{eghC}	860.50 ± 0.14 ^{eghC}	916.25 ± 0.37 ^{eghC}
	5	670.46 ± 0.14 ^{eghC}	1003.23 ± 0.10 ^{eghC}	984.38 ± 0.16 ^{eghC}
	10	719.57 ± 0.28 ^{eghC}	1146.06 ± 0.05 ^{eghC}	1187.11 ± 0.81 ^{eghC}
	15	769.32 ± 0.17 ^{eghC}	1288.26 ± 0.34 ^{eghC}	1525.60 ± 0.71 ^{eghC}
Ratio number	0	2.12 ± 0.01 ^{abciD}	3.14 ± 0.04 ^{abciD}	3.73 ± 0.04 ^{abciD}
	5	3.94 ± 0.02 ^{abciD}	7.00 ± 0.08 ^{abciD}	8.19 ± 0.07 ^{abciD}
	10	5.76 ± 0.01 ^{abciD}	10.80 ± 0.07 ^{abciD}	12.14 ± 0.05 ^{abciD}
	15	7.45 ± 0.12 ^{abciD}	14.66 ± 0.05 ^{abciD}	15.44 ± 0.05 ^{abciD}

Means followed by different lowercase superscripts within similar column are significantly different ($p < 0.05$). Means followed by different uppercase superscripts within similar row are significantly different ($p < 0.05$).

Discussion

pH, temperature, TTA, and PCA

Following 5-d fermentation, the pH of SC1 with 100% carob flour decreased from 5.0 to 4.0 (-18.8% change), while that of SC5 with 100% wheat flour decreased from 5.8 to 3.6 (-37.9% change). The pH values of carob and wheat flour blends (SC2, SC3, and SC4) decreased by 23.9, 20.6, and 30.3%, respectively. The changes in pH were significantly different between SC2 and SC3 ($p < 0.05$). However, the pH changes were not significantly different between the other groups ($p \geq 0.05$). The regulation of the metabolic activities of LAB species is dependent on the pH. Previous studies have reported that the pH decreases by approximately 4.0 during sourdough fermentation (Oshiro *et al.*, 2020). Generally, the pH of sourdough is in the range of 3.4 - 6.4, depending on the type of flour, duration of fermentation, and specific microorganisms (Pejcz *et al.*, 2021). One study reported that the initial pH of sourdough at 0 h ranged from 5.52 to 5.32, which decreased to 4.22 after 6 h of fermentation (Tomić *et al.*, 2023). A survey revealed that the pH of wheat sourdough was 3.89 after 24 h of fermentation (Şerban *et al.*, 2023). Meanwhile, another study reported that the pH of wheat sourdough decreased from 6.2 to 3.4 (50.0% change) after 72 h of fermentation (Pejcz *et al.*, 2021). The pH of carob sourdough decreased to 4.2 - 4.5 after 24 h of fermentation (Novotni *et al.*, 2020). A recent study demonstrated that the pH of carob and wheat sourdough did not significantly vary during 5-d fermentation when compared with that of wheat-carob sourdough ($p \geq 0.05$). This was attributed to LAB-mediated phytate-degrading activity (Leenhardt *et al.*, 2004; Anastasio *et al.*, 2010).

In the present work, the temperature of the whole sourdoughs did not increase significantly during the fermentation period (3.3 to 5.6%) ($p \geq 0.05$). Temperature is a critical parameter for sourdough fermentation. Low-temperature fermentation may increase the synthesis of secondary aromatic compounds (Xu *et al.*, 2020). The temperature of dough with a high percentage of whole grains is optimally maintained at 23°C to avoid rapid fermentation and acid production (Salovaara and Valjakka, 1987). Additionally, both time and temperature influence the formation of pungent

flavour in the baked products (Katina *et al.*, 2006). A recent survey revealed that the mean temperature of the sourdough samples was close to 23.0°C, which is the recommended temperature for fermentation and increases acidity during 5-d fermentation.

Carob flour dose-dependently increased TTA. Consistently, the TTA of SC1 with 100% carob changed from 5.5 to 8.8 (in mL NaOH consumed; 60.0% decrease), which was 2.1-fold lower than that of SC5 with 100% wheat flour during 5-d fermentation. This indicated that the TTA of sourdough samples was not significantly different ($p \geq 0.05$). Acidity generated during fermentation can be attributed to the breakdown of starch into monosaccharides and disaccharides by LAB species. The increased acidity influences properties, such as flavour profile (Najafi *et al.*, 2012; Fekri *et al.*, 2024), regulates the amylase-mediated breakdown of starch into fermentable sugars (González-Alonso *et al.*, 2024), and stimulates the fermentation by LAB species (D'Amico *et al.*, 2023). The TTA rate (166.9%) (4.8 to 11.1 mL) significantly increased during 6-h fermentation for wheat sourdough (Dapčević-Hadnađev *et al.*, 2022). Meanwhile, one study reported that the TTA rate increased by 24.2% (9.9 to 12.3 mL) for carob sourdough (Novotni *et al.*, 2020). The authors demonstrated that lactic acid production in carob sourdough was significantly slower than that in wheat sourdough, which was consistent with the results of Novotni *et al.* (2020) and Dapčević-Hadnađev *et al.* (2022).

In the present work, the PAC values were significantly different between SC1 with 100% carob flour and SC5 with 100% wheat ($p < 0.05$). In contrast, the PAC values were not significantly different between the other composite groups ($p \geq 0.05$). The mean PAC values of SC1 and SC5 were 6.0 ± 2.9 (12.0 to 3.5 mg/100 g, 70.6% decrease) and 20.1 ± 8.1 mg/100 g (44.8 to 11.8 mg/100 g, 73.7% decrease), respectively, during the fermentation period. Phytic acid, also called *myo*-inositol hexaphosphoric acid, is a micronutrient inhibitor occurring in many cereal grains and legumes (Anastasio *et al.*, 2010). Sourdough fermentation significantly degrades phytic acid and increases the bioavailability of free amino acids, minerals, and proteins (Gobbetti *et al.*, 2019). Previous studies have reported that the PAC of wheat sourdough is in the range of 3.3 - 5.1 mg/100 g (Longin *et al.*, 2023) or in

the range of 5.0 - 79.0 mg/100 g with a decrease of 42.9 - 96.6% after fermentation (Fang *et al.*, 2023). Meanwhile, the PAC of carob sourdough is reported to be 142 mg/100 g after fermentation (79% decrease), which was 3.4-fold lower than that of wheat sourdough after fermentation (Haros *et al.*, 2001). In the present work, carob sourdough improved the nutritional value of baked products by reducing antinutritive phytic acid levels during dough fermentation. However, future studies must evaluate the effect of carob and its sourdough on iron and zinc deficiencies, which are major public health problems (Chondrou *et al.*, 2024).

Vitamins B₁, B₂, B₃, and B₆

The present work investigated the levels of vitamins B₁, B₂, B₃, and B₆ in the sourdough samples. The levels of B-group vitamins (2,258 µg/100 g) in SC1 with 100% carob flour were significantly higher (1.9-fold) than those in the other samples ($p < 0.05$), which was consistent with the increasing quantity of carob flour. The fermentation duration with method and microbial composition affect the levels of B-group vitamins in sourdough. For instance, prolonged fermentation enhanced B₁ levels (250 µg/100 g) when compared with short fermentation duration (140 µg/100 g) (Batifoulier *et al.*, 2005). Batifoulier *et al.* (2006) also reported that the B₁, B₂, and B₆ levels in wheat sourdough were in the range of 260 - 613, 48 - 106, and 145 - 316 µg/100 g, respectively. Ikram *et al.* (2023) revealed that the levels of B₂, B₃, and B₆ in carob sourdough were 0.380, 185.6, and 23.8 mg/100 g, respectively. These findings indicated that the increased nutritive and health values of carob sourdough relative to wheat sourdough could be attributed to the increased levels of B-group vitamins.

TPC, TFC, and AC

Compared with those in wheat and carob-wheat composite sourdoughs, TPC (555.3 mg FAE/100 g) and TFC (38.14 mg CE/100 g) levels were 4-fold higher, and the AC levels were 1.2-fold higher (545.7 mg TE/100 g) in carob sourdough. This indicated that the TPC, TFC, and AC levels in carob sourdough were significantly different from those in carob-wheat sourdoughs ($p < 0.05$), with the variations depending on the amount of wheat flour. Ivanova *et al.* (2023) determined that the concentrations of TPC, TFC, and AC (DPPH) in fermented wheat sourdough were 150 mg FAE/100 g,

980 mg CE/100 g, and 350 mg TE/100 g, respectively. Previous studies have reported that the TPC, TFC, and AC levels of C sourdough were 811 mg FAE/100 g (Ioannou *et al.*, 2023), 98.7 mg CE/100 g (Goulas and Georgiou, 2019), and 52.6 mg TE/100 g (Novotni *et al.*, 2020), respectively. The findings of the present work on carob sourdough were consistent with those reported in previous studies. However, the levels reported in the present work varied from those reported in Goulas and Georgiou (2019), Novotni *et al.* (2020), and Ioannou *et al.* (2023), which can be attributed to fermenting conditions, composition of sourdough, and origin and nutritive quality of carob. Fermentation significantly improved TPC and TFC, as well as *in vitro* and *ex vivo* AC (Gabriele *et al.*, 2024). Zahorec *et al.* (2024) revealed that the addition of 3.5% of carob flour to wheat sourdough increased *in vitro* antioxidant activity (DPPH) by 31-fold. Sebai *et al.* (2013) demonstrated that *in vivo*-subacute dose-dependent intervention with carob for 7 d significantly decreased cerebral and myocardial lipid peroxidation, and the level of hydrogen peroxide in the kidney, liver, and brain. The findings of the present work suggested that carob sourdough comprised high TPC and TFC, and exerted antioxidative effects in baked products.

Characterisation of LAB

A recent study identified *Lp. plantarum* as the predominant strain in carob and carob-wheat sourdoughs. In the present work, the bacterial sequences exhibited 100% homology with *Lp. plantarum* strain JCM 1149 16S ribosomal RNA (NCBI Reference Sequence: NR_115605.1). The biochemical changes during sourdough fermentation can be attributed to the metabolic activity of microorganisms from the raw materials, affecting the quality of the bakery product (Fekri *et al.*, 2024). The supplementation of carob flour to millet flour promoted the growth of lactobacilli and the production of organic acids (Voučko *et al.*, 2024). The common LAB strains in sourdoughs are *Lp. plantarum*, *L. casei*, *L. curvatus*, *L. paracasei*, *L. brevis*, and *L. uvarum* (Bartkiene *et al.*, 2022). Among the LAB species, *Lp. plantarum* is mainly found in the fermented plant-derived ingredients (Martins-Loução *et al.*, 2024). Paramithiotis *et al.* (2010) reported that *Lp. plantarum* is the most common LAB strain in sourdough. Subsequent supplementation during fermentation can increase the

relative abundance of *Lp. plantarum* strain in sourdough samples.

LAB can hydrolyse macromolecular compounds, such as polysaccharides with α -(1 \rightarrow 4) glycosidic bonds. *Lp. plantarum* (Oguntoyinbo and Narbad, 2012) can degrade proteins, related amino acids, and other non-nutritive and harmful substances (Wang *et al.*, 2021), contributing to the functional benefits of sourdough (Fraberger *et al.*, 2020). LAB can also metabolise maltose into lactic and acetic acids, decreasing pH (Oshiro *et al.*, 2020). *Lp. plantarum* can produce lactic acid, acetic acid, and/or ethanol (Moore *et al.*, 2007; Sanmartín *et al.*, 2025). Similar to the fermentation of carob, sorghum, and millet, the microbial metabolism of phenolic compounds in sourdough fermentation is associated with the production of bioactive substances, such as phenolic compounds (Wang and Wang, 2024). Microbial amino acid metabolites promote the accumulation of antioxidant metabolites (Pérez-Alvarado *et al.*, 2022). The metabolic process of *Lactobacillus* spp. can promote lipid oxidation, indicating a potent antioxidant impact during sourdough fermentation (Kirchhoff and Schieberle, 2002). Future studies must examine the synergistic interaction of carob and LAB community structure with antioxidant release mechanisms during fermentation.

Cytotoxicity results

The MTS assay results demonstrated that the sourdough samples were not cytotoxic to L929 cells but were cytotoxic to HCT-116 cells ($p < 0.05$). Fermented foods are associated with a reduced risk of cancer due to the presence of antioxidant molecules (Gabriele *et al.*, 2024). However, further studies are needed to elucidate the mechanism underlying the cytotoxic effects of carob sourdough containing LAB species (Krishnan *et al.*, 2024). The carob extract exhibits strong cytotoxic activity against human breast cancer cells (Javadi *et al.*, 2019). Klenow *et al.* (2009) demonstrated that the carob extract promoted the death of LT97 adenoma cells, but not HT29 carcinoma cells. Khazaei *et al.* (2023) revealed that carob extract suppressed the proliferation of prostate cancer cells. Thus, further studies are needed to determine the effects of carob sourdough with microbial and antioxidative composition on the apoptotic and proliferative pathways of specific cancer types.

Effect of carob flour supplementation on farinograph and extensograph indices

Farinograph indices

Increasing the proportion of carob (5, 10, and 15%) gradually increased the WA from $57.34 \pm 0.23\%$ (with 0% carob flour) to $61.32 \pm 0.39\%$ (with 5% carob flour), $62.33 \pm 0.05\%$ (with 10% carob flour), and $60.20 \pm 0.02\%$ (with 15% carob flour). This indicated that carob flour exhibited increased WA ability owing to its high fibre content (18.0% in carob) when compared with whole wheat flour (10.7%) (Goulas *et al.*, 2016; Buzzanca *et al.*, 2025). The high fibre content of carob can be attributed to a large number of hydrophilic groups (hydroxyl, OH-groups) in the fibre structure, which interact with water *via* hydrogen (H) bonding (Zhu *et al.*, 2019). WA is a critical quality property in dough that is closely linked to the yield and quality of bakery products (Sapirstein *et al.*, 2018). Turfani *et al.* (2016) demonstrated that wheat dough supplemented with 10 - 20% carob flour slightly increased the WA. Thus, carob flour may positively affect dough behaviour during mixing, fermentation, and baking *via* hydration effects.

DT is the time needed for the dough to reach maximum consistency. In the present work, the DT value of sourdough increased from 3.65 ± 0.05 (0% carob flour) to 25.50 ± 0.02 min (15% carob flour), which was approximately 7-fold higher than that of wheat flour. This indicated strong gluten and dough characteristics (MacRitchie, 2016). Cui *et al.* (2023) reported that the dough DT is related to the protein content. Thus, prolonged DT is required for strong flour. The protein contents of carob and whole wheat flour are approximately 4.55 and 13.2%, respectively (Papaefstathiou *et al.*, 2018). The rheological properties of carob flour or sourdough depend on the chemical composition (Novotni *et al.*, 2020). Recent studies have demonstrated that carob can improve the DT characteristics of dough as it exhibits higher hydration rates than wheat.

The addition of carob flour at concentrations of 0, 10, and 15% increased the ST (min) by 1.7-fold. A dough enriched with fibre-rich ingredients (such as carob) exhibited increased stability and WA (Pycia and Juszczak, 2023). Moreover, the ST value provides information about the strength of a flour (Li *et al.*, 2023). The replacement of wheat flour with an alternative flour decreased the strength of the dough due to the dilution of gluten, negatively affecting the

development of the gluten network in the dough. The sum of DT and ST indicates the resistance of dough to mixing (Sadowska *et al.*, 2003). The present work demonstrated that carob increased the stability of a dough formulation depending on the quantity and quality of its gluten content.

Extensograph indices

Extensograph data indicated that energy was significantly correlated with resistance ($p < 0.05$). However, energy value was not significantly associated with extensibility and ratio number ($p \geq 0.05$).

The wheat flour (without carob flour addition) exhibited higher energy values than the blends, except for the energy values of blends with 5, 10, and 15% carob flour at 90 min. This indicated unit extension and processability of dough (Yavuz *et al.*, 2021). Previous studies have reported an increase in energy values in wheat-carob blends (from 142.9 to 196.4 cm²) (Sorolja-Simovic *et al.*, 2016) with an average value of up to 129 cm² (Miš *et al.*, 2012), which was mainly due to gluten protein-associated changes (Hrušková *et al.*, 2006). The findings of the present work agreed with those of previous studies. Thus, carob can be used to improve the processability of doughs.

The extensibility at different time points was negatively affected by carob flour supplementation. Extensibility is the length of the swelling curve and a critical factor that determines the quality and purpose of the final flour product (Luo *et al.*, 2023). Issaoui *et al.* (2021), Karlidağ *et al.* (2022), and Zahorec *et al.* (2024) demonstrated that carob flour supplementation (5, 10, and 20%) to wheat flour decreased extensibility. In the present work, the highest proportion of carob flour (15%) decreased extensibility at the three time points by 3-fold when compared with wheat flour. Thus, increasing the amount of carob flour decreased extensibility and increased resistance. The resistance to extension in the wheat-carob flour blends increased by 24, 49, and 66% at 45, 90, and 135 min, respectively, which was consistent with a 70% increase in resistance reported by Sorolja-Simovic *et al.* (2016). The optimal ratio of resistance to extensibility was reported to be 1.5 to 2.5 (Zahorec *et al.*, 2024). Zahorec *et al.* (2025) determined that supplementation with 5% carob flour significantly decreased extensibility by 5%. Additionally, the resistance to extensibility ratio value was 2.9. The present work demonstrated that

the resistance to extensibility ratio was 4.6 in the blend supplemented with 5% carob flour, which was above the upper limit of the optimal range. This indicated that carob flour must be supplemented in an optimal proportion for preparing dough used for baking purposes. Thus, the addition of carob flour led to a significant cause-and-effect association between extensibility and dough resistance ($p < 0.05$) but not between energy and extensibility ($p \geq 0.05$).

The ratio number increased in blends supplemented with 15% carob flour (from 2.12 ± 0.01 (0% carob flour) to 7.45 ± 0.12 , 14.66 ± 0.05 , and 15.44 ± 0.05 at 45, 90, and 135 min, respectively). The resistance was significantly and positively correlated with the ratio number. Thus, the farinograph and extensograph indices were partially dependent on the duration.

Conclusion

The present work compared the effects of carob and wheat flours on the nutritional, health, and pre-bake rheological properties of spontaneously fermented sourdough. Results indicated that carob is a natural, non-wheat, healthy, and nutritious ingredient for dough formulations of baked products. However, further studies are needed to examine the biochemical function of dough formulations and validate the final product. Therefore, the elucidation of the mode of action of carob during sourdough fermentation can aid in ensuring the manufacture of customised and healthy fermented foods, especially baked products, for the specific health needs of consumers.

Acknowledgement

The authors gratefully acknowledge the Hygiene Laboratory of Tipaza and its staff in Algeria, Dr. Mustafa Yaman, Dr. Ömer Faruk Mızrak, Dr. Yasemin Yılmaz, Exp. Aleyna Çavdar, Full-Gen Biotechnology and Ek-Im Life Sciences, and Dr. Ömer Akineden for their support in the analyses.

References

- Abdik, H. 2022. Antineoplastic effects of erufosine on small cell and non-small cell lung cancer cells through induction of apoptosis and cell cycle arrest. *Molecular Biology Reports* 49(4): 2963-2971.

- American Association of Cereal Chemists (AACC). 2000. International standard methods no: 54-21 and no: 54-10. United States: AACC.
- Anastasio, M., Pepe, O., Cirillo, T., Palomba, S., Blaiotta, G. and Villani, F. 2010. Selection and use of phytate-degrading LAB to improve cereal-based products by mineral solubilization during dough fermentation. *Journal of Food Science* 75(1): M28-M35.
- Association of Official Agricultural Chemists (AOAC). 2009a. Official methods of analysis no: UNI EN ISO 10993-5 - Biological evaluation of medical devices - part 5: *In vitro* cytotoxicity testing. United States: AOAC.
- Association of Official Agricultural Chemists (AOAC). 2009b. Official methods of analysis no: UNI EN ISO 10993-12 - Biocompatibility testing of medical devices. United States: AOAC.
- Ayad, R., Ayad, R., Zineddine, L. and Berghida, N. E. H. 2023. Algerian wild green carob (*Ceratonia siliqua* L.): Physicochemical characteristics and antioxidant potency. *Bulletin of the Chemical Society of Ethiopia* 38(1): 187-198.
- Aydın, S., Özdemir, Y. and Keçeli, M. 2022. Enrichment of wheat bread with carob molasses source: Impact on bread quality and acceptability. *Toros University Journal of Food Nutrition and Gastronomy* 1(1): 17-26.
- Azab, A. 2022. D-Pinitol—Active natural product from carob with notable insulin regulation. *Nutrients* 14(7): 1453.
- Azaizeh, H., Tayeh, H. N. A., Schneider, R. and Venus, J. 2022. Pilot scale for production and purification of lactic acid from *Ceratonia siliqua* L. (carob) bagasse. *Fermentation* 8(9): 424.
- Banu, I., Vasilean, I. and Aprodu, I. 2010. Effect of lactic fermentation on antioxidant capacity of rye sourdough and bread. *Food Science and Technology Research* 16(6): 571-576.
- Bartkiene, E., Özogul, F. and Rocha, J. M. 2022. Bread sourdough lactic acid bacteria—Technological, antimicrobial, toxin-degrading, immune system-, and faecal microbiota-modelling biological agents for the preparation of food, nutraceuticals and feed. *Foods* 11(3): 452.
- Batifoulier, F., Verny, M., Chanliaud, E., Rémésy, C. and Demigné, C. 2005. Effect of different breadmaking methods on thiamine, riboflavin and pyridoxine contents of wheat bread. *Journal of Cereal Science* 42(1): 101-108.
- Batifoulier, F., Verny, M., Chanliaud, E., Rémésy, C. and Demigné, C. 2006. Variability of B vitamin concentrations in wheat grain, milling fractions and bread products. *European Journal of Agronomy* 25(2): 163-169.
- Beta, T., Nam, S., Dexter, J. E. and Sapiirstein, H. D. 2005. Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry* 82(4): 390-393.
- Brassescos, M. E., Vilas-Boas, A. M., Brandão, T. R. S., Silva, C. L. M., Azevedo, M. and Pintado, M. 2023. Impact of roasting temperature and seed presence on carob flour (*Ceratonia siliqua* L.): Physical, chemical, and functional properties. *ACS Food Science and Technology* 3(11): 1890-1902.
- Buzzanca, C., D'Amico, A., Pistorio, E., Di Stefano, V. and Melilli, M. G. 2025. Carob-based functional beverages: Nutritional value and health properties. *Beverages* 11(1): 1.
- Cakir, E., Arici, M. and Durak, M. Z. 2021. Effect of starter culture sourdough prepared with Lactobacilli and *Saccharomyces cerevisiae* on the quality of hull-less barley-wheat bread. *LWT - Food Science and Technology* 152: 112230.
- Cannella, V., Altomare, R., Leonardi, V., Russotto, L., Di Bella, S., Mira, F. and Guercio, A. 2020. *In vitro* biocompatibility evaluation of nine dermal fillers on L929 cell line. *BioMed Research International* 2020: 8676343.
- Cardinali, F., Garofalo, C., Reale, A., Boscaino, F., Osimani, A., Milanović, V., ... and Aquilanti, L. 2022. Liquid sourdough from stone-ground soft wheat (*Triticum aestivum*) flour: Development and exploitation in the breadmaking process. *Food Research International* 161: 111796.
- Chondrou, T., Adamidi, N., Lygouras, D., Hirota, S. A., Androutsos, O. and Svolos, V. 2024. Dietary phytic acid, dephytinization, and phytase supplementation alter trace element bioavailability—A narrative review of human interventions. *Nutrients* 16(23): 4069.
- Christou, A., Stavrou, I. J. and Kapnissi-Christodoulou, C. P. 2021. Continuous and pulsed ultrasound-assisted extraction of carob's antioxidants: Processing parameters

- optimization and identification of polyphenolic composition. *Ultrasonics Sonochemistry* 76: 105630.
- Cui, C., Caporaso, N., Chen, J. and Fearn, T. 2023. Farinograph characteristics of wheat flour predicted by near infrared spectroscopy with an ensemble modelling method. *Journal of Food Engineering* 359: 111689.
- D'Amico, V., Gänzle, M., Call, L., Zwirzitz, B., Grausgruber, H., D'Amico, S. and Brouns, F. 2023. Does sourdough bread provide clinically relevant health benefits? *Frontiers in Nutrition* 10: 1230043.
- Dapčević-Hadnađev, T., Stupar, A., Stevanović, D., Škrobot, D., Maravić, N., Tomić, J. and Hadnađev, M. 2022. Ancient wheat varieties and sourdough fermentation as a tool to increase bioaccessibility of phenolics and antioxidant capacity of bread. *Foods* 11(24): 3985.
- Dewanto, V., Wu, X., Adom, K. K. and Liu, R. H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* 50(10): 3010-3014.
- Doğan, M. and Tekiner, İ. H. 2019. Assessment of probiotic properties of lactic acid bacteria from traditional sourdoughs for bread-making in Turkey against some gut conditions. *Journal of Applied Food Technology* 6(2): 34-40.
- Eraslan, H., Wehbeh, J. and Ermis, E. 2023. Effect of sourdough prepared with the combination of chickpea and carob on bread properties. *International Journal of Gastronomy and Food Science* 32: 100753.
- Fang, L., Wang, W., Dou, Z., Chen, J., Meng, Y., Cai, L. and Li, Y. 2023. Effects of mixed fermentation of different lactic acid bacteria and yeast on phytic acid degradation and flavor compounds in sourdough. *LWT - Food Science and Technology* 174: 114438.
- Fekri, A., Abedinzadeh, S., Torbati, M., Azadmard-Damirchi, S. and Savage, G. P. 2024. Considering sourdough from a biochemical, organoleptic, and nutritional perspective. *Journal of Food Composition and Analysis* 125: 105853.
- Fernández-Peláez, J., Paesani, C. and Gómez, M. 2020. Sourdough technology as a tool for the development of healthier grain-based products: An update. *Agronomy* 10(12): 1962.
- Fraberger, V., Ladurner, M., Nemeč, A., Grunwald-Gruber, C., Call, L. M., Hochegger, R., ... and D'Amico, S. 2020. Insights into the potential of sourdough-related lactic acid bacteria to degrade proteins in wheat. *Microorganisms* 8(11): 1689.
- Gabriele, M., Cavallero, A., Tomassi, E., Arouna, N., Árvay, J., Longo, V. and Pucci, L. 2024. Assessment of sourdough fermentation impact on the antioxidant and anti-inflammatory potential of pearl millet from Burkina Faso. *Foods* 13(5): 704.
- Gioxari, A., Amerikanou, C., Nestoridi, I., Gourgari, E., Pratsinis, H., Kalogeropoulos, N., ... and Kaliora, A. C. 2022. Carob: A sustainable opportunity for metabolic health. *Foods* 11(14): 2154.
- Gobbetti, M., de Angelis, M., di Cagno, R., Calasso, M., Archetti, G. and Rizzello, C. G. 2019. Novel insights on the functional/nutritional features of the sourdough fermentation. *International Journal of Food Microbiology* 302: 103-113.
- González-Alonso, V., Pradal, I., Wardhana, Y. R., Cnockaert, M., Wieme, A. D., Vandamme, P. and De Vuyst, L. 2024. Microbial ecology and metabolite dynamics of backslopped triticale sourdough productions and the impact of scale. *International Journal of Food Microbiology* 408: 110445.
- Goulas, V. and Georgiou, E. 2019. Utilization of carob fruit as sources of phenolic compounds with antioxidant potential: Extraction optimization and application in food models. *Foods* 9(1): 20.
- Goulas, V., Stylos, E., Chatziathanasiadou, M., Mavromoustakos, T. and Tzakos, A. 2016. Functional components of carob fruit: Linking the chemical and biological space. *International Journal of Molecular Sciences* 17(11): 1875.
- Hara, K., Someya, T., Sano, K., Sagane, Y., Watanabe, T. and Wijesekara, R. 2018. Antioxidant activities of traditional plants in Sri Lanka by DPPH free radical-scavenging assay. *Data in Brief* 17: 870-875.
- Haros, M., Rosell, C. M. and Bedito, C. 2001. Fungal phytase as a potential breadmaking

- additive. *European Food Research and Technology* 213(4-5): 317-322.
- Hernández-Figueroa, R. H., Mani-López, E., Palou, E. and López-Malo, A. 2023. Sourdoughs as natural enhancers of bread quality and shelf life: A review. *Fermentation* 10(1): 7.
- Hrušková, M., Švec, I. and Jirsa, O. 2006. Correlation between milling and baking parameters of wheat varieties. *Journal of Food Engineering* 77(3): 439-444.
- Igreja, W. S., De Andrade Maia, F., Lopes, A. S. and Chisté, R. C. 2021. Biotechnological production of carotenoids using low cost-substrates is influenced by cultivation parameters: A review. *International Journal of Molecular Sciences* 22(16): 8819.
- Ikram, A., Khalid, W., Zafar, K. W., Ali, A., Afzal, M. F., Aziz, A., ... and Koraqi, H. 2023. Nutritional, biochemical, and clinical applications of carob: A review. *Food Science and Nutrition* 11(7): 3641-3654.
- İlgin, M., Germec, M. and Turhan, I. 2019. Inulinase production and mathematical modeling from carob extract by using *Aspergillus niger*. *Biotechnology Progress* 36(1): e2919.
- Ioannou, G. D., Savva, I. K., Christou, A., Stavrou, I. J. and Kapnissi-Christodoulou, C. P. 2023. Phenolic profile, antioxidant activity, and chemometric classification of carob pulp and products. *Molecules* 28(5): 2269.
- Issaoui, M., Flamini, G. and Delgado, A. 2021. Sustainability opportunities for Mediterranean food products through new formulations based on carob flour (*Ceratonia siliqua* L.). *Sustainability* 13(14): 8026.
- Ivanova, P., Chochkov, R., Zlateva, D. and Stefanova, D. 2023. Total phenolic and flavonoid content, and antioxidant activity of wheat bread enriched with pumpkin, chestnut and rosehip flour. *Carpathian Journal of Food Science and Technology* 15(2): 33-41.
- Javadi, F., Yazdi, M. E. T., Baghani, M. and Es-Haghi, A. 2019. Biosynthesis, characterization of cerium oxide nanoparticles using *Ceratonia siliqua* and evaluation of antioxidant and cytotoxicity activities. *Materials Research Express* 6(6): 065408.
- Karlidağ, S., Arici, M. and Özülkü, G. 2022. Carob flour addition to sourdough: Effect of sourdough fermentation, dough rheology and bread quality. *Journal of Tekirdag Agricultural Faculty* 19(3): 541-553.
- Katina, K., Heiniö, R., Autio, K. and Poutanen, K. 2006. Optimization of sourdough process for improved sensory profile and texture of wheat bread. *LWT - Food Science and Technology* 39(10): 1189-1202.
- Khazaei, F., Bozorgi, M. and Khazaei M. 2023. Anticancer effect of carob bean extract on human prostate cancer cell lines: Apoptosis induction and Bax/Bcl-2 ratio improvement. *Middle East Journal of Cancer* 14(3): 363-369.
- Kirchhoff, E. and Schieberle, P. 2002. Quantitation of odor-active compounds in rye flour and rye sourdough using stable isotope dilution assays. *Journal of Agricultural and Food Chemistry* 50(19): 5378-5385.
- Klenow, S., Jahns, F., Pool-Zobel, B. L. and Glej, M. 2009. Does an extract of carob (*Ceratonia siliqua* L.) have chemopreventive potential related to oxidative stress and drug metabolism in human colon cells? *Journal of Agricultural and Food Chemistry* 57(7): 2999-3004.
- Krishnan, S. V., Nampoothiri, K. M., Suresh, A., Linh, N. T., Balakumaran, P. A., Pócsi, I. and Pusztahelyi, T. 2024. Fusarium biocontrol: Antagonism and mycotoxin elimination by lactic acid bacteria. *Frontiers in Microbiology* 14: 1260166.
- Kuhnert, P., Capaul, S. E., Nicolet, J. and Frey, J. 1996. Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. *International Journal of Systematic Bacteriology* 46(4): 1174-1176.
- Kulcan, A. A., Assoumou, U. Z., Aygün, M., Kuzu, Ş., Yildiz, D., Kaya, N., ... and Karhan, M. 2021. Impact of carob extract supplementation on chemical and sensory properties of yogurt and ice cream. *The Journal of Food* 46(4): 980-991.
- Laaraj, S., Salmaoui, S., Addi, M., El-Rhouttais, C., Tikent, A., Elbouzidi, A., ... and Elfazazi, K. 2023. Carob (*Ceratonia siliqua* L.) seed constituents: A comprehensive review of composition, chemical profile, and diverse applications. *Journal of Food Quality* 2023: 1-14.
- Leenhardt, F., Levrat-Verny, M., Chanliaud, E. and Rémésy, C. 2004. Moderate decrease of pH by

- sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *Journal of Agricultural and Food Chemistry* 53(1): 98-102.
- Li, R., Wang, C., Wang, Y., Xie, X., Sui, W., Liu, R., ... and Zhang, M. 2023. Extrusion modification of wheat bran and its effects on structural and rheological properties of wheat flour dough. *Foods* 12(9): 1813.
- Lim, S. B., Tingirikari, J. M. R., Seo, J. S., Li, L., Shim, S., Seo, J. and Han, N. S. 2017. Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough fermentation. *Food Science and Biotechnology* 27(1): 73-78.
- Lima, T. T. M., de Oliveira Hosken, B., de Dea Lindner, J., Menezes, L. A. A., Pirozi, M. R. and Martin, J. G. P. 2023. How to deliver sourdough with appropriate characteristics for the bakery industry? The answer may be provided by microbiota. *Food Bioscience* 56: 103072.
- Longin, C. F. H., Afzal, M., Pfanstiel, J., Bertsche, U., Melzer, T., Ruf, A., ... and Rodehutschord, M. 2023. Mineral and phytic acid content as well as phytase activity in flours and breads made from different wheat species. *International Journal of Molecular Sciences* 24(3): 2770.
- Luo, X., Yu, H., An, J., Wang, H. and Tang, X. 2023. Determination of extensibility and toughness of wheat-flour dough based on bubbles blown by sheeted dough and airflow-3D imaging technique. *Journal of Cereal Science* 113: 103754.
- MacRitchie, F. 2016. Seventy years of research into breadmaking quality. *Journal of Cereal Science* 70: 123-131.
- Martínez-Villaluenga, C., Peñas, E., Rico, D., Martín-Diana, A., Portillo, M., Macarulla, M., ... and Miranda, J. 2018. Potential usefulness of a wakame/carob functional snack for the treatment of several aspects of metabolic syndrome: From *in vitro* to *in vivo* studies. *Marine Drugs* 16(12): 512.
- Martins-Loução, M. A., Correia, P. J. and Romano, A. 2024. Carob: A Mediterranean resource for the future. *Plants* 13(9): 1188.
- Mateeva, A., Kondeva-Burdina, M., Peikova, L., Guncheva, S., Zlatkov, A. and Georgieva, M. 2023. Simultaneous analysis of water-soluble and fat-soluble vitamins through RP-HPLC/DAD in food supplements and brewer's yeast. *Heliyon* 9(1): e12706.
- Miś, A., Grundas, S., Dziki, D. and Laskowski, J. 2012. Use of farinograph measurements for predicting extensograph traits of bread dough enriched with carob fibre and oat wholemeal. *Journal of Food Engineering* 108(1): 1-12.
- Moore, M. M., Bello, F. D. and Arendt, E. K. 2007. Sourdough fermented by *Lactobacillus plantarum* FST 1.7 improves the quality and shelf life of gluten-free bread. *European Food Research and Technology* 226(6): 1309-1316.
- Najafi, M. A., Rezaei, K., Safari, M. and Razavi, S. H. 2012. Use of sourdough to reduce phytic acid and improve zinc bioavailability of a traditional flat bread (sangak) from Iran. *Food Science and Biotechnology* 21(1): 51-57.
- Novotni, D., Mutak, N., Nanjara, L., Drakula, S., Mustač, N. Č., Voučko, B. and Čurić, D. 2020. Sourdough fermentation of carob flour and its application to wheat bread. *Food Technology and Biotechnology* 58(4): 465-474.
- Ognean, C. F. 2015. The technological evaluation of sourdoughs prepared in different conditions. *Management of Sustainable Development* 7(1): 33-36.
- Oguntoyinbo, F. A. and Narbad, A. 2012. Molecular characterization of lactic acid bacteria and *in situ* amylase expression during traditional fermentation of cereal foods. *Food Microbiology* 31(2): 254-262.
- Oshiro, M., Tanaka, M., Zendo, T. and Nakayama, J. 2020. Impact of pH on succession of sourdough lactic acid bacteria communities and their fermentation properties. *Bioscience of Microbiota Food and Health* 39(3): 152-159.
- Papaefstathiou, E., Agapiou, A., Giannopoulos, S. and Kokkinofa, R. 2018. Nutritional characterization of carobs and traditional carob products. *Food Science and Nutrition* 6(8): 2151-2161.
- Paramithiotis, S., Tsiasiotou, S. and Drosinos, E. H. 2010. Comparative study of spontaneously fermented sourdoughs originating from two regions of Greece: Peloponnesus and Thessaly. *European Food Research and Technology* 231(6): 883-890.
- Pejcz, E., Lachowicz-Wiśniewska, S., Nowicka, P., Wojciechowicz-Budzisz, A., Szychaj, R. and Gil, Z. 2021. Effect of inoculated lactic acid

- fermentation on the fermentable saccharides and polyols, polyphenols and antioxidant activity changes in wheat sourdough. *Molecules* 26(14): 4193.
- Pérez-Alvarado, O., Zepeda-Hernández, A., Garcia-Amezquita, L. E., Requena, T., Vinderola, G. and García-Cayuela, T. 2022. Role of lactic acid bacteria and yeasts in sourdough fermentation during breadmaking: Evaluation of postbiotic-like components and health benefits. *Frontiers in Microbiology* 13: 969460.
- Pycia, K. and Juszcak, L. 2023. Rheological characteristics of wheat dough containing powdered hazelnuts or walnuts oil cakes. *Foods* 13(1): 140.
- Rašković, A., Martić, N., Tomas, A., Andrejić-Višnjić, B., Bosanac, M., Atanasković, M., ... and Stilinović, N. 2023. Carob extract (*Ceratonia siliqua* L.): Effects on dyslipidemia and obesity in a high-fat diet-fed rat model. *Pharmaceutics* 15(11): 2611.
- Rehagel, C., Akineden, Ö. and Usleber, E. 2022. Microbiological and mycotoxicological analyses of processed cereal-based complementary foods for infants and young children from the German market. *Journal of Food Science* 87(4): 1810-1822.
- Sadowska, J., Błaszczak, W., Fornal, J., Vidal-Valverde, C. and Frias, J. 2003. Changes of wheat dough and bread quality and structure as a result of germinated pea flour addition. *European Food Research and Technology* 216(1): 46-50.
- Salovaara, H. and Valjakka, T. 1987. The effect of fermentation temperature, flour type, and starter on the properties of sour wheat bread. *International Journal of Food Science and Technology* 22(6): 591-597.
- Sánchez-Adriá, I. E., Sanmartín, G., Prieto, J. A., Estruch, F., Fortis, E. and Randez-Gil, F. 2023. Technological and acid stress performance of yeast isolates from industrial sourdough. *LWT - Food Science and Technology* 184: 114957.
- Sanmartín, G., Prieto, J. A., Morard, M., Estruch, F., Blasco-García, J. and Randez-Gil, F. 2025. The effects of sourdough fermentation on the biochemical properties, aroma profile and leavening capacity of carob flour. *Foods* 14(10): 1677.
- Sapirstein, H., Wu, Y., Koksel, F. and Graf, R. 2018. A study of factors influencing the water absorption capacity of Canadian hard red winter wheat flour. *Journal of Cereal Science* 81: 52-59.
- Sebai, H., Souli, A., Chehimi, L., Rtibi, K., Amri, M., El-Benna, J. and Sakly, M. 2013. *In vitro* and *in vivo* antioxidant properties of Tunisian carob (*Ceratonia siliqua* L.). *Journal of Medicinal Plants Research* 7(2): 85-90.
- Şerban, L. R., Păucean, A., Chiş, M. S., Pop, C. R., Man, S. M., Puşcaş, A., ... and Mureşan, V. 2023. Metabolic profile of einkorn, spelt, emmer ancient wheat species sourdough fermented with strain of *Lactiplantibacillus plantarum* ATCC 8014. *Foods* 12(5): 1096.
- Shongwe, S. G., Kidane, S. W., Shelembe, J. S. and Nkambule, T. P. 2022. Dough rheology and physicochemical and sensory properties of wheat-peanut composite flour bread. *Legume Science* 4(3): e138.
- Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3): 144-158.
- Soronja-Simovic, D., Smole-Mozina, S., Raspor, P., Maravic, N., Zahorec, J., Luskar, L. and Seres, Z. 2016. Carob flour and sugar beet fiber as functional additives in bread. *Acta Periodica Technologica* 47: 83-93.
- Tomić, J., Dapčević-Hadnađev, T., Škrobot, D., Maravić, N., Popović, N., Stevanović, D. and Hadnađev, M. 2023. Spontaneously fermented ancient wheat sourdoughs in breadmaking: Impact of flour quality on sourdough and bread physico-chemical properties. *LWT - Food Science and Technology* 175: 114482.
- Tsatsaragkou, K., Mandala, I. and Stoforos, N. G. 2023. Fermentation kinetics of gluten-free breads: The effect of carob fraction and water content. *Foods* 12(9): 1809.
- Turfani, V., Narducci, V., Durazzo, A., Galli, V. and Carcea, M. 2016. Technological, nutritional and functional properties of wheat bread enriched with lentil or carob flours. *LWT - Food Science and Technology* 78: 361-366.
- Voučko, B., Čukelj Mustač, N., Nanjara, L., Drakula, S., Grgić, T., Čurić, D. and Novotni, D. 2024. Fermentation performance of carob flour,

- proso millet flour and bran for gluten-free flatbread. *Foods* 13(21): 3458.
- Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., ... and Geng, W. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology* 9: 612285.
- Wang, Z. and Wang, L. 2024. Impact of sourdough fermentation on nutrient transformations in cereal-based foods: Mechanisms, practical applications, and health implications. *Grain and Oil Science and Technology* 7(2): 124-132.
- Xu, D., Zhang, H., Xi, J., Jin, Y., Chen, Y., Guo, L., ... and Xu, X. 2020. Improving bread aroma using low-temperature sourdough fermentation. *Food Bioscience* 37: 100704.
- Yavuz, Z., Tornuk, F. and Durak, M. Z. 2021. Effect of oleaster flour addition as a source of dietary fiber on rheological properties of wheat dough. *European Food Science and Engineering* 2(1): 7-12.
- Ydjedd, S., Bouriche, S., López-Nicolás, R., Sánchez-Moya, T., Frontela-Saseta, C., Ros-Berruezo, G., ... and Kati, D. 2017. Effect of *in vitro* gastrointestinal digestion on encapsulated and nonencapsulated phenolic compounds of carob (*Ceratonia siliqua* L.) pulp extracts and their antioxidant capacity. *Journal of Agricultural and Food Chemistry* 65(4): 827-835.
- Yildirim, R. M. and Arici, M. 2019. Effect of the fermentation temperature on the degradation of phytic acid in whole-wheat sourdough bread. *LWT - Food Science and Technology Journal* 112: 108224.
- Zahorec, J., Šoronja-Simović, D., Petrović, J., Nikolić, I., Pavlić, B., Bijelić, K., ... and Fišteš, A. 2025. Fortification of bread with carob extract: A comprehensive study on dough behavior and product quality. *Foods* 14(10): 1821.
- Zahorec, J., Šoronja-Simović, D., Petrović, J., Šereš, Z., Pavlić, B., Sterniša, M., ... and Jozinović, A. 2024. The effect of carob extract on antioxidant, antimicrobial and sensory properties of bread. *Applied Sciences* 14(9): 3603.
- Zhu, B. J., Zayed, M. Z., Zhu, H. X., Zhao, J. and Li, S. P. 2019. Functional polysaccharides of carob fruit: A review. *Chinese Medicine* 14: 40.