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RESEARCH

Targeted Profiling of Tryptophan- and Tyrosine-Derived Metabolites in Traditionally Fermented Foods

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Abstract

This study aimed to investigate the tryptophan and tyrosine-derived metabolites in traditional fermented foods. For that purpose, kynurenine, kynurenic acid, indole-3-acetic acid, indole-3-propionic acid, phenol, and *p*-cresol levels were analyzed using High-performance liquid chromatography (HPLC). Tryptophan metabolite levels ranged from 62.4 to 718.5 µg/100 g for kynurenine, 1.1 to 340.0 µg/100 g for kynurenic acid, 0.0 to 36.6 µg/100 g for indole-3-acetic acid, and 0.0 to 72.5 µg/100 g for indole-3-propionic acid. Tyrosine metabolite levels ranged from 4.4 to 115.7 µg/100 g for phenol and 0.0 to 7.2 µg/100 g for *p*-cresol. Traditional, fermented, and aged products such as tarhana appear to contain high levels of various metabolites of tryptophan metabolism. Phenol levels were also found to be high in tarhana products and aged dairy products. *p*-cresol levels remained generally low, with significantly high levels found only in aged cheddar cheese. These results suggest that microbial fermentation and long-term maturation may be associated with differences in the biochemical composition of the fermented foods. It is of great importance to evaluate fermented products in terms of metabolites with neuroactive and inflammatory potential.

Keywords Fermented foods · Kynurenines · Indole-derived acids phenol · *p*-cresol

Introduction

The human gut microbiota is a dynamic and complex ecosystem of microorganisms that has multifaceted effects on host health. Beyond digestion, it plays a role in many vital functions, such as regulating the immune system, controlling inflammation, protecting against pathogens, and producing various bioactive metabolites [1]. Disruptions of the structural and functional integrity of the microbiota (dysbiosis) have been associated with many health issues, including inflammatory bowel diseases, metabolic syndrome, neurodegenerative diseases, and even certain types of cancer [2]. It is reported that there are approximately 10^{12} microorganisms in the large intestine of an adult human, with the number of microbial cells being approximately 30% greater than that of human cells. This microbial community consists of 500 to 1,000 different species, the majority of which are composed of *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* bacteria [3]. The microbiota exhibits a wide range of diversity, and one of the most significant factors shaping this diversity is said to be indigestible food components, such as carbohydrates, lipids, proteins and some bioactive compounds [4].

Indigestible carbohydrates cannot be broken down by digestive enzymes and reach the large intestine, where they are fermented by the microbiota, promoting the production of short-chain fatty acids (SCFAs). These carbohydrates also support the proliferation of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, increasing microbial diversity, reducing the risk of dysbiosis, regulating the immune system, and exerting effects on immune cells via SCFAs, supporting the activation of regulatory T cells and reducing pro-inflammatory responses [5]. The interaction between dietary fiber and gut health is a well-established topic that

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has been extensively studied in the literature. In addition, increasing attention has been directed toward understanding the role of proteins and their metabolic byproducts in modulating intestinal functions [6].

However, when fiber intake is insufficient or protein-rich diets are consumed, the microbiota increasingly turns to proteolytic fermentation as an energy source. This process primarily occurs in the distal sections of the large intestine, resulting in the microbial breakdown of dietary or endogenous proteins [7]. This breakdown produces various microbial protein metabolites, such as indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), kynurine, kynurenic acid, phenol, *p*-cresol and others [3]. These compounds, derived from aromatic amino acids including tryptophan and tyrosine, have significant effects on the gut-brain axis, the immune system, and epithelial cell health [3, 7–10]. Tryptophan is a precursor to serotonin, melatonin, and niacin; therefore, it has indirect effects on mood regulation, sleep, and immune function [11]. Tyrosine is the primary substrate in the biosynthesis of catecholamine neurotransmitters, such as dopamine, adrenaline, and noradrenaline and is associated with stress response, motivation, and cognitive function. The metabolic conversion products of these amino acids are considered important biomarkers in terms of both host physiology and gut microbiota interactions. The metabolic fate of tryptophan and tyrosine-derived molecules in fermented foods may have implications for nutrition, microbial fermentation and inflammatory processes [12].

Fermented foods are products that have been developed through the controlled activity of microorganisms, resulting in improved shelf-life, nutritional content, and sensory characteristics. During fermentation, microorganisms break down carbohydrates and/or proteins in food to produce various bioactive compounds. This process enhances the sensory properties and also provides health benefits, including probiotic effects, immune system support, improved digestion, and antimicrobial and anti-inflammatory potential [13]. In this context, the examination of microbial metabolites in fermented products is of great importance in terms of evaluating the microbial profile of the product and the biological effects that may occur depending on the fermentation process. Although previous studies have quantified individual kynurenine-pathway metabolites in selected fermented foods [14–16], there is still a lack of comparative data simultaneously covering both tryptophan- and tyrosine-derived metabolites across different traditional products. For that purpose, this study aims to identify proteolytic fermentation products in fermented foods, including cheese, kefir, yogurt, tarhana, and sucuk. We hypothesized that traditionally fermented and aged foods would contain higher levels of selected tryptophan- and tyrosine-derived microbial metabolites compared with fresh or less-fermented products. By simultaneously quantifying kynurenine-pathway metabolites and phenolic fermentation products, this study provides novel insight into how traditional fermentation and aging practices shape metabolite composition in commonly consumed foods.

Materials and Methods

Samples

A total of 24 different fermented foods including cheese, yogurt, kefir, tarhana and sucuk were purchased from commercial markets in Istanbul, Türkiye. The energy and nutrient values, including fat, carbohydrates, sugar, protein, salt, and fiber, declared on the label are presented in Table 1. All samples were analyzed immediately after purchase without any freezing or long-term storage to minimize the degradation of labile metabolites. Each sample was analyzed in triplicate (technical replicates), and the mean values were used for statistical evaluation.

Chemicals

All chemicals used in this study were of analytical or HPLC grade. Methanol (≥99.9%), acetonitrile (≥99.9%), potassium dihydrogen phosphate (KH_2PO_4), and sodium dihydrogen phosphate (NaH_2PO_4) were obtained from Merck (Darmstadt, Germany). Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7\ H_2O$) and potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6] \cdot 3\ H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All aqueous solutions were prepared using ultrapure water (resistivity≥18.2 MΩ·cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). CAREZ I solution was prepared by dissolving 15 g of potassium hexacyanoferrate(II) trihydrate in distilled water. CAREZ II solution was prepared as a 30% (w/v) aqueous solution of zinc sulfate heptahydrate. Due to their limited solubility, both solutions were dissolved using an ultrasonic water bath prior to use. Standard compounds of kynurenine, kynurenic acid, phenol, *p*-cresol, indole-3-acetic acid, and indole-3-propionic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA) and prepared in appropriate solvents as stock solutions, then diluted to working concentrations for HPLC analysis. Syringe filters (0.45 µm, cellulose acetate) used for sample clarification prior to injection into the HPLC system were purchased from Sartorius (Göttingen, Germany).

Sample Preparation for Tyrosine and Tryptophan Metabolites

The extraction procedure described by Yılmaz and Gökmen [16] was applied with minor modifications. Five grams of homogenized food sample were transferred into a 50 mL

Table 1 The energy and macronutrient contents of the fermented-food samples as declared on the respective labels. *n.d: not detected

Number	Sample	Energy (kcal/100 g)	Fat (g/100 g)	Saturated Fat (g/100 g)	Carbohydrate (g/100 g)	Sugar (g/100 g)	Protein (g/100 g)	Salt (g/100 g)	Fiber (g/100 g)
1	White cheese	215	16.5	10.7	3.5	3.5	13	2	n.d.
2	white cheese with a soft, creamy texture	225	18	5	4.1	4.1	15	2.5	n.d.
3	Aged cow cheese	311	26	17.9	0.5	0.5	18.8	3.1	n.d.
4	Full-fat aged white cheese with herbs	362	30	20	0.5	0	21	5.4	n.d.
5	Full-fat aged Tulum cheese	353	28	18	0.5	0	26	5	n.d.
6	Full-fat aged Kashar cheese	323	25	9	2.6	2.5	22	2	n.d.
7	Full-fat aged Kashar cheese	350	16.7	16.5	4.19	0.8	24.91	2.6	n.d.
8	Cecil (traditional Turkish string) cheese	331	25	15	1.5	0	25	2.2	n.d.
9	Full-fat fresh string cheese	325	25	15	2	2	22	1.5	n.d.
10	String cheese	284	20	13.4	2.2	0.7	23.9	2	n.d.
11	Yogurt	84	3.8	2.5	7.1	7.1	4.5	0	n.d.
12	Probiotic yogurt	73	3.8	2.3	6	6	3.8	0	n.d.
13	Non-fat yogurt	73	3.8	2.5	5.8	5.8	4	0.1	n.d.
14	Plain kefir	55	3	1.9	3.7	1.7	3	0.2	n.d.
15	lactose-free kefir	55	3	1.9	3.7	1.7	3	0.2	n.d.
16	Probiotic kefir shot	34	0	0	4.6	4.6	3.7	0.1	n.d.
17	Plain kefir with triple probiotics	31	0	0	4.6	4.6	3	0	n.d.
18	Kefir with triple probiotics, elderberry, and blueberry	42	0	0	7.6	7.3	2.7	0	n.d.
19	Kefir with celery, banana, and pineapple	52	2.3	1.5	5	4.5	2.7	0.2	n.d.
20	Instant tarhana soup	349	4.8	0.8	65	4.9	11.4	9.4	3.5
21	Sweetened tarhana	338	2.2	0.3	70	2.6	13	5.3	5
22	Organic tarhana	320	n.d.	n.d.	68.3	7.8	10.7	0.8	6.2
23	Spicy Turkish sausage	444	40	25	5	0	16	2.6	n.d.
24	Spicy Turkish sausage	389	40	20	1.5	0.2	16	1.5	n.d.

polypropylene Falcon tube and extracted with 20 mL of a 50% methanol–water (v/v) solution using an Ultra-Turrax homogenizer for 1 min. The mixture was then centrifuged at 10,000 rpm, and 5 mL of the resulting supernatant was collected. Subsequently, 0.5 mL each of CAREZ I and CAREZ II solutions was added to precipitate proteins. The solution was filtered through a 0.45 µm cellulose acetate syringe filter and transferred into HPLC vials for analysis.

CAREZ I was prepared by dissolving 15 g of potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6]\cdot 3\ H_2O$) in distilled water. CAREZ II consisted of a 30% (w/v) solution of zinc sulfate heptahydrate ($ZnSO_4\cdot 7\ H_2O$) in water. Due to their low solubility, both solutions were dissolved using an ultrasonic water bath [17].

HPLC Analysis of Kynurenine and Kynurenic Acid

The HPLC conditions described by Badawy and Morgan [18] were used with some modifications [18]. Analyses were performed using a Shimadzu Nexera series HPLC system equipped with UV/VIS and fluorescence detectors (Shimadzu Corporation, Kyoto, Japan). The system was operated under isocratic conditions with a total run time of 20 min. Kynurenine was detected by UV at 220 nm, while kynurenic acid was monitored using fluorescence detection at an excitation wavelength of 254 nm and an emission wavelength of 404 nm. Chromatographic separation was carried out on an ACE C18 column (250 × 4.6 mm, 5 µm) maintained at 30 °C. The mobile phase consisted of methanol and 10 mM sodium dihydrogen phosphate buffer (27:73, v/v), adjusted to pH 2.8 with orthophosphoric acid. The total run time was 20 min.

HPLC Analysis of Phenol, *p*-cresol, Indole-3-Acetic Acid, and Indole-3-Propionic Acid

The analysis of phenol, -cresol, indole-3-acetic acid, and indole-3-propionic acid was performed using a modified method based on Calaf et al. [19]. The HPLC system consisted of a Shimadzu Nexera series instrument equipped with a fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Phenol and p-cresol were detected using fluorescence at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. Indole-3-acetic acid and indole-3-propionic acid were detected with an excitation wavelength of 280 nm and an emission wavelength of 360 nm. Chromatographic separation was performed on a Phenomenex Luna C18 column (150 × 4.6 mm, 5 µm), maintained at 30 °C. The mobile phase was composed of solvent A (0.05 M potassium dihydrogen phosphate, adjusted to pH 3.2 with orthophosphoric acid) and solvent B (acetonitrile) in a ratio of 80:20 (v/v), and was delivered isocratically. The total run time was 20 min.

Statistical Analysis

Statistical evaluation of tyrosine and tryptophan metabolites analyses was performed to determine the reliability of the data. The normal distribution of the data was checked by the Shapiro-Wilk test. The mean and standard deviation (SD) were calculated for normally distributed data, and differences between groups were analyzed using one-way analysis of variance (ANOVA). In cases where the ANOVA test showed significant differences, Tukey’s HSD (Hhonest Ssignificant Ddifference) test was used to determine the differences between pairs. The relationships between metabolite levels and nutritional values were evaluated using the Spearman rank correlation test. Results with a *p*-value below 0.05 were considered statistically significant.

Results and Discussion

The HPLC chromatograms of kynurenine and kynurenic acid in the sample 22 (organic tarhana) are shown in Fig. 1(A) and Fig. 1(B), respectively, and the chromatograms of indole-3-acetic acid, indole-3-propionic acid, phenol, and *p*-cresol in Sample 1 (white cheese) are presented in Fig. 2. These chromatograms are shown as representative examples. Figures 1 and 2 demonstrate good peak resolution and baseline separation for all analytes. No significant interfering peaks were observed at the retention times of the target compounds, indicating that the method exhibits adequate selectivity. The chromatographic profile was stable and reproducible across repeated measurements. The chromatograms of all analytical standards and a mixed-standard solution are provided in the Supplementary Material to document retention times, peak shapes, and chromatographic resolution.

The declared energy and nutrient composition on the label, including fat, saturated fat, carbohydrate, sugar, protein, salt, and fiber, for the fermented food samples analyzed

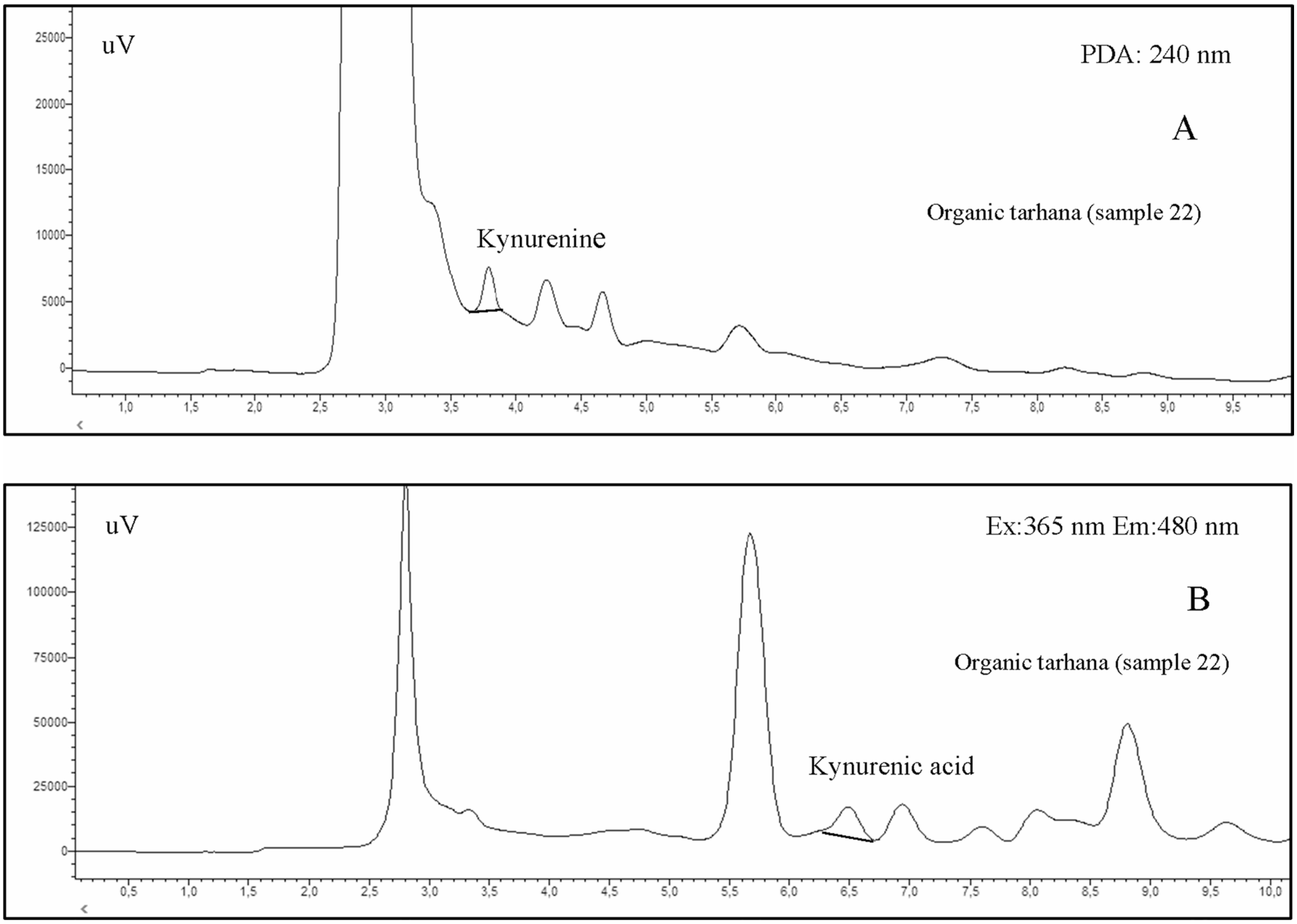


Fig. 1 HPLC chromatograms representation of kynurenine (**A**) and kynurenic acid (**B**) detected in Organic tarhana (sample 22)

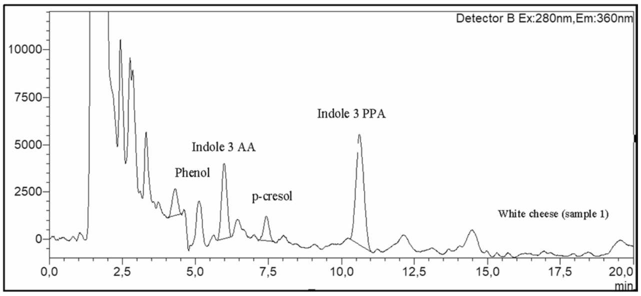


Fig. 2 HPLC chromatograms representation of indole-3-acetic acid (Indole 3 AA), indole-3-propionic acid (Indole 3 PPA), phenol, and p-cresol detected in white cheese (sample 1)

are given in Table 1. The nutritional composition of 24 fermented food products varied widely, with energy ranging from 31 to 444 kcal/100 g, carbohydrates 0.5–70 g, sugar 0–7.8 g, fat 0–40 g, protein 2.7–26 g, and fiber 0–6.2 g.

Tryptophan metabolite amounts, including Kynurenine, Kynurenic acid, Indole-3-acetic acid, and IPA, in the fermented-food samples are presented in Table 2. As observed, the levels of protein-derived microbial metabolites in the analyzed fermented food products exhibited a wide variation. Kynurenine concentrations of fermented-foods ranged from 62.4 to 718.5 µg/100 g; according to product type, the amount of kynurenine in cheese varies between 155 and 324 µg/100 g, in yogurt it ranges from 62 to 158 µg/100 g, in kefir from 62 to 175 µg/100 g, in tarhana it ranges from 135 to 719 µg/100 g, and in sausage it ranges from 196 to 204 µg/100 g. Yılmaz and Gökmen [16] found that dairy products such as yogurt, white cheese, and kefir, also contained kynurenine, with concentrations ranging from 30.3 to 763.8 µg/kg dry weight [16]. In the present study, kynurenine levels are seen to increase in products that are highly fermented and aged, especially those with high

Table 2 Tryptophan metabolite amounts in the fermented-food samples

Number	Sample	Kynurenine	Kynurenic acid	Indole-3-acetic acid	Indole-3-propionic acid
1	White cheese	187.5 ± 5.2^{hi}	2.9 ± 0.2^{ghi}	7.5 ± 0.3^{ef}	7.8 ± 0.1^{f}
2	White cheese with a soft, creamy texture	184.0 ± 2.6^{hi}	1.1 ± 0.1^{i}	32.6 ± 1.9^{b}	3.2 ± 0.1^{ijk}
3	Aged cow cheese	253.5 ± 3.6^{e}	3.8 ± 0.1^{ghi}	6.9 ± 0.2^{efg}	6.7 ± 0.2^{fg}
4	Full-fat aged white cheese with herbs	206.3 ± 6.3^{g}	7.2 ± 0.2^{ghi}	0.0 ± 0.0^{k}	5.4 ± 0.1^{gh}
5	Full-fat aged Tulum cheese	206.7 ± 4.3^{g}	32.0 ± 2.0^{e}	1.7 ± 0.2^{jk}	11.2 ± 0.4^{de}
6	Full-fat aged Kashar cheese	155.2 ± 3.7^{kl}	5.3 ± 0.2^{ghi}	27.6 ± 1.0^{c}	11.9 ± 0.7^{d}
7	Full-fat aged Kashar cheese	305.3 ± 7.0^{d}	7.1 ± 0.2^{ghi}	3.1 ± 0.2^{ij}	9.8 ± 0.2^{e}
8	Cecil (traditional Turkish string) cheese	233.8 ± 3.9^{f}	10.3 ± 0.2^{g}	1.8 ± 0.1^{jk}	3.4 ± 0.1^{ij}
9	Full-fat fresh string cheese	323.7 ± 7.2^{c}	6.1 ± 0.1^{ghi}	3.1 ± 0.2^{ij}	2.8 ± 0.1^{ijkl}
10	String cheese	163.1 ± 4.1^{jk}	7.6 ± 0.3^{ghi}	4.1 ± 0.2^{hi}	3.5 ± 0.1^{ij}
11	Yogurt	134.1 ± 3.6^{mno}	2.0 ± 0.2^{hi}	0.6 ± 0.2^{k}	4.3 ± 0.0^{hi}
12	Probiotic yogurt	147.7 ± 3.7^{lm}	4.9 ± 0.2^{ghi}	1.2 ± 0.1^{k}	0.5 ± 0.1^{no}
13	Non-fat yogurt	158.0 ± 6.2^{kl}	38.8 ± 1.8^{de}	3.2 ± 0.1^{ij}	9.7 ± 0.2^{e}
14	Plain kefir	122.7 ± 3.9^{no}	4.2 ± 0.1^{ghi}	0.6 ± 0.2^{k}	2.7 ± 0.1^{jkl}
15	lLactose-free kefir	119.7 ± 4.4^{o}	9.3 ± 0.1^{gh}	< LOD	< LOD
16	Probiotic kefir shot	175.1 ± 3.8^{ij}	6.2 ± 0.1^{ghi}	0.6 ± 0.1^{k}	5.5 ± 0.1^{gh}
17	Plain kefir with triple probiotics	62.4 ± 2.0^{p}	2.2 ± 0.1^{hi}	0.6 ± 0.1^{k}	0.2 ± 0.1^{no}
18	Kefir with triple probiotics, elderberry, and blueberry	64.5 ± 2.7^{p}	9.6 ± 0.6^{gh}	0.6 ± 0.1^{k}	0.7 ± 0.1^{mno}
19	Kefir with celery, banana, and pineapple	174.5 ± 4.3^{ij}	20.5 ± 1.0^{f}	0.3 ± 0.1^{k}	1.3 ± 0.1^{lmno}
20	Instant tarhana soup	389.4 ± 7.1^{b}	219.1 ± 8.6^{b}	14.5 ± 0.8^{d}	57.5 ± 1.4^{b}
21	Sweetened tarhana	134.9 ± 3.1^{mn}	340.0 ± 8.2^{a}	36.6 ± 1.8^{a}	72.5 ± 1.1^{a}
22	Organic tarhana	718.5 ± 6.1^{a}	23.3 ± 1.2^{f}	7.7 ± 0.1^{e}	54.1 ± 1.4^{c}
23	Spicy Turkish sausage	196.1 ± 4.4^{gh}	61.4 ± 0.8^{c}	5.8 ± 0.2^{fgh}	2.3 ± 0.2^{jkl}
24	Spicy Turkish sausage	204.1 ± 5.3^{g}	45.1 ± 2.0^{d}	5.6 ± 0.2^{gh}	1.8 ± 0.1^{klm}

Different letters in the same column show statistical differences between the groups ($p < 0.05$). Results are presented as mean values ± standard deviation ($n = 3$)

LOD (limit of detection) and LOQ (limit of quantification) (µg/g) were 0.06 and 0.20 for indole-3-acetic acid, 0.045 and 0.15 for indole-3-propionic acid, 0.30 and 1.00 for kynurenine, and 0.24 and 0.80 for kynurenic acid. Values reported as < LOD indicate concentrations below the analytical detection limit protein content. Organic tarhana, fresh string cheese, and aged cheddar cheese are notable products in this regard. In contrast, kynurenine levels are pretty low in products with low protein content or limited fermentation processes. The highest kynurenine content in tarhana may be related to its grain and milk content, which are tryptophan-rich raw materials, the organic product is being less refined, containing no additives, and undergoing a long natural fermentation process. Fermentation enhances microbial activation of the kynurenine pathway through enzymes, such as indolamine 2,3-dioxygenase analogs found in lactic acid bacteria and yeasts. These results may reflect microbial involvement in tryptophan catabolism, while prolonged fermentation may be linked to kynurenine accumulation [20, 21].

Kynurenic acid was detected between 1.1 and 340.0 µg/100 g. The amount of kynurenic acid in cheeses varies between 1.1 and 32.0 µg/100 g, in yogurt it ranged from 2.0 to 38.8 µg/100 g, in kefir from 2.2 to 20.5 µg/100 g, in tarhana from 23.3 to 340.0 µg/100 g, and in sausage from 45.1 to 61.4 µg/100 g. The detection of high kynurenic acid levels in tarhana and low-fat yogurt can be explained by the combined effect of factors related to the raw material structure and the fermentation process. Additionally, lower fat content may be associated with the predominance of bacterial populations that favor kynurenic acid formation. Conversely, kynurenic acid levels tended to be lower in products with limited fermentation or potentially reduced microbial activity. The high levels of kynurenic acid observed in low-fat yogurt may be related to the fact that some lactic acid bacteria (*e.g.*, *Lactobacillus* species) that are dominant during fermentation can affect tryptophan metabolism. In addition, low fat content may have indirectly facilitated kynurenic acid formation by affecting microbial growth and metabolic activity [20, 22]. From a food-science perspective, this findings highlights the role of traditional fermentation in shaping the metabolite profile of fermented foods.

Indole-3-acetic acid levels varied from < limit of detection (LOD) to 36.6 µg/100 g; the amount of indole-3-acetic acid in cheeses ranged from <LOD to 32.6 µg/100 g, in yogurt it ranged from 0.6 to 3.2 µg/100 g, in kefir from <LOD to 0.6 µg/100 g, in tarhana from 7.7 to 36.6 µg/100 g, and in sausage from 5.6 to 5.8 µg/100 g. In the present study, the highest Indole-3-acetic acid levels were detected in products, such as sweet tarhana (36.6 µg/100 g), creamy white cheese (32.6 µg/100 g), and aged cheddar cheese (27.6 µg/100 g). The higher IAA values observed in tarhana samples may be related to the product's grain-milk mixture structure and the natural fermentation processes, which make tryptophan more readily available for microbial metabolism. Similarly, the detection of relatively high IAA levels in some cheeses, such as creamy white cheese and aged cheddar, suggests that increased proteolysis and accompanying microbial activity during ripening may have supported IAA formation. In contrast, lower IAA levels in yogurt and especially kefir may be related to factors, such as shorter fermentation times, different starter culture compositions, or the product matrix being less conducive to the accumulation of indole derivatives.

Indole-3-propionic acid ranged from < LOD to 72.5 µg/100 g. Indole-3-propionic acid levels range from 2.8 to 11.9 µg/100 g in cheese, 0.5–9.7 µg/100 g in yogurt, < LOD − 5.5 µg/100 g in kefir, 54.1–72. 5 µg/100 g, and in sucuk samples, they range from 1.8 to 2.3 µg/100 g. Indole-3-propionic acid, produced from tryptophan by the gut microbiota, is an important metabolite that has positive effects similar to tryptophan on energy balance and the cardiovascular system. Indole-3-propionic acid prevents the harmful effects of oxidative stress at the cellular level, inhibits lipoperoxidation, and reduces inflammation by suppressing the production of pro-inflammatory cytokines [23]. In particular, Indole-3-propionic acid levels are quite high in fermented grain-based products, such as sweet tarhana, instant tarhana, and organic tarhana. This indicates that tarhana's rich composition of plant fiber, protein, and fermentable carbohydrates supports microbial production. Also, high IPA levels in tulum cheese may be related to maturation time and proteolysis, which increase the availability of tryptophan [24].

Tryptophan can be converted by microorganisms via two main metabolic pathways. In the kynurenine pathway, tryptophan is metabolized to kynurenine via enzymes, such as indolamine-2,3-dioxygenase or tryptophan-2,3-dioxygenase, which have been reported in Lactobacillus, Bifidobacterium, Pseudomonas, and Bacillus species. The fermentation process and proteolysis may increase the amount of available tryptophan. The tryptophanase enzyme converts tryptophan to indole, which is then metabolized to IAA and IPA. This pathway has been identified in *Bacteroides*, *Clostridium*, *Escherichia coli*, *Lactobacillus*, and *Enterococcus* species. These metabolic pathways biologically explain the detection of kynurenine and indole derivatives in fermented foods [22]. The content of the products, fermentation time, microbial strains used, and fat-to-protein ratios may be associated with the observed differences in the levels of these compounds. These variations may be associated with differences in fermentation conditions, ingredient composition, and microbial diversity across fermented food matrices. These data can be used to evaluate the potential health effects of functional foods and guide new product development processes [13].

Tryosine metabolite levels including phenol and *p*-cresol in the fermented-food samples are presented in Table 3. Phenol levels ranged from 4.4 to 115.7 µg/100 g; according to food types, ranged from 4.4 to 86.8 µg/100 g in cheese,

Table 3 Tryosine metabolite amounts in the fermented-food samples

Number	Sample	Phenol	*p*-cresol
1	White cheese	4.4 ± 0.2^{l}	2.2 ± 0.1^{b}
2	white cheese with a soft, creamy texture	6.0 ± 0.2^{kl}	< LOD
3	Aged cow cheese	11.9 ± 0.4^{lk}	< LOD
4	Full-fat aged white cheese with herbs	31.4 ± 0.8^{f}	0.5 ± 0.1^{gh}
5	Full-fat aged Tulum cheese	32.1 ± 2.3^{f}	< LOD
6	Full-fat aged Kashar cheese	86.8 ± 3.1^{c}	7.2 ± 0.1^{a}
7	Full-fat aged Kashar cheese	21.0 ± 1.4^{hi}	1.8 ± 0.1^{c}
8	Cecil (traditional Turkish string) cheese	21.5 ± 2.2^{gh}	0.5 ± 0.1^{fgh}
9	Full-fat fresh string cheese	12.8 ± 0.7^{j}	0.8 ± 0.1^{e}
10	String cheese	32.1 ± 1.6^{f}	< LOD
11	Yogurt	42.1 ± 2.9^{e}	< LOD
12	Probiotic yogurt	11.8 ± 0.3^{jk}	< LOD
13	Non-fat yogurt	17.9 ± 0.4^{hij}	< LOD
14	Plain kefir	27.7 ± 1.2^{fg}	0.7 ± 0.1^{ef}
15	Lactose-free kefir	11.8 ± 0.2^{jk}	< LOD
16	Probiotic kefir shot	13.3 ± 0.8^{j}	< LOD
17	Plain kefir with triple probiotics	16.1 ± 0.5^{ij}	0.6 ± 0.1^{efg}
18	Kefir with triple probiotics, elderberry, and blueberry	13.4 ± 1.2^{j}	< LOD
19	Kefir with celery, banana, and pineapple	24.3 ± 1.2^{gh}	0.3 ± 0.1^{h}
20	Instant tarhana soup	52.5 ± 2.8^{d}	1.2 ± 0.1^{d}
21	Sweetened tarhana	107.6 ± 3.6^{b}	< LOD
22	Organic tarhana	115.7 ± 6.5^{a}	< LOD
23	Spicy Turkish sausage	11.4 ± 0.8^{jk}	1.1 ± 0.1^{d}
24	Spicy Turkish sausage	13.9 ± 0.4^{j}	0.4 ± 0.1^{gh}

Different letters in the same column show statistical differences between the groups ($p < 0.05$). Results are presented as mean values ± standard deviation ($n = 3$)

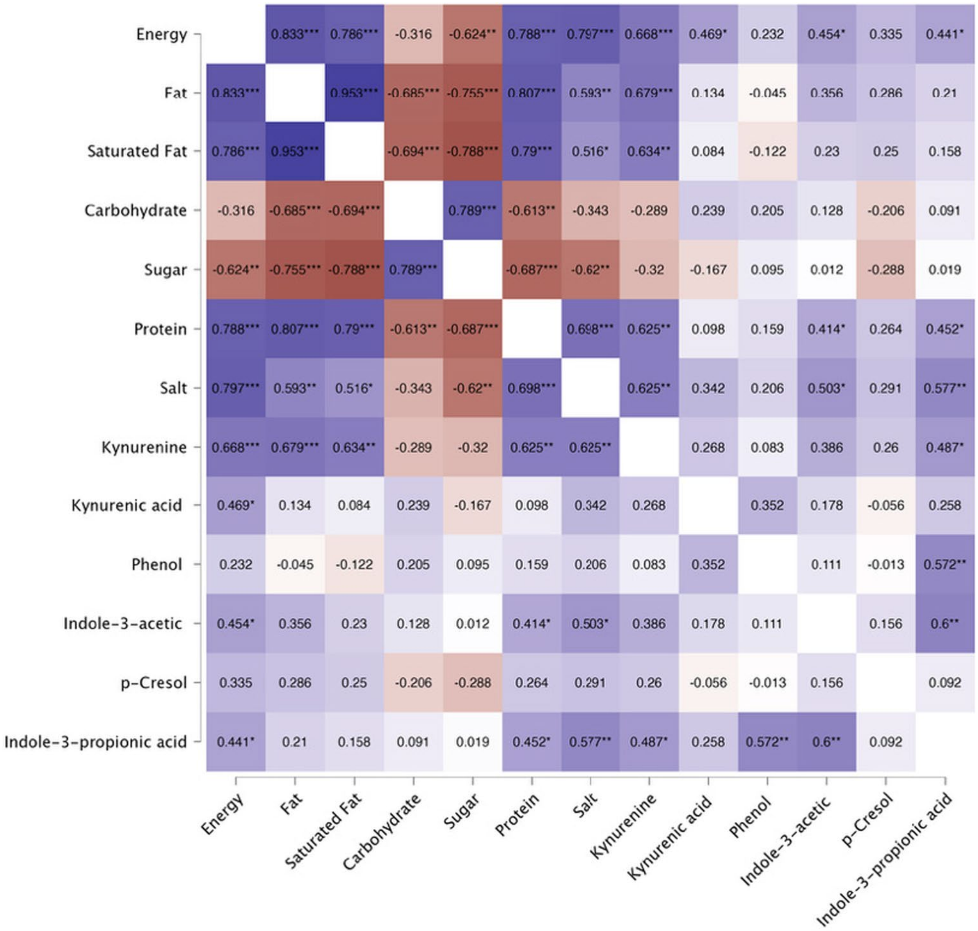
LOD (limit of detection) and LOQ (limit of quantification) values (µg/g) were 0.12 and 0.40 for phenol, and 0.06 and 0.20 for *p*-cresol, respectively. Values reported as <LOD indicate concentrations below the analytical detection limit

11.8–42.1 µg/100 g in yogurt, 11.8–63.2 µg/100 g in kefir, 52.5–115 µg/100 g in tarhana products, and 11.4–13.9 µg/100 g. in sucuk samples. The highest phenol contents were observed in organic tarhana (115.7 µg/100 g) and sweetened tarhana (107.6 µg/100 g), followed by plain kefir with triple probiotics (63.2 µg/100 g) and yogurt (42.1 µg/100 g), which may reflect higher levels of microbial metabolism in these matrices. Additionally, phenol levels in dairy products, such as kefir and yogurt, vary depending on the product type, but are generally high, making them an important parameter to consider when evaluating the effects of fermented dairy products on gut health. The accumulation of these compounds in products like tarhana, which are based on a mixture of grains and milk and undergo a long fermentation process, may be associated with more extensive microbial activity and a deeper degree of fermentation.

p-cresol was either below the LOD or present in relatively low amounts, ranging from < LOD to 7.2 µg/100 g. According to food type, p-cresol levels ranged from < LOD to 7.2 µg/100 g in cheese, < LOD to 0.7 µg/100 g in kefir, < LOD to 1.2 µg/100 g in tarhana products, and 0.4 to 1.1 µg/100 g in sausage, while it was below the LOD in yogurt. The highest p-cresol level was found in aged full-fat cheddar cheese, which may be associated with the maturation time, and high fat and protein composition that could favor microbial metabolism. This product also has a high phenol content (86.8 µg/100 g), which may be related to microbial activity and proteolytic degradation. While phenol levels are high in some kefir and yogurt varieties (plain kefir: 63.2 µg/100 g), p-cresol levels are mostly below the LOD or very low. These findings are consistent with the possibility that the cultures selected for probiotic products may contain strains with limited *p*-cresol–producing ability. Both phenol and p-cresol were detected at low levels in fermented meat products such as sausage. This may be related to limited fermentation or thermal processing that could restrict microbial activity, despite the presence of animal protein. p-cresol levels in tarhana products were generally below the detection limit. This may be associated with the starter cultures or fermentation conditions used, which may not favor the formation of p-cresol. Tyrosine derivatives, such as phenol and *p*-cresol, are primarily produced by anaerobic bacteria, particularly *Clostridium* cluster XIVa species, via para-hydroxyphenylacetate intermediates; this may explain the generally low p-cresol levels observed in most fermented foods [25]. From a nutritional perspective, the direct presence of these metabolites in foods suggests that fermented foods could be a potential dietary source of bioactive tryptophan derivatives and contribute to the evaluation of the health-related effects of these compounds.

Spearman correlation analysis revealed several significant associations between nutritional composition parameters and metabolite levels, which are summarized in Fig. 3. Spearman correlation analysis has shown significant relationships between the energy and macronutrient content of products and certain aromatic amino acid derivative metabolites. Energy, fat, saturated fat, protein, and salt content showed moderate to high positive correlations, particularly with kynurenine ($p < 0.01$). Similarly, protein and salt content showed weak to moderate positive relationships with IAA and IPA ($p < 0.05$). In contrast, carbohydrate and sugar content did not show a significant relationship with metabolite levels in general. Between metabolites, IPA showed significant positive correlations with kynurenine, phenol, and IAA, whereas p-cresol levels were not significantly related to either nutrient content or other metabolites.

Although this study has revealed significant findings regarding tryptophan and tyrosine metabolites in



	Energy	Fat	Saturated Fat	Carbohydrate	Sugar	Protein	Salt	Kynurenine	Kynurenic acid	Phenol	Indole-3-acetic	p-Cresol	Indole-3-propionic acid
Energy		0.833***	0.786***	-0.316	-0.624**	0.788***	0.797***	0.668***	0.469*	0.232	0.454*	0.335	0.441*
Fat	0.833***		0.953***	-0.685***	-0.755***	0.807***	0.593**	0.679***	0.134	-0.045	0.356	0.286	0.21
Saturated Fat	0.786***	0.953***		-0.694***	-0.788***	0.79***	0.516*	0.634**	0.084	-0.122	0.23	0.25	0.158
Carbohydrate	-0.316	-0.685***	-0.694***		0.789***	-0.613**	-0.343	-0.289	0.239	0.205	0.128	-0.206	0.091
Sugar	-0.624**	-0.755***	-0.788***	0.789***		-0.687***	-0.62**	-0.32	-0.167	0.095	0.012	-0.288	0.019
Protein	0.788***	0.807***	0.79***	-0.613**	-0.687***		0.698***	0.625**	0.098	0.159	0.414*	0.264	0.452*
Salt	0.797***	0.593**	0.516*	-0.343	-0.62**	0.698***		0.625**	0.342	0.206	0.503*	0.291	0.577**
Kynurenine	0.668***	0.679***	0.634**	-0.289	-0.32	0.625**	0.625**		0.268	0.083	0.386	0.26	0.487*
Kynurenic acid	0.469*	0.134	0.084	0.239	-0.167	0.098	0.342	0.268		0.352	0.178	-0.056	0.258
Phenol	0.232	-0.045	-0.122	0.205	0.095	0.159	0.206	0.083	0.352		0.111	-0.013	0.572**
Indole-3-acetic	0.454*	0.356	0.23	0.128	0.012	0.414*	0.503*	0.386	0.178	0.111		0.156	0.6**
p-Cresol	0.335	0.286	0.25	-0.206	-0.288	0.264	0.291	0.26	-0.056	-0.013	0.156		0.092
Indole-3-propionic acid	0.441*	0.21	0.158	0.091	0.019	0.452*	0.577**	0.487*	0.258	0.572**	0.6**	0.092	

Fig. 3 The results of the Spearman correlation analysis between nutritional composition parameters and metabolite levels

fermented products, it also has certain limitations. First, since such studies are not usually conducted *in vitro*, it is challenging to draw direct conclusions about the biological effects of metabolites on the gut microbiota or their interactions with the host organism. Also, microbial composition, fermentation duration, and the characteristics of the starter culture were not directly assessed. Therefore, the associations observed between metabolite concentrations and fermentation-related factors should be interpreted as associative rather than causal. Additionally, analyzing only free-form metabolites may overlook bound or conjugated forms and may not fully reflect the total biological effect. Since the fermentation conditions of the products, including starter culture type, duration or temperature are not standardized, comparisons between data may be limited. Further studies should integrate in vitro digestion and fermentation models to evaluate the post-digestion bioavailability and interaction of these metabolites with the microbiota, and then also focus on in vivo human or animal studies. However, the effects of different fermentation techniques and microbial profiles on metabolite production should be investigated more systematically. Correlation analysis with functional biomarkers to understand the potential health effects of metabolites is also important for future research.

Conclusion

In conclusion, the present study revealed that fermented food products such as cheese, yogurt, kefir, tarhana, and sucuk contain tryptophan and tyrosine metabolites, including kynurenine, kynurenic acid, indole-3-acetic acid, indole-3-propionic acid, phenol, and *p*-cresol. Tryptophan and tyrosine metabolites can be formed in fermented foods through microbial metabolism, specifically via the tryptophan, kynurenine, and indole pathways, as well as through tyrosine metabolism via phenolic compounds. High levels of kynurenic acid were detected in tarhana, aged cheese, yogurt, and kefir, suggesting that both raw material composition and fermentation-related microbial diversity may contribute to its formation. Similarly, elevated phenol levels in tarhana, kefir, and yogurt indicate active microbial metabolism and highlight phenol as a potentially relevant compound when considering the gut-health impact of fermented foods. In contrast, p-cresol was generally detected at low levels, which may be related to its production by specific microorganisms only under certain anaerobic fermentation conditions, as well as potential losses during processing and storage due to its volatility. The metabolite profile mapped in this study suggest that traditional fermentation and aging processes can directionally enrich specific amino acid metabolites with potential bioactivity. This can provide a scientific perspective for evaluating and optimizing the nutritional and functional quality of fermented foods from a metabolomics perspective.

These findings provide an important basis for understanding the potential effects of amino acid metabolites in fermented foods on health, and highlight the need for further studies to investigate in more detail the effects of microbial diversity, fermentation conditions, and product characteristics on the formation of these metabolites.

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Data Availability Data derived from public sources are included in the article; additional processed data are available from the corresponding author upon request.

Declarations

Use of Artificial Intelligence Artificial intelligence–assisted tools (ChatGPT, OpenAI; and DeepL Translator) were used during manuscript preparation to improve language quality and clarity. All scientific content, data interpretation, and conclusions were generated and verified by the authors, who take full responsibility for the integrity and originality of the work.

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare that they have no conflict of interest.

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