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Gastric survival of lactic acid bacteria in probiotic-labelled products from the Turkish market: An *in vitro* study

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Abstract: The resilience of lactic acid bacteria (LAB) in commercial probiotic products remains a critical area of investigation, particularly regarding their capacity to survive the harsh gastric environment. Scientific guidelines indicate that at least 6 log CFU·g^{-1} of viable probiotics must reach the intestines to achieve therapeutic benefits, which often requires an initial concentration of 8–9 log CFU·g^{-1} in the product. However, national regulations may specify lower thresholds; for example, Turkish Food Legislation requires 6 log CFU·g^{-1} for probiotic products and 7 log CFU·g^{-1} for kefir products. This study evaluates the *in vitro* gastric survival of LAB in 20 probiotic-labelled foods and 5 supplements available in the Turkish market using a simulated gastric model. Results reveal that 75% of the marketed probiotic-labelled foods comply with their label claims. Additionally, 55% of the samples demonstrate LAB strains fully resistant to gastric acidity. Dairy-based products exhibit significantly better survival rates under simulated gastric conditions compared to supplements, highlighting their potential for enhanced therapeutic efficacy.

Keywords: probiotics; probiotics; gastrointestinal conditions; legislation; viability

Probiotics, particularly lactic acid bacteria (LAB), have been widely studied for their health benefits, including their ability to modulate intestinal microbiota and enhance immunity (Avci et al. 2020). The range of commercial probiotic-labelled products, such as dairy products, dietary supplements, and fermented foods, has grown significantly. However, ensuring the survival of microorganisms through the acidic gastric environment remains a major challenge (Altun and Yildiz 2017).

To deliver therapeutic benefits, probiotics must survive gastrointestinal conditions, such as stomach acid, pepsin, and bile salts. The required viable LAB count for efficacy is 6–7 log CFU·g^{-1}, necessitating 8–9 log CFU·g^{-1} in products (Millette et al. 2013; Shori 2017). Turkish Food Codex regulations specify 6 log CFU·g^{-1} for probiotic products and 7 log CFU·g^{-1} for kefir at point of sale (Turkish Food Codex 2006/34, Official Gazette Issue: 26221; Turkish Food Codex 2022/44, Official Gazette Issue: 32029)

It has been stated that even in developed countries, the gastric protection warranty of licensed probiotics is not higher than 10% (Caillard and Lapointe 2017). International organisations such as the Food and Agriculture Organization (FAO) and World Health Organization (WHO), recommend a minimum LAB count of 6 log CFU·g^{-1} at the point of consumption (Morelli and Capurso 2012). However, many commercial probiotics fail to meet viability requirements post-gastric exposure, reducing their functional benefits (Stasiak-Rozanska et al. 2021).

Global regulations on probiotics exhibit significant variability. In the U.S., the Food and Drug Administra-

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tion (FDA) classifies probiotics as dietary supplements, requiring accurate labelling (mandates labelling accuracy) [FDA, Published Document: 2018-19367 (83 FR 45454)]. The EU requires scientific evidence for probiotic health claims but lacks unified regulations, leaving countries such as Italy and the Czech Republic to set their own standards. In Asia, countries like China and India have implemented regulations for probiotics, while Japan's Food for Specified Health Uses (FOSHU) system allows gastrointestinal health claims for specific strains (Siong et al. 2021). In Turkey, legislation mandates a minimum of 6 log CFU·g^{-1} in probiotics and 7 log CFU·g^{-1} in kefir (Turkish Food Codex 2006/34, Official Gazette Issue: 26221; Turkish Food Codex 2022/44, Official Gazette Issue: 32029). However, there is no requirement to demonstrate survival through the gastrointestinal tract.

Advancements like microencapsulation, prebiotic co-encapsulation, and dairy protein matrices have been developed to enhance probiotic resilience, protecting against low pH and digestive enzymes (Chen et al. 2018; Vivek et al. 2023). However, their consistent application in commercial products, particularly in emerging markets like Turkey, remains uncertain.

In this study we use the term 'probiotic-labelled products' to reflect the commercial labelling of the products at the time of purchase. While international definitions of probiotics emphasise strain-specific characterisation and documented health benefits, Turkish regulations currently allow products to be marketed as probiotic without mandatory strain identification. This discrepancy highlights an important regulatory gap.

This study evaluates the gastric survival of LAB in Turkish probiotic products using a simulated *in vitro* model, providing the first insights into their gastric resilience and ability to fulfil health claims.

MATERIAL AND METHODS

Probiotic-labelled samples. Twenty industrially produced probiotic-containing food products were collected from the market, along with oral suspensions and powdered supplements from five different brands obtained from pharmacies. These products were selected for comparative analysis (see Table 1 for sample details). All samples were stored at 4 °C until analysis. Analyses were conducted within the expiry dates of the products, and each sample was freshly opened on the day of its respective analysis. Consumption instructions, such as taking the products with water or with/without meals, were followed accordingly.

Simulated gastric fluid. The simulated gastric fluid (SGF) used in this study was based on a previously described *in vitro* model (Yuk and Schneider 2006), adapted to include key gastric components such as pepsin, lysozyme, and bile salts, with some modifications: porcine bile extract (Sigma-Aldrich, St. Louis, MO, USA) was used instead of ox bile. The conditions (gastric fluid volume, pH, and transit time) were established to mimic human physiological conditions. Gastric digestion was performed in a shaking water bath for 2 h at 37 °C at 175 rpm. SGF was freshly prepared daily by dissolving 8.3 g·L^{-1} proteose–peptone (Sigma-Aldrich, St. Louis, MO, USA), 3.5 g·L^{-1} D-glucose (Anhydrous, Merck, Darmstadt, Germany), 2.05 g·L^{-1} NaCl (extra pure for analysis, Tekkim), 0.6 g·L^{-1} KH_2PO_4 (Sigma-Aldrich, St. Louis, MO, USA), 0.11 g·L^{-1} $CaCl_2$ (Merck, Darmstadt, Germany), 0.37 g·L^{-1} KCl (Merck) in distilled water. This mixture was autoclaved at 121 °C, 15 min. After cooling, 0.05 g·L^{-1} porcine bile extract (Sigma-Aldrich, St. Louis, MO, USA), 0.1 g·L^{-1} lysozyme (from hen egg White, Roche, Mannheim, Germany) and 13.3 mg·L^{-1} pepsin (porcine gastric mucosa powder, Sigma Aldrich, St. Louis, MO, USA) were added via filter sterilisation (0.45 µm; M & Nagel, Düren, Germany). The pH was adjusted to 1.5 with 25% HCl. While *in vitro* systems cannot fully replicate the complexity of the human gastrointestinal tract, the model provides standardised and reproducible conditions for comparative survival studies under gastric exposure.

Sample preparation and microbial analysis. The pH values of all samples and SGF were measured in triplicate. To simulate the interaction between gastric fluid and food products, 25 g or 25 mL of each sample was mixed with 25 mL of SGF. For supplements requiring consumption 'with meals', 25 mL of fruit juice was included. The samples labelled as 23 and 25 were powdered sachets intended for direct consumption without liquid. To simulate this under *in vitro* conditions, these powders were rehydrated in 9 mL of sterile MRS broth before being added to SGF. This step was used to standardise hydration prior to simulated gastric exposure, approximating oral cavity rehydration while avoiding additional acid stress prior to SGF exposure.

Probiotic food products were incubated in SGF within a shaking water bath at 37 °C and 75 rpm for 2 h. Samples were collected from the stomacher bags both before the addition of SGF and after incubation in the water bath. Bacterial enumeration was performed using the spread plate method on MRS and M-17 Agar (Merck, Germany), both commonly used

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Table 1. Sample details

Sample number	Product form	Probiotic strain stated on the label	Consuming instructions	Encapsulation information	Claimed culture concentration (CFU·1 g^{-1} or mL^{-1})
1	probiotic smoothie	NA	1 portion per day	NA	10^6
2	probiotic dairy product	*Bifidobacterium animalis strain* DN-173 010	1 portion per day	NA	10^6
3	probiotic yogurt	*Bifidobacterium animalis* strain DN-173 010	1 portion per day	NA	2×10^6
4	kefir	kefir yeast	1 portion per day	NA	10^6
5	probiotic yogurt	NA	NA	NA	NA
6	kefir	NA	NA	NA	NA
7	probiotic yogurt	*Lactobacillus delbrueckii subs. bulgaricus, Streptococcus thermophillus, Lactobacillus acidophillus*	NA	NA	10^7
8	probiotic ayran	*Lactobacillus bulgaricus, Streptococcus thermophillus*	NA	NA	10^6
9	kefir	*Bifidobacterium, Lactobacillus acidophillus*	NA	NA	10^6
10	probiotic kefir	starter cultures	NA	NA	10^8
11	probiotic kefir	*Bifidobacterium lactis* (BB-12), *Lactobacillus acidophillus* (LA-5)	1 portion per day	NA	10^6
12	probiotic yogurt	*Bifidobacterium*, BB-12® and *Lactobacillus acidophilus*, LA-5®	NA	NA	NA
13	probiotic milk	NA	NA	NA	10^6
14	probiotic kefir	kefir yeast	for expected effect 1 portion per day/ drink cold, shake, keep + 2–4 °C	NA	10^6
15	probiotic yogurt	NA	NA	NA	NA
16	powder yeast	starter cultures	NA	NA	10^9
17	probiotic kefir	kefir yeast	drink cold, shake, store in the refrigerator.	NA	
18	probiotic yogurt	NA	NA	NA	NA
19	probiotic tea	NA	NA	NA	NA
20	probiotic chocolate	NA	NA	NA	NA
21	powdered sachet	*Saccharomyces boulardii* (CNCM I-745)	with water	NA	NA
22	powdered sachet	*Lacticaseibacillus casei, Lactiplantibacillus rhamnosus, Lactiplantibacillus plantarum, Bifidobacterium lactis*	with or after meal	double capsulation	2×10^9
23	powdered sachet	*Lactobacillus acidophillus, Lactiplantibacillus plantarum, Bifidobacterium lactis, Limosilactobacillus reuteri, Streptococcus thermophillus*	consume directly	NA	10^9

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Table 1. To be continued

Sample number	Product form	Probiotic strain stated on the label	Consuming instructions	Encapsulation information	Claimed culture concentration (CFU·1 g^{-1} or mL^{-1})
24	capsule	*Lactobacillus acidophillus* (LA-5), *Bifidobacterium animalis* subs. *Lactis* (BB-12)	with meal	coated	10^9
25	powdered sachet	*Bifidobacterium animalis* ssp. *Lactis* B94	NA	NA	10^{10}

NA – not available

for LAB enumeration. Although more selective media exist for bifidobacteria, yeasts and other microorganisms, these two media were selected to allow consistent total LAB viability comparisons across all product types. Since the Turkish Food Codex specifies only the 'total probiotic count' without separate limits for yeast, no specific enumeration of yeasts was performed. All plates were incubated anaerobically at 37 °C for 48 h.

Statistical analysis. The analysis was conducted in duplicate. Bacterial counts obtained before and after simulated digestion were statistically evaluated to determine the survival rates of lactic acid bacteria in the gastric environment. Statistical analysis was performed using SAS 9.0 software (SAS Institute Inc., Cary, NC, USA). Comparisons between samples were conducted using the *t*-test, with the least significant difference (LSD) method applied at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The survival of LAB in commercially available probiotic food products was analysed before and after simulated gastric digestion, as presented in Figure 1. The initial LAB count was 8.13 ± 0.93 log CFU·g^{-1}, which decreased to 7.15 ± 1.17 log CFU·g^{-1} following exposure to SGF. This represents a statistically significant reduction of approximately 1 log CFU·g^{-1} ($P < 0.05$), highlighting the impact of gastric conditions on LAB viability. These findings align with previous studies,

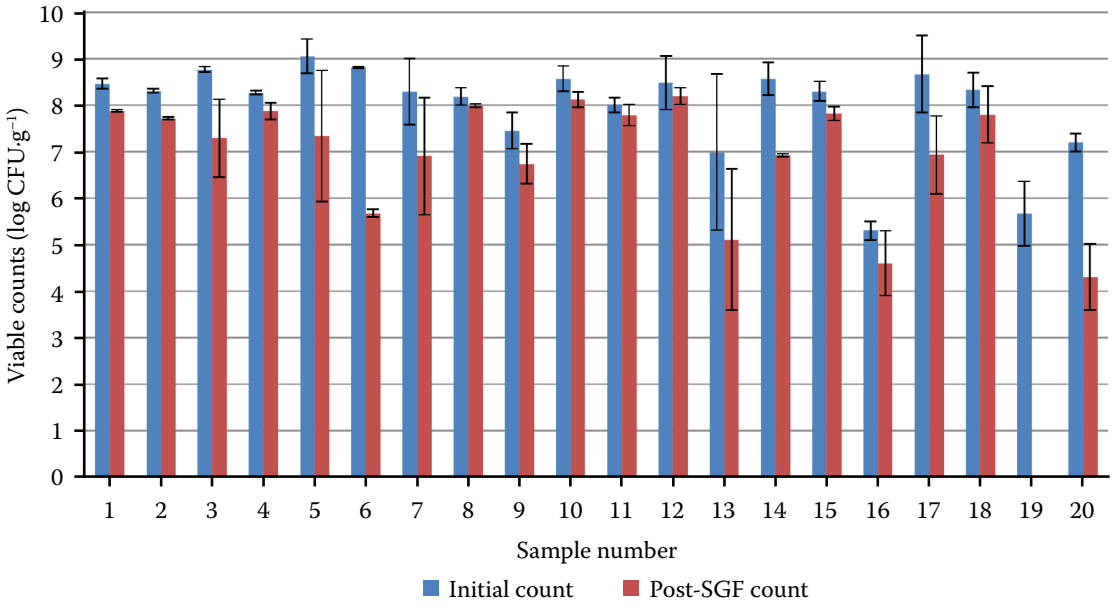

Figure 1. Lactic acid bacteria (LAB) counts in commercial probiotic food products before and after incubation in simulated gastric fluid. The bars show ± SD

SGF – simulated gastric fluid

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suggesting that LAB strains present in fermented food matrices may exhibit greater resistance to gastric conditions compared to those in powdered supplements (Vinderola et al. 2011).

Among the food samples analysed, eight lacked clear labelling of probiotic strains, while all pharmacy-sourced supplements specified their strains. Food products predominantly contained species such as *Bifidobacterium animalis*, *Lactobacillus acidophilus*, and *Bifidobacterium lactis*, whereas supplements included *Saccharomyces boulardii* and *Lactiplantibacillus rhamnosus*. Strain differences significantly influenced survival rates. LAB species such as *Lactobacillus acidophilus* and *Bifidobacterium lactis*, commonly found in dairy products, exhibited higher gastric tolerance compared to *Saccharomyces boulardii*, a yeast often used in supplements. This discrepancy in bacterial species between product types may influence their gastric survival rates, as the robustness of the strain may impact viability post-digestion. Advances in strain engineering, such as genetic modifications to enhance acid tolerance, offer promising solutions to improve probiotic functionality (Stasiak-Rozanska et al. 2021; Spacova et al. 2023).

Labelling inconsistencies were also apparent, with 40% of food product labels lacking strain details and most products providing no information on encapsulation. Encapsulation technology, present in only two supplement samples, is known to enhance LAB survival by forming a protective barrier against stomach acidity. Materials like alginate and chitosan have been shown to improve survival in simulated gastric environments (Abbas et al. 2023). While countries like Canada enforce encapsulation and labelling criteria (Canada Gazette 2011, Part II: Vol. 145, Registration SOR/2011-28), Turkey does not currently regulate these aspects. Establishing clearer standards could enhance probiotic efficacy and increase consumer trust.

Turkish Food Legislation mandates that the probiotic microorganism count in a product must be at least 6 log CFU·g^{-1} (Turkish Food Codex 2006/34, Official Gazette Issue: 26221). In this study, 18 out of 20 food samples met this requirement based on their initial LAB count, with only two samples slightly below the threshold. Following exposure to SGF, 11 of the 20 samples showed no statistically significant difference in LAB count ($P > 0.05$), indicating that more than half of the products maintained probiotic viability during gastric transit. However, five samples fell below the required 6 log CFU·g^{-1} after SGF exposure, while 15 maintained LAB counts above this threshold. These findings suggest that 75% of the commercial probiotic products tested are reliable in terms of delivering viable probiotics after gastric transit.

Internationally, regulatory frameworks vary considerably. For instance, the European Food Safety Authority (EFSA) employs the Qualified Presumption of Safety (QPS) system to assess probiotic strains, while the U.S. FDA assigns Generally Recognized as Safe (GRAS) status to specific strains for use in food. These frameworks emphasise that survival claims must be supported by rigorous testing, reflecting the high standards established globally for functional foods (Spacova et al. 2023).

Among the 20 probiotic food samples, seven were kefir or probiotic-labelled kefir. According to Turkish Food Legislation (Turkish Food Codex 2022/44, Official Gazette Issue: 32029), the LAB count in kefir products must be at least 7 log CFU·g^{-1}. In this study, the initial LAB counts for kefir samples were found to be within the required limits. However, after exposure to SGF, one kefir sample had a LAB count below 7 log CFU·g^{-1}, indicating a decline in viability. This was observed for sample No. 14 (Table 1).

Food supplements, which were also analysed, showed more variable results. Out of five samples tested, only two initially met the claimed LAB content, while one product contained no viable bacteria. These samples were sample No. 21 and sample No. 23, with the latter showing no detectable viable bacteria (Table 2). After gastric transit, none of the supplements retained LAB counts above the required threshold. However, supplements with instructions to be consumed with meals demonstrated better survival rates than those intended for direct consumption. This suggests that probiotic supplements have lower survivability post-SGF when consumed without food, highlighting the importance of including meal-related instructions on labels. While

Table 2. Survival of lactic acid bacteria in probiotic food supplements before and after incubation in simulated gastric fluid (SGF)

Sample number	Initial count	Post-SGF count	Post-SGF pH
21	5.90 ± 0.31^{a}	4.60 ± 0.20^{b}	4.59 ± 0.21
22	9.22 ± 0.16^{a}	2.96 ± 0.65^{b}	4.39 ± 0.19
23	< 2	< 2	1.66 ± 0.06
24	9.60 ± 0.09^{a}	3.61 ± 0.59^{b}	4.67 ± 0.10
25	7.80 ± 0.73^{a}	< 2	1.55 ± 0.05

^{a,b}values within a row with different superscripts differ significantly at $P < 0.05$; SGF – simulated gastric fluid

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some studies suggest that probiotics perform better without food (Agyeman and Gaisford 2015), others indicate that food buffers stomach acidity, thereby improving bacterial survival (Vinderola et al. 2011). Clearer labelling practices, implemented in countries like Canada and Japan, could enhance consumer outcomes (Spacova et al. 2023).

The initial and post-SGF pH of the samples were evaluated. The average initial pH of the probiotic food products was 4.21 ± 0.15. The pH of the SGF, initially adjusted to 1.58 ± 0.21, increased to 3.15 ± 0.42 after the addition of probiotic products. While commercial probiotic food samples showed relatively consistent pH values, food supplements exhibited high pH variability depending on their consumption instructions. Supplements with no specific consumption instructions and those intended for direct consumption exhibited post-SGF pH values as low as 1.61 ± 0.09, which may explain their poor viability.

In our study, probiotic food supplements showed very low survival rates after the gastric phase. This finding aligns with the results of Caillard and Lapointe (2017), who reported that most oral formulations fail to ensure the survival of LAB strains. In our study, although none of the samples achieved more than 6 log CFU·g^{-1} of viable LAB, those consumed with a meal showed some degree of viability. In contrast, the supplement intended for direct consumption showed no survival, highlighting the importance of consuming probiotics with or after meals and following the instructions on the product label. The impact of meals on LAB survival might be attributed to the stomach pH at the time of bacterial entry. The post-SGF pH of food supplements consumed directly was significantly lower than that of other samples, which may explain the complete absence of viable counts. The observed differences in survival likely result from both strain properties and food matrix effects. Dairy-based products contained strains such as *Lactobacillus acidophilus*, known for higher acid resistance, while supplements included strains like *Lactiplantibacillus rhamnosus*, *Saccharomyces boulardii* and others, which may vary in gastric tolerance. Additionally, dairy matrices may buffer gastric pH and protect microorganisms during digestion. In our study, food products increased SGF pH, while supplements consumed directly maintained low pH, correlating with reduced survival. Although we could not directly separate strain and matric effects, both likely contribute to the observed outcomes. Further targeted studies are needed to clarify those factors.

The probiotic bacterial species found in the analysed food samples differed significantly from those in food supplements. One possible reason for the lower gastric survival of food supplements might be the variation in bacterial strains. Using more resilient bacterial strains is recommended for both fermented foods and food supplements. This study also reveals a regulatory gap in the current Turkish Food Codex, where probiotic-labelled products are only required to meet a minimum bacterial count, without any obligation to declare the specific probiotic strains. A previous study indicated that *Bifidobacterium* spp. exhibit poor growth and survival in gastrointestinal conditions unless genetically improved (Egan et al. 2018). The analysed food supplements in this study contained *Bifidobacterium* spp., *Limosilactobacillus rhamnosus*, *Lactobacillus acidophilus* and *S. boulardii*, among others – some of which have been recognised as successful probiotic strains (Pais et al. 2020). However, these supplements did not achieve the desired survival rates in this study. Factors such as the specific bacterial strain, encapsulation, genetic modification, adaptation, and strain enhancement may significantly influence survival rates (Yao et al. 2020).

The buffering effect of food matrices was also evident. Dairy-based products demonstrated a stabilising effect on gastric pH, which may support LAB survival. All non-dairy food products and food supplements resulted in LAB counts below 6 log CFU·g^{-1} after gastric transit. Sumeri et al. (2008) showed that the same probiotic bacteria can behave differently depending on the food matrix. Although Silva et al. (2017) indicated that chocolate improves probiotic survival in simulated gastrointestinal fluids, our study found a nearly 3 log CFU·g^{-1} reduction in LAB count in probiotic chocolate product. This discrepancy may be due to differences in bacterial strains, but unfortunately, no strain information was provided on the product label. Additionally, previous studies have shown that probiotics stored in milk exhibit better survival due to milk's buffering capacity (Tompkins et al. 2011). Moreover, the advantage of dairy products may result from their fermentation process, which not only supports probiotic growth but also creates a prebiotic, protective, and nutritionally favourable environment that enhances bacterial viability (Millette et al. 2013).

Millette et al. (2013) also reported that the majority of probiotic products available in the Canadian and U.S. markets failed to meet the required viability standards. In our study, 75% of probiotic-labelled food products in the Turkish market were found to be reli-

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able, indicating improvements in probiotic production technology in recent years.

This study primarily aimed to assess total LAB survival in probiotic-labelled products, without species- or strain-level resolution. The lack of selective cultivation, species-specific media, and molecular identification represents a methodological limitation. Similarly, the applied *in vitro* gastric model represents a simplified simulation of gastric conditions and does not fully reflect the dynamic and complex environment of the entire gastrointestinal tract.

Future investigations should integrate molecular identification methods, comprehensive characterisation of food matrix composition, and experimental designs capable of distinguishing between matrix-related and strain-dependent survival effects. Such integrated approaches would enable a more precise elucidation of the factors determining probiotic viability during gastrointestinal passage. Moreover, the findings of this study are restricted to the specific products analysed and cannot be generalised to all probiotic-labelled products available on the market.

CONCLUSION

This study aimed to evaluate the survival of LAB in commercially available food products containing probiotics after simulated gastric conditions. Products with post-SGF LAB counts below 6 log CFU·g^{-1} were those without any specific probiotic strain listed on the label. Thus, probiotic-labelled food products in the Turkish market were found to be 75% reliable, indicating that labelling provides a reasonable level of confidence in these products. Overall, probiotic food products demonstrated significantly higher gastric survival compared to food supplements, with probiotic dairy products showing the best survival rates, likely due to the bacterial strains used. While many *in vitro* studies assess bacterial survival, they often do not examine commercial products. To our knowledge, this is the first study to verify the gastric survival rate of commercially available probiotic products in the Turkish market.

The results of this study highlight the importance of evaluating the gastric resilience of probiotic-containing foods. Notably, dairy-based probiotic products demonstrated superior tolerance to gastric conditions, with a higher capacity to deliver live bacteria to the intestines. From a public health perspective, this suggests that prioritising dairy-based probiotic products can maximise benefits, such as immune system support, digestive regulation and microbiota balance. For the food industry, these findings underscore the need to develop improved formulations that enhance the gastric acid resilience of probiotics. Furthermore, transparent and regulatory-compliant labelling of probiotic strains and quantities is essential for building trust and ensuring a better understanding of their health benefits. In conclusion, this study provides valuable insights for both consumers and the food industry, promoting more effective and informed probiotic use.

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