





## Glycemic response and starch digestibility in traditional and industrial breads from the galician market (NW Spain)

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### ABSTRACT

In Galicia, bread is not only a dietary staple but also a cultural and high-value product. The increasing replacement of traditional bread by industrial alternatives has raised nutritional concerns. This study assessed *in vitro* starch digestibility and estimated glycemic index (eGI) in 72 breads from the Galician market, including industrial and artisan traditional samples made with wheat, rye, or corn. Significant differences ( $p < 0.05$ ) were observed across samples in terms of starch fractions and glycemic index. Traditional breads made with local corn or rye flours showed lower eGI values and higher resistant starch contents, whereas industrial wheat breads exhibited higher eGI and rapidly digestible starch fractions. Both compositional and technological factors influenced glycemic response, including cereal type, sourdough use, and fermentation duration. Overall, traditional breads produced with local flours and long fermentation displayed a slower starch digestibility pattern and more favorable nutritional profile than industrial counterparts, supporting their promotion as healthier options within a balanced diet.

### 1. Introduction

Bread is a staple food consumed daily worldwide, playing a fundamental role in human diets (Arp et al., 2018; Paciulli et al., 2021). In Galicia (NW Spain), bread holds not only nutritional but also cultural significance, with a long-standing artisanal tradition that continues to this day (Carballo-Casla et al., 2021; Vaz Velho et al., 2016). One of the most emblematic products of this region is “Pan Galego”, which has been granted the Protected Geographical Indication (PGI) label (European Commission, 2019). Moreover, the Galician baking heritage includes other types of artisanal breads made from rye and corn flours, also widely appreciated at a local level (Leis Trabazo et al., 2019; Pérez Samper, 2014; Seabra et al., 2018).

Thus, despite a general decline in bread consumption in Spain over recent decades, Galicia continues to show a higher-than-average consumption rate (MAPA, 2025), reflecting its deep-rooted in the regional food culture. However, data from the 2025 report of the Spanish

Ministry of Agriculture, Fisheries and Food (MAPA) also reveal a progressive decrease in the consumption of fresh and frozen bread, which includes most artisanal made products, while the share of industrial bread has increased within the overall declining trend. This shift raises concerns about the potential nutritional implications associated with changes in bread quality (Shakhman et al., 2025).

One of the key nutritional attributes of bread is its glycemic index (GI), which reflects the rate at which the carbohydrates in a food are digested and absorbed, thereby influencing postprandial blood glucose levels (Jenkins et al., 1981). A high GI is associated with rapid glucose release into the bloodstream, which may contribute to insulin resistance, obesity, type 2 diabetes, and other non-communicable diseases (Dong et al., 2011; Hardy et al., 2020; Reynolds et al., 2019). According to international classifications, foods can be categorized based on their GI values as low ( $\leq 55$ ), medium (56–69), or high ( $\geq 70$ ) (Augustin et al., 2015; Kanter et al., 2022). Given that bread is an important contributor to dietary glycemic load in many regions, evaluating the GI of different

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types of bread, particularly those differing in formulation and processing, is of great interest.

The *in vivo* determination of GI involves human testing and is time-consuming, costly, and ethically constrained (Argyri et al., 2016; Germaine et al., 2008). As an alternative, *in vitro* models simulating gastrointestinal digestion have been developed and widely applied. Among them, the protocol proposed by Goñi et al. (1997) and further adapted in recent studies (Ferrer-Mairal et al., 2012; Kahraman et al., 2019; Koksel et al., 2023; Sulung et al., 2023) enables the calculation of the hydrolysis index (HI) and estimation of the glycemic index (eGI).

Starch digestibility is not only affected by the amount of total starch but also by the proportion of its different fractions: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). These fractions are known to have distinct physiological effects: RDS leads to a sharp rise in blood glucose, SDS contributes to a more gradual release, and RS resists digestion in the small intestine, behaving more like dietary fiber and promoting colonic health (Englyst et al., 1992; Fuentes-Zaragoza et al., 2010; Magallanes-Cruz et al., 2017). Thus, the composition and proportion of starch fractions in bread play a central role in its glycemic impact (Demirkesen-Bicak et al., 2021; Shumoy & Raes, 2017).

To date, no comprehensive assessment has been reported on the starch digestibility and glycemic response of bread produced and commercialized in Galicia, one of the Spanish regions with the strongest breadmaking tradition. Therefore, this study aimed to evaluate *in vitro*

starch digestibility and eGI of a representative selection of traditional and industrial breads from the Galician market, to elucidate the main factors influencing glycemic response and to identify formulations with potential benefits for glycemic control.

## 2. Materials and methods

### 2.1. Bread samples

A total of 72 bread samples from the Galician market were analyzed. Of these, 49 were traditional Galician breads and 23 were industrial breads. Traditional breads were collected directly from local bakeries and processed within 24 h after baking to preserve their original characteristics. Industrial breads were purchased in various supermarkets and immediately processed upon arrival at the laboratory. All bread samples were dehydrated by oven-drying, then hermetically sealed and kept at room temperature until subsequent analysis.

Among the traditional samples, 42 breads were produced by Grupo Da Cunha company under fully controlled conditions. These samples, whose main characteristics are summarized in Table 1, include a subset of wheat breads produced in accordance with the specifications outlined in the Protected Geographical Indication (PGI) “Pan Galego” regulation. This certification applies exclusively to wheat-based breads and is defined by the use of sourdough, a minimum of 25 % flour from local wheat cultivars (‘Caaveiro’ and ‘Callobre’), high water content, and long

**Table 1**  
Characterized traditional bread samples (CTB) of wheat, rye and corn in two cultivation systems.

Sample	Cereal Type	Cultivar/Ecotype	Harvest Year	Percentage of Local Flour	Cultivation System
CTB-W100-1	Wheat	‘Caaveiro’	2023	100 %	Organic
CTB-W100-2	Wheat	‘Caaveiro’	2023	100 %	Conventional
CTB-W100-3	Wheat	‘Callobre’	2023	100 %	Organic
CTB-W100-4	Wheat	‘Callobre’	2023	100 %	Conventional
CTB-W25-1	Wheat	‘Caaveiro’	2023	25 %	Organic
CTB-W25-2	Wheat	‘Caaveiro’	2023	25 %	Conventional
CTB-W25-3	Wheat	‘Callobre’	2023	25 %	Organic
CTB-W25-4	Wheat	‘Callobre’	2023	25 %	Conventional
CTB-W100-5	Wheat	‘Caaveiro’	2024	100 %	Organic
CTB-W100-6	Wheat	‘Caaveiro’	2024	100 %	Conventional
CTB-W100-7	Wheat	‘Callobre’	2024	100 %	Organic
CTB-W100-8	Wheat	‘Callobre’	2024	100 %	Conventional
CTB-W100-9	Wheat	‘Caaveiro’	2024	100 %	Conventional
CTB-W25-5	Wheat	‘Caaveiro’	2024	25 %	Organic
CTB-W25-6	Wheat	‘Caaveiro’	2024	25 %	Conventional
CTB-W25-7	Wheat	‘Callobre’	2024	25 %	Organic
CTB-W25-8	Wheat	‘Callobre’	2024	25 %	Conventional
CTB-W25-9	Wheat	‘Caaveiro’	2024	25 %	Conventional
CTB-R100-1	Rye	‘Palas’	2023	100 %	Organic
CTB-R100-2	Rye	‘Palas’	2023	100 %	Conventional
CTB-R100-3	Rye	‘Trevinca’	2023	100 %	Organic
CTB-R100-4	Rye	‘Trevinca’	2023	100 %	Conventional
CTB-R100-5	Rye	‘Trevinca’	2023	100 %	Conventional
CTB-R75-1	Rye	‘Palas’	2023	75 %	Organic
CTB-R75-2	Rye	‘Palas’	2023	75 %	Conventional
CTB-R75-3	Rye	‘Trevinca’	2023	75 %	Organic
CTB-R75-4	Rye	‘Trevinca’	2023	75 %	Conventional
CTB-R75-5	Rye	‘Trevinca’	2023	75 %	Conventional
CTB-R100-6	Rye	‘Palas’	2024	100 %	Organic
CTB-R100-7	Rye	‘Palas’	2024	100 %	Conventional
CTB-R100-8	Rye	‘Trevinca’	2024	100 %	Organic
CTB-R100-9	Rye	‘Trevinca’	2024	100 %	Conventional
CTB-R75-6	Rye	‘Palas’	2024	75 %	Organic
CTB-R75-7	Rye	‘Palas’	2024	75 %	Conventional
CTB-R75-8	Rye	‘Trevinca’	2024	75 %	Organic
CTB-R75-9	Rye	‘Trevinca’	2024	75 %	Conventional
CTB-C70-1	Corn	‘Carballeira’	2020	70 %	ND
CTB-C70-2	Corn	‘Oroso’	2020	70 %	ND
CTB-C70-3	Corn	‘Ribadumia’	2020	70 %	ND
CTB-C70-4	Corn	‘Rebordane’	2020	70 %	ND
CTB-C70-5	Corn	‘EPS27’	2020	70 %	ND
CTB-C70-6	Corn	‘Tuy’	2020	70 %	ND

ND: Not Determined.

fermentation and baking times, with baking performed exclusively in stone ovens (MAPA, 2018).

The specific formulation and processing conditions used for the characterized traditional breads are detailed in Table 2. This includes wheat, rye, and corn breads made under standardized protocols to allow comparative analysis.

In addition, seven traditional bread samples were purchased from various artisanal bakeries, recognized for their traditional production practices and local relevance. Selection was guided by the availability of products marketed as “traditional” or “artisan” bread, ensuring inclusion of samples perceived by consumers as representative of the category. Although the ingredients were known (Table S1, Supplementary material), detailed nutritional composition data were not available. These samples were therefore classified as non-characterized traditional breads (NCTB). Among them, one sample (NCTB-R-1) was made with rye flour, while the remaining six (NCTB-W-1 to NCTB-W-6) were wheat-based.

Finally, a total of 23 industrial bread samples were also analyzed, as detailed in Table 3. These breads were selected to represent the main categories and brands most consumed in the Galician market, based on their availability in large supermarket chain. The information provided on the product labels is included in Table S2 (Supplementary material). For the purposes of comparative analysis, industrial breads were grouped into two categories: industrial tin loaf breads (ITLB), typically sold pre-sliced and differing substantially in formulation, texture, and processing method; and other industrial breads (OIB), including baguette-style, ring-shaped, and round loaves, which, despite differences in shape, share more similar crumb structure, crust characteristics, and baking profiles.

To minimize the impact of storage time on starch retrogradation and digestibility, all characterized traditional breads produced under controlled conditions were dehydrated on the same day of baking. For industrial breads, samples were purchased and processed within 24 h of

**Table 2**

Formulation and processing parameters of characterized traditional breads (CTB) made with wheat, rye and corn.

Sample Type	Percentage of Local Flour	Formulation	Fermentation Stages	Baking Conditions
CTB-W100	100 %	7 kg local wheat, 4.1 L water, 2.5 L sourdough, 70 g yeast, 140 g salt	1 h bulk + 20 min ball + 2 h piece	1 h at 220 °C
CTB-W25	25 %	5.25 kg commercial wheat + 1.75 kg local wheat, 4.1 L water, 2.5 L sourdough, 70 g yeast, 140 g salt	1 h bulk + 20 min ball + 2 h piece	1 h at 220 °C
CTB-R100	100 %	7 kg local rye, 3.7 L water, 2.5 L sourdough, 70 g yeast, 140 g salt	2 h bulk + 20 min ball + 2 h piece	1 h at 220 °C
CTB-R75	75 %	5.25 kg local rye + 1.75 kg commercial wheat, 3.7 L water, 2.5 L sourdough, 70 g yeast, 140 g salt	2 h bulk + 20 min ball + 2h	1 h at 220 °C
CTB-C70	70 %	4 kg local corn + 1 kg wheat + 650 g rye, 5.6 L water, 1.225 L sourdough, 33 g yeast, 221 g salt	1 h bulk	50 min at 220 °C

CTB-W100: Characterized Traditional Bread with 100 % local wheat flour; CTB-W25: Characterized Traditional Bread with 25 % local wheat flour; CTB-R100: Characterized Traditional Bread with 100 % local rye flour; CTB-R75: Characterized Traditional Bread with 75 % local rye flour; CTB-C70: Characterized Traditional Bread with 70 % corn flour.

**Table 3**

Industrial bread samples (IB) from the Galician market.

Sample	Type
ITLB-1	Tin Loaf
ITLB-2	Tin Loaf
ITLB-3	Tin Loaf
ITLB-4	Tin Loaf
ITLB-5	Tin Loaf
ITLB-6	Tin Loaf
ITLB-7	Tin Loaf
ITLB-8	Tin Loaf
ITLB-9	Tin Loaf
ITLB-10	Tin Loaf
ITLB-11	Tin Loaf
ITLB-12	Tin Loaf
ITLB-13	Tin Loaf
ITLB-14	Tin Loaf
ITLB-GF-1	Gluten Free Tin Loaf
ITLB-GF-2	Gluten Free Tin Loaf
ITLB-GF-3	Gluten Free Tin Loaf
ITLB-GF-4	Gluten Free Tin Loaf
OIB-1	Baguette-style
OIB-2	Wholemeal Baguette-style
OIB-3	Baguette-style
OIB-4	Ring-shaped
OIB-5	Round loaf

GF: Gluten Free; ITLB: Industrial Tin Loaf Breads; OIB: Other Industrial Breads.

acquisition. Similarly, the non-characterized traditional breads obtained from bakeries were oven-dried within 24 h after baking.

## 2.2. *In vitro* starch digestibility

The *in vitro* digestibility of starch in bread samples was evaluated using the K-DSTRS assay kit, purchased from Megazyme International (Wicklow Ireland Ltd). The procedure allowed for the quantification of rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and total digestible starch (TDS) contents.

For each analysis, 500 mg of dried bread sample was weighed into a 250 mL bottle. A mixture of 17.5 mL of 50 mM sodium maleate buffer (pH 6.0) and 0.5 mL of 95 % ethanol was added. After homogenization, the suspension was preincubated in a shaking water bath at 37 °C for 5 min. Then, 2.5 mL of a freshly prepared pancreatic  $\alpha$ -amylase/amyloglucosidase (PAA/AMG) enzyme solution was added, and the incubation continued under agitation. Aliquots of 1 mL were removed at 20, 120 and 240 min. Each aliquot was transferred to 20 mL of 50 mM acetic acid solution to stop enzymatic activity, then centrifuged at 13,000 rpm for 5 min. A 0.1 mL aliquot of the supernatant was incubated with diluted AMG solution and GOPOD reagent. After color development (20 min at 50 °C), absorbance was read at 510 nm against a reagent blank.

The amount of digestible starch was calculated using:

$$\text{Starch (mg)} = \Delta A \times F \times \frac{EV}{W} \quad (1)$$

Where  $\Delta A$  is the difference in absorbance between the sample and the blank, F is the conversion factor, EV is the extraction volume (mL), and W is the sample weight (g).

Fragmented starch fractions were calculated as:

$$\text{RDS (mg)} = G_{20} \times 0.9 \quad (2)$$

$$\text{SDS (mg)} = (G_{120} - G_{20}) \times 0.9 \quad (3)$$

$$\text{TDS (mg)} = G_{240} \times 0.9 \quad (4)$$

Where  $G_{20}$ ,  $G_{120}$ , and  $G_{240}$  are the glucose amounts (mg) at 20 and 120 min, respectively, and 0.9 is the conversion factor from glucose to starch.

For RS, 4 mL of the hydrolysate remaining after 240 min were mixed

with 4 mL of 95 % ethanol, centrifuged (4000 rpm, 10 min), washed with 50 % ethanol, and the pellet was dissolved in 1.7 M NaOH under stirring in an ice bath for 20 min. After neutralization with 1 M sodium acetate buffer (pH 3.8), the sample was incubated with AMG and GOPOD as above. The RS content was calculated using:

$$RS \text{ (mg)} = \Delta A \times F \times \frac{EV}{W} \times \frac{FV}{1000} \times 0.9 \quad (5)$$

Where FV is the final volume of the RS extract (mL).

Finally, total starch (TS) was estimated as:

$$TS = TDS + RS \quad (6)$$

All measurements were performed in quadruplicate for each bread sample.

### 2.3. *In vitro* glycemic index

The estimation of *in vitro* glycemic index (eGI) of bread samples was determined following the methodology described by Goñi et al., 1997, with adaptations based on subsequent studies (Ferrer-Mairal et al., 2012; Kahraman et al., 2019; Koksel et al., 2023; Sulung et al., 2023), in order to simulate gastrointestinal digestion. An overview of the sequential steps involved in the protocol is presented in Fig. 1.

For this purpose, 100 mg of dried bread samples were placed in 50 mL centrifuge tubes containing 10 glass beads (5 mm). For the gastric phase, 2 mL of 0.05 M hydrochloric acid and 10 mg of pepsin (Sigma-Aldrich, P7000) were added, followed by incubation at 37 °C for 30 min in a shaking water bath. The intestinal phase was initiated by adding 4 mL of 0.5 M sodium acetate buffer (pH 5.2) and 1 mL of an enzyme solution prepared with 0.139 g of pancreatin (Sigma-Aldrich, P7545) and 14.26 U of amyloglucosidase (AMG, 3300 U/mL, Megazyme, Wicklow, Ireland). Tubes were incubated at 37 °C, and 100 µL aliquots

were withdrawn at 0, 30, 60, 90, 120, and 180 min.

Each aliquot was mixed with 96 % ethanol and centrifuged (10,000 rpm, 6 min). From the supernatant, 100 µL were transferred in duplicate to test tubes. All tubes were treated with 3 mL of GOPOD reagent (Megazyme, Wicklow, Ireland) and incubated at 50 °C for 20 min. Absorbance was measured at 510 nm.

The glucose released over time was used to calculate the area under the hydrolysis curve (AUC) using the trapezoidal method:

$$AUC = \sum_{i=1}^n \left[ \frac{C_i + C_{i-1}}{2} \times (t_i - t_{i-1}) \right] \quad (7)$$

Where  $C_i$  and  $C_{i-1}$  are the glucose concentrations (mg/mL) at consecutive time points, and  $t_i$  and  $t_{i-1}$  are the respective sampling times.

The hydrolysis index (HI) was calculated as:

$$HI = \left( \frac{AUC \text{ sample}}{AUC \text{ reference}} \right) \times 100 \quad (8)$$

Glucose was used as reference. The eGI was then estimated according to Granfeldt et al., 1994:

$$eGI = 0,862 \times HI + 8,198 \quad (9)$$

All measurements were performed in quadruplicate for each bread sample.

### 2.4. Statistical analysis

Descriptive statistics (mean ± standard deviation) were calculated using Microsoft Excel.

Inferential analyses were performed using SPSS (version 29.0.2). A two-way analysis of variance (ANOVA) was conducted to assess the effects of cereal type and processing category, as well as their interaction, on the dependent variables (starch fractions, HI, and eGI). When significant interaction effects were identified, stratified analyses were performed, followed by independent samples *t*-tests to explore specific group differences. In addition, the effect of the proportion of local flour was evaluated independently using one-way ANOVA. Statistical significance was set at  $p < 0.05$ .

Additionally, Principal Component Analysis (PCA) and graphical output were performed in R (version 4.3.2), with the packages “Factoextra” (Version: 1.0.7) (Kassambara & Mundt, 2016) and “Tidyverse” (Version: 2.0.0) (Wickham et al., 2019).

## 3. Results and discussion

### 3.1. Samples characterization

The nutritional characteristics of all bread samples, based on starch digestibility and glycemic response parameters (mean ± standard deviation), are detailed in Table 4.

### 3.2. Principal component analysis (PCA)

To explore the relationships between starch fractions and glycemic response, a PCA (Fig. 2) was conducted. The first two principal components accounted for 74.8 % of the total variance, with PC1 explaining 52.8 % and PC2 explaining 22.0 %.

The analysis revealed that HI, eGI and TDS clustered closely, suggesting a strong positive association among these variables and indicating that breads with greater amounts of digestible starch tend to elicit higher glycemic responses. RDS and TS also showed a positive trend in the same direction, though their contribution appeared less prominent and with a positive contribution to PC2. In contrast, RS and SDS loaded in the opposite direction (negative PC1), reinforcing their recognized contribution to lowering glycemic response (Englyst et al., 199;

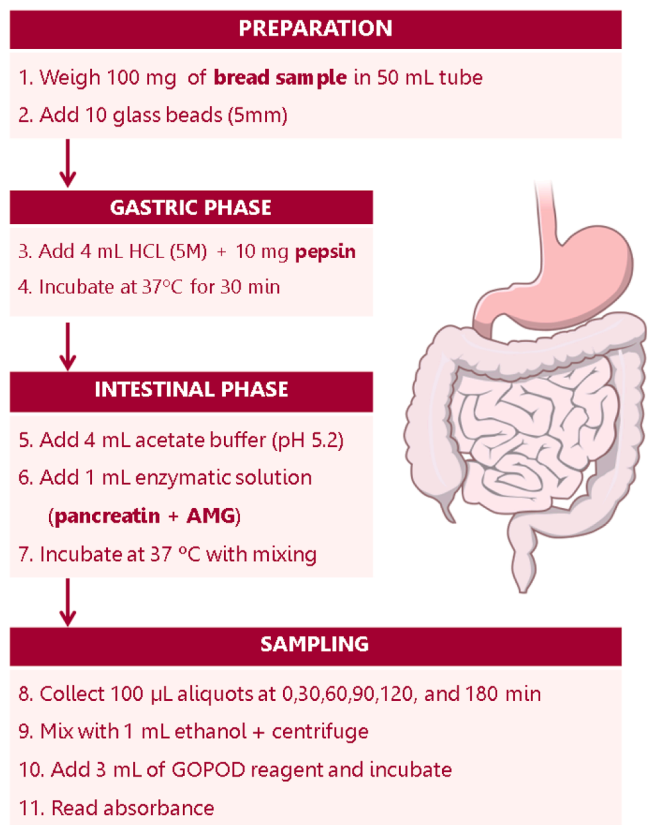


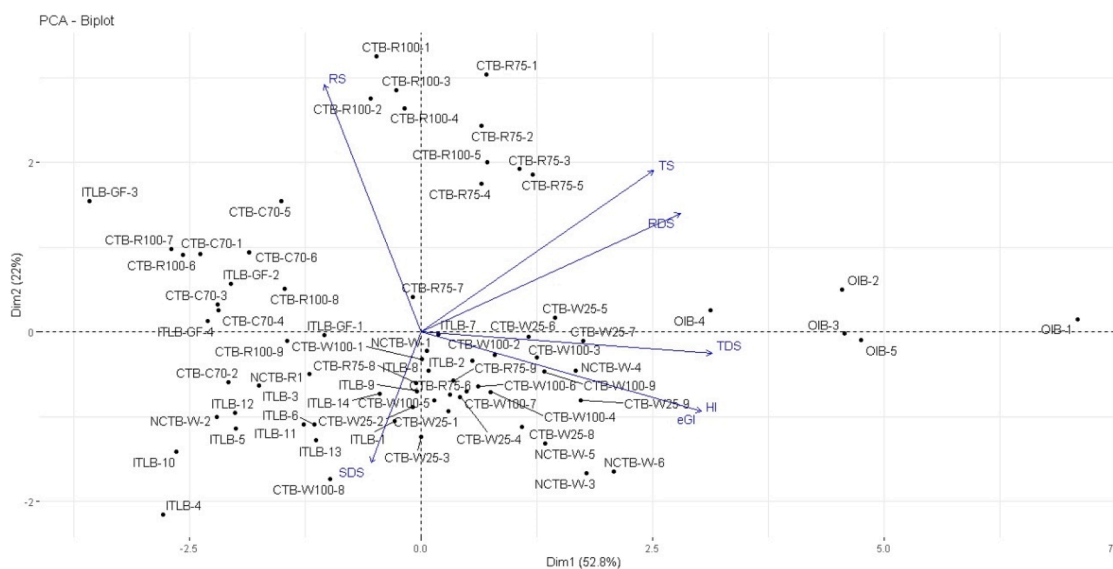
Fig. 1. Schematic representation of the *in vitro* digestion protocol used for glycemic index estimation (eGI) in bread samples.

Table 4

Descriptive statistics (mean  $\pm$  standard deviation) of starch digestibility fractions, hydrolysis index (HI), and estimated glycemic index (eGI) in analyzed bread samples wheat (W), rye (R) and corn (C).

Sample	RDS (g/100 g)	SDS (g/100 g)	TDS (g/100 g)	RS (g/100 g)	TS (g/100 g)	HI (%)	eGI (%)
CTB-W100-1	26.40 $\pm$ 6.55	0.59 $\pm$ 0.78	33.56 $\pm$ 1.09	4.12 $\pm$ 1.55	37.68 $\pm$ 0.46	66.75 $\pm$ 1.24	65.74 $\pm$ 1.06
CTB-W100-2	27.06 $\pm$ 5.02	0.94 $\pm$ 0.58	36.58 $\pm$ 1.51	3.67 $\pm$ 1.06	40.24 $\pm$ 0.44	68.74 $\pm$ 1.42	67.45 $\pm$ 1.22
CTB-W100-3	27.98 $\pm$ 6.59	1.59 $\pm$ 0.20	37.89 $\pm$ 1.64	3.51 $\pm$ 1.34	41.40 $\pm$ 0.31	70.51 $\pm$ 3.17	68.98 $\pm$ 2.73
CTB-W100-4	27.20 $\pm$ 6.00	0.73 $\pm$ 0.95	34.83 $\pm$ 0.45	3.11 $\pm$ 0.81	37.94 $\pm$ 0.36	72.41 $\pm$ 1.36	70.61 $\pm$ 1.17
CTB-W25-1	25.28 $\pm$ 4.81	0.75 $\pm$ 0.01	33.53 $\pm$ 1.11	3.19 $\pm$ 0.65	36.71 $\pm$ 0.46	72.11 $\pm$ 0.41	70.35 $\pm$ 0.35
CTB-W25-2	23.75 $\pm$ 4.89	0.91 $\pm$ 0.28	32.13 $\pm$ 0.35	4.19 $\pm$ 0.39	36.32 $\pm$ 0.74	72.13 $\pm$ 4.54	70.37 $\pm$ 3.91
CTB-W25-3	23.98 $\pm$ 5.16	1.34 $\pm$ 0.44	32.15 $\pm$ 1.22	3.41 $\pm$ 1.10	35.55 $\pm$ 0.11	73.50 $\pm$ 1.01	71.56 $\pm$ 0.87
CTB-W25-4	25.61 $\pm$ 4.02	0.73 $\pm$ 0.78	33.09 $\pm$ 0.32	4.00 $\pm$ 0.08	37.08 $\pm$ 0.39	73.96 $\pm$ 3.03	71.95 $\pm$ 2.61
CTB-W100-5	29.29 $\pm$ 2.04	3.04 $\pm$ 0.25	32.36 $\pm$ 2.64	3.68 $\pm$ 0.13	36.04 $\pm$ 2.77	68.97 $\pm$ 0.39	67.65 $\pm$ 0.33
CTB-W100-6	31.63 $\pm$ 1.94	4.72 $\pm$ 0.08	33.40 $\pm$ 1.10	4.27 $\pm$ 0.31	37.67 $\pm$ 1.42	69.87 $\pm$ 0.17	68.42 $\pm$ 0.15
CTB-W100-7	28.50 $\pm$ 4.75	5.91 $\pm$ 1.06	33.58 $\pm$ 0.42	5.59 $\pm$ 0.16	39.17 $\pm$ 0.59	71.61 $\pm$ 0.52	69.92 $\pm$ 0.45
CTB-W100-8	22.82 $\pm$ 2.44	7.65 $\pm$ 0.48	29.09 $\pm$ 0.13	5.81 $\pm$ 0.97	34.90 $\pm$ 0.84	71.64 $\pm$ 0.27	69.95 $\pm$ 0.24
CTB-W100-9	31.62 $\pm$ 2.73	4.99 $\pm$ 1.59	36.04 $\pm$ 0.26	4.51 $\pm$ 0.08	40.55 $\pm$ 0.34	72.34 $\pm$ 0.75	70.56 $\pm$ 0.64
CTB-W25-5	33.14 $\pm$ 1.37	3.07 $\pm$ 0.12	35.53 $\pm$ 0.58	5.36 $\pm$ 0.39	40.89 $\pm$ 0.19	71.97 $\pm$ 1.28	70.23 $\pm$ 1.10
CTB-W25-6	30.59 $\pm$ 0.10	2.89 $\pm$ 0.69	35.15 $\pm$ 0.02	5.31 $\pm$ 0.30	40.46 $\pm$ 0.28	72.43 $\pm$ 0.83	70.63 $\pm$ 0.72
CTB-W25-7	29.70 $\pm$ 0.77	4.69 $\pm$ 0.08	38.25 $\pm$ 0.14	5.57 $\pm$ 0.23	43.82 $\pm$ 0.38	74.02 $\pm$ 0.28	72.01 $\pm$ 0.24
CTB-W25-8	26.86 $\pm$ 1.81	7.18 $\pm$ 0.68	36.09 $\pm$ 0.28	5.06 $\pm$ 0.12	41.15 $\pm$ 0.39	75.75 $\pm$ 0.72	73.50 $\pm$ 0.62
CTB-W25-9	29.92 $\pm$ 0.22	5.74 $\pm$ 0.43	38.35 $\pm$ 0.78	3.82 $\pm$ 0.20	42.17 $\pm$ 0.98	74.40 $\pm$ 0.72	72.33 $\pm$ 0.62
CTB-R100-1	32.66 $\pm$ 0.33	1.80 $\pm$ 0.16	34.52 $\pm$ 0.61	11.91 $\pm$ 0.07	46.42 $\pm$ 0.54	47.83 $\pm$ 0.71	49.43 $\pm$ 0.61
CTB-R100-2	31.27 $\pm$ 0.03	1.21 $\pm$ 0.07	33.31 $\pm$ 0.05	11.19 $\pm$ 0.68	44.50 $\pm$ 0.63	51.61 $\pm$ 0.88	52.69 $\pm$ 0.76
CTB-R100-3	32.43 $\pm$ 0.30	1.76 $\pm$ 0.01	34.31 $\pm$ 0.15	11.20 $\pm$ 0.44	45.51 $\pm$ 0.28	51.48 $\pm$ 1.30	52.57 $\pm$ 1.12
CTB-R100-4	33.24 $\pm$ 0.14	1.61 $\pm$ 0.12	34.10 $\pm$ 0.33	10.41 $\pm$ 0.57	44.52 $\pm$ 0.24	52.39 $\pm$ 1.38	53.36 $\pm$ 1.19
CTB-R100-5	33.17 $\pm$ 0.19	0.91 $\pm$ 0.01	33.54 $\pm$ 0.16	9.77 $\pm$ 0.20	43.32 $\pm$ 0.03	64.73 $\pm$ 3.04	64.00 $\pm$ 2.62
CTB-R75-1	32.65 $\pm$ 0.02	1.93 $\pm$ 0.08	33.17 $\pm$ 0.11	13.77 $\pm$ 0.22	46.94 $\pm$ 0.11	64.66 $\pm$ 4.19	63.93 $\pm$ 3.62
CTB-R75-2	32.48 $\pm$ 0.25	1.91 $\pm$ 0.05	32.97 $\pm$ 1.27	12.13 $\pm$ 1.23	44.87 $\pm$ 0.37	65.69 $\pm$ 4.31	64.82 $\pm$ 3.72
CTB-R75-3	34.04 $\pm$ 0.15	0.24 $\pm$ 0.05	32.83 $\pm$ 0.10	9.85 $\pm$ 0.11	42.45 $\pm$ 0.11	69.49 $\pm$ 0.14	68.10 $\pm$ 0.12
CTB-R75-4	32.30 $\pm$ 0.32	0.71 $\pm$ 0.04	32.17 $\pm$ 0.18	9.89 $\pm$ 0.14	42.05 $\pm$ 0.04	68.11 $\pm$ 4.71	66.91 $\pm$ 4.06
CTB-R75-5	34.05 $\pm$ 0.06	0.53 $\pm$ 0.24	32.72 $\pm$ 0.20	9.94 $\pm$ 0.03	42.67 $\pm$ 0.24	71.28 $\pm$ 1.59	69.65 $\pm$ 1.37
CTB-R100-6	25.62 $\pm$ 0.66	5.44 $\pm$ 0.21	28.64 $\pm$ 1.39	9.53 $\pm$ 1.80	38.17 $\pm$ 0.41	47.31 $\pm$ 2.04	48.98 $\pm$ 1.76
CTB-R100-7	23.94 $\pm$ 0.75	4.88 $\pm$ 0.21	28.82 $\pm$ 0.03	9.82 $\pm$ 0.58	38.64 $\pm$ 0.55	47.00 $\pm$ 1.87	48.71 $\pm$ 1.61
CTB-R100-8	25.12 $\pm$ 0.60	2.60 $\pm$ 0.91	30.53 $\pm$ 0.04	7.63 $\pm$ 0.41	38.15 $\pm$ 0.45	56.55 $\pm$ 1.66	56.94 $\pm$ 1.43
CTB-R100-9	26.21 $\pm$ 0.26	5.57 $\pm$ 0.60	30.37 $\pm$ 0.42	7.00 $\pm$ 0.25	37.37 $\pm$ 0.66	57.40 $\pm$ 0.21	57.68 $\pm$ 0.19
CTB-R75-6	28.34 $\pm$ 0.67	4.40 $\pm$ 0.41	36.89 $\pm$ 0.23	2.69 $\pm$ 0.11	39.59 $\pm$ 0.34	62.49 $\pm$ 1.03	62.07 $\pm$ 0.88
CTB-R75-7	29.69 $\pm$ 0.13	1.82 $\pm$ 1.07	32.60 $\pm$ 0.20	6.11 $\pm$ 0.28	38.71 $\pm$ 0.48	63.37 $\pm$ 1.92	62.82 $\pm$ 1.65
CTB-R75-8	30.05 $\pm$ 2.48	2.29 $\pm$ 0.75	33.27 $\pm$ 0.25	2.86 $\pm$ 0.29	36.13 $\pm$ 0.54	63.54 $\pm$ 1.17	62.97 $\pm$ 1.01
CTB-R75-9	30.69 $\pm$ 1.91	2.55 $\pm$ 2.15	34.04 $\pm$ 0.38	2.99 $\pm$ 0.65	37.03 $\pm$ 0.27	65.94 $\pm$ 2.26	65.04 $\pm$ 1.94
CTB-C70-1	23.58 $\pm$ 0.68	5.73 $\pm$ 2.09	30.05 $\pm$ 0.55	9.95 $\pm$ 1.70	40.00 $\pm$ 1.15	48.56 $\pm$ 3.42	50.06 $\pm$ 2.95
CTB-C70-2	22.58 $\pm$ 1.17	8.41 $\pm$ 0.84	33.00 $\pm$ 0.42	5.98 $\pm$ 0.39	38.98 $\pm$ 0.03	49.56 $\pm$ 0.32	50.92 $\pm$ 0.27
CTB-C70-3	23.93 $\pm$ 2.31	5.65 $\pm$ 1.64	31.36 $\pm$ 0.11	7.57 $\pm$ 0.53	38.92 $\pm$ 0.63	48.48 $\pm$ 1.04	49.99 $\pm$ 0.90
CTB-C70-4	24.83 $\pm$ 2.58	5.33 $\pm$ 1.69	30.51 $\pm$ 0.17	7.35 $\pm$ 0.29	37.87 $\pm$ 0.45	49.52 $\pm$ 1.97	50.88 $\pm$ 1.70
CTB-C70-5	28.31 $\pm$ 1.60	2.59 $\pm$ 0.11	31.32 $\pm$ 0.77	9.06 $\pm$ 1.62	40.38 $\pm$ 0.85	49.74 $\pm$ 0.08	51.07 $\pm$ 0.07
CTB-C70-6	26.64 $\pm$ 2.38	4.29 $\pm$ 2.02	31.28 $\pm$ 0.02	8.26 $\pm$ 0.29	39.54 $\pm$ 0.31	48.69 $\pm$ 1.12	50.17 $\pm$ 0.97
NCTB-R-1	26.12 $\pm$ 0.35	4.57 $\pm$ 0.44	33.29 $\pm$ 1.38	4.11 $\pm$ 0.78	37.39 $\pm$ 2.16	54.57 $\pm$ 1.03	55.24 $\pm$ 0.89
NCTB-W-1	30.35 $\pm$ 0.72	4.14 $\pm$ 0.59	35.21 $\pm$ 0.85	3.92 $\pm$ 0.50	39.14 $\pm$ 0.35	60.36 $\pm$ 1.22	60.23 $\pm$ 1.05
NCTB-W-2	23.84 $\pm$ 1.21	4.52 $\pm$ 0.36	29.42 $\pm$ 0.36	4.11 $\pm$ 0.14	33.52 $\pm$ 0.50	54.19 $\pm$ 1.31	54.91 $\pm$ 1.13
NCTB-W-3	26.80 $\pm$ 0.73	9.44 $\pm$ 0.72	39.15 $\pm$ 0.42	3.94 $\pm$ 1.04	43.09 $\pm$ 0.63	78.58 $\pm$ 1.05	75.93 $\pm$ 0.90
NCTB-W-4	26.95 $\pm$ 0.14	6.26 $\pm$ 0.31	36.27 $\pm$ 0.21	7.27 $\pm$ 0.62	43.54 $\pm$ 0.41	80.76 $\pm$ 1.08	77.82 $\pm$ 0.93
NCTB-W-5	30.23 $\pm$ 0.13	2.60 $\pm$ 0.24	34.14 $\pm$ 0.69	2.34 $\pm$ 0.03	36.47 $\pm$ 0.66	78.64 $\pm$ 1.05	75.99 $\pm$ 0.91
NCTB-W-6	28.23 $\pm$ 0.96	11.01 $\pm$ 0.59	39.78 $\pm$ 2.47	4.33 $\pm$ 0.14	44.11 $\pm$ 2.61	79.43 $\pm$ 3.04	76.66 $\pm$ 2.62
ITLB-1	27.64 $\pm$ 1.11	7.53 $\pm$ 0.51	36.21 $\pm$ 0.11	3.12 $\pm$ 0.38	39.33 $\pm$ 0.50	58.78 $\pm$ 1.17	58.87 $\pm$ 1.01
ITLB-2	30.50 $\pm$ 0.07	2.76 $\pm$ 0.51	36.38 $\pm$ 0.99	3.31 $\pm$ 0.95	39.69 $\pm$ 0.04	58.60 $\pm$ 0.80	58.72 $\pm$ 0.69
ITLB-3	28.13 $\pm$ 0.89	4.98 $\pm$ 1.00	37.91 $\pm$ 0.82	4.07 $\pm$ 0.66	41.98 $\pm$ 0.16	62.89 $\pm$ 1.64	62.41 $\pm$ 1.41
ITLB-4	31.32 $\pm$ 1.95	4.25 $\pm$ 0.52	35.65 $\pm$ 0.33	2.72 $\pm$ 0.71	38.36 $\pm$ 0.39	59.03 $\pm$ 0.25	59.08 $\pm$ 0.21
ITLB-5	21.49 $\pm$ 0.41	4.04 $\pm$ 1.25	27.37 $\pm$ 1.00	1.55 $\pm$ 0.15	28.92 $\pm$ 0.84	55.82 $\pm$ 1.19	56.31 $\pm$ 1.02
ITLB-6	22.80 $\pm$ 0.57	5.76 $\pm$ 0.29	28.79 $\pm$ 0.54	5.32 $\pm$ 0.73	34.11 $\pm$ 0.19	59.34 $\pm$ 3.30	59.35 $\pm$ 2.85
ITLB-7	22.90 $\pm$ 0.66	7.68 $\pm$ 1.97	33.06 $\pm$ 0.16	5.31 $\pm$ 0.40	38.37 $\pm$ 0.24	60.31 $\pm$ 0.83	60.19 $\pm$ 0.71
ITLB-8	24.60 $\pm$ 0.22	7.83 $\pm$ 2.33	32.04 $\pm$ 0.77	5.82 $\pm$ 1.11	37.86 $\pm$ 0.33	53.31 $\pm$ 1.08	54.16 $\pm$ 0.93
ITLB-9	29.54 $\pm$ 0.06	4.35 $\pm$ 1.72	34.71 $\pm$ 0.35	3.04 $\pm$ 0.23	37.74 $\pm$ 0.12	61.73 $\pm$ 0.86	61.41 $\pm$ 0.74
ITLB-10	24.31 $\pm$ 1.63	3.46 $\pm$ 0.57	28.15 $\pm$ 1.67	2.23 $\pm$ 0.10	30.38 $\pm$ 1.77	51.77 $\pm$ 0.62	52.82 $\pm$ 0.53
ITLB-11	27.12 $\pm$ 0.14	3.78 $\pm$ 1.20	32.67 $\pm$ 0.66	1.83 $\pm$ 0.49	34.49 $\pm$ 0.16	54.85 $\pm$ 0.50	55.48 $\pm$ 0.43
ITLB-12	26.44 $\pm$ 0.36	3.96 $\pm$ 0.45	31.39 $\pm$ 0.54	2.21 $\pm$ 0.03	33.60 $\pm$ 0.57	49.49 $\pm$ 1.24	50.86 $\pm$ 1.07
ITLB-13	27.72 $\pm$ 0.18	5.46 $\pm$ 1.87	34.26 $\pm$ 0.36	1.23 $\pm$ 0.22	35.49 $\pm$ 0.58	53.03 $\pm$ 1.46	53.91 $\pm$ 1.25
ITLB-14	30.06 $\pm$ 0.00	4.15 $\pm$ 0.83	36.25 $\pm$ 0.80	1.24 $\pm$ 0.16	37.49 $\pm$ 0.64	53.36 $\pm$ 1.35	54.20 $\pm$ 1.16
ITLB-GF-1	27.00 $\pm$ 0.05	6.21 $\pm$ 0.12	35.23 $\pm$ 0.06	5.17 $\pm$ 0.03	40.40 $\pm$ 0.10	51.07 $\pm$ 0.27	52.22 $\pm$ 0.23
ITLB-GF-2	24.72 $\pm$ 0.92	5.74 $\pm$ 0.73	28.80 $\pm$ 0.49	9.65 $\pm$ 0.49	38.44 $\pm$ 0.00	54.33 $\pm$ 4.02	55.03 $\pm$ 3.47
ITLB-GF-3	22.75 $\pm$ 0.37	4.49 $\pm$ 0.87	22.99 $\pm$ 0.87	13.65 $\pm$ 0.69	36.64 $\pm$ 0.18	49.33 $\pm$ 1.37	50.72 $\pm$ 1.17
ITLB-GF-4	25.55 $\pm$ 0.09	4.71 $\pm$ 0.12	28.87 $\pm$ 0.53	7.12 $\pm$ 1.44	35.99 $\pm$ 0.91	50.93 $\pm$ 0.64	52.10 $\pm$ 0.56
OIB-1	42.17 $\pm$ 0.30	4.19 $\pm$ 0.80	52.23 $\pm$ 1.18	1.52 $\pm$ 0.70	53.75 $\pm$ 0.48	92.95 $\pm$ 3.13	88.32 $\pm$ 2.70
OIB-2	36.22 $\pm$ 1.26	6.16 $\pm$ 0.38	47.85 $\pm$ 2.19	4.17 $\pm$ 0.23	52.01 $\pm$ 1.96	77.90 $\pm$ 1.42	75.35 $\pm$ 1.22
OIB-3	40.99 $\pm$ 1.37	2.26 $\pm$ 0.64	43.80 $\pm$ 0.42	1.45 $\pm$ 0.19	45.25 $\pm$ 0.23	82.67 $\pm$ 2.48	79.46 $\pm$ 2.13
OIB-4	35.65 $\pm$ 1.59	1.15 $\pm$ 1.37	39.88 $\pm$ 0.55	3.73 $\pm$ 0.23	43.60 $\pm$ 0.78	79.12 $\pm$ 1.42	76.40 $\pm$ 1.22
OIB-5	37.63 $\pm$ 0.67	3.59 $\pm$ 0.64	45.12 $\pm$ 0.55	2.65 $\pm$ 0.40	47.78 $\pm$ 0.15	85.77 $\pm$ 3.53	82.13 $\pm$ 3.04

eGI: Estimated Glycemic Index; HI: Hydrolysis Index; RDS: Rapidly Digestible Starch; RS: Resistant Starch; SDS: Slowly Digestible Starch; TDS: Total Digestible Starch; TS: Total Starch; CTB-C70: Characterized Traditional Bread with 70 % corn flour; CTB-R100: Characterized Traditional Bread with 100 % rye flour; CTB-R75: Characterized Traditional Bread with 75 % rye flour; CTB-W100: Characterized Traditional Bread with 100 % wheat flour; CTB-W25: Characterized Traditional Bread with 25 % local wheat flour; ITLB: Industrial Tin Loaf Bread; ITLB-GF: Gluten-Free Industrial Tin Loaf Bread; NCTB: Non-Characterized Traditional Bread; OIB: Other Industrial Bread.



**Fig. 2.** Principal component analysis (PCA) of bread samples (C, corn; R, rye; W, wheat) based on starch fractions and glycemic response variables. eGI: Estimated Glycemic Index; HI: Hydrolysis Index; RDS: Rapidly Digestible Starch; RS: Resistant Starch; SDS: Slowly Digestible Starch; TDS: Total Digestible Starch; TS: Total Starch; CTB-C70: Characterized Traditional Bread with 70 % corn flour; CTB-R100: Characterized Traditional Bread with 100 % rye flour; CTB-R75: Characterized Traditional Bread with 75 % rye flour; CTB-W100: Characterized Traditional Bread with 100 % wheat flour; CTB-W25: Characterized Traditional Bread with 25 % local wheat flour; ITLB: Industrial Tin Loaf Bread; ITLB-GF: Gluten-Free Industrial Tin Loaf Bread; NCTB: Non-Characterized Traditional Bread; OIB: Other Industrial Bread.

Fuentes-Zaragoza et al., 2010; Magallanes-Cruz et al., 2017).

Furthermore, PCA enabled the identification of distinct clusters of bread samples based on their starch digestibility profiles and predicted glycemic response. Characterized traditional breads formulated with 70 % corn flour (CTB-C70) consistently exhibited the lowest glycemic response, as reflected by their minimal eGI values across all bread categories. This may be attributed to the compositional characteristics of corn starch, which includes a higher proportion of slowly digestible and resistant fractions (Singh et al., 2019). Traditional rye breads (CTB-R100 and CTB-R75) are also clustered in regions associated with elevated RS content and low glycemic response, albeit slightly higher than that observed for corn-based samples.

In contrast, many industrial bread samples, particularly those classified as OIB clustered in the quadrant associated with high HI, eGI, RDS, and TS. These samples generally reflect formulations with refined wheat flour, low fiber content, and fast fermentation processes, all of which contribute to rapid starch digestion and higher glycemic response (Borcak et al., 2018). Moreover, ITLB group occupied an intermediate position in the PCA space. Although they generally showed higher HI and eGI values than traditional corn-based breads, they did not reach the extremes observed in OIB. Their nutritional profile was characterized by moderate levels of RDS and TS, along with measurable amounts of SDS and RS, likely attributable to the inclusion of added fibers or legume flours in some formulations (Pasqualoni et al., 2024).

On the other hand, NCTB displayed greater variability. While some showed compositional similarities to the characterized traditional wheat breads (CTB-W), others clustered closer to OIB based on their digestibility profiles. This suggests that artisanal origin alone does not ensure a favorable glycemic response, unless supported by specific ingredients, such as local cereal varieties, and processing techniques like extended fermentation or the use of sourdough, which have been associated with increased levels of SDS and RS (Costantini et al., 2022;

Temkov et al., 2024; Terrazas-Avila et al., 2024).

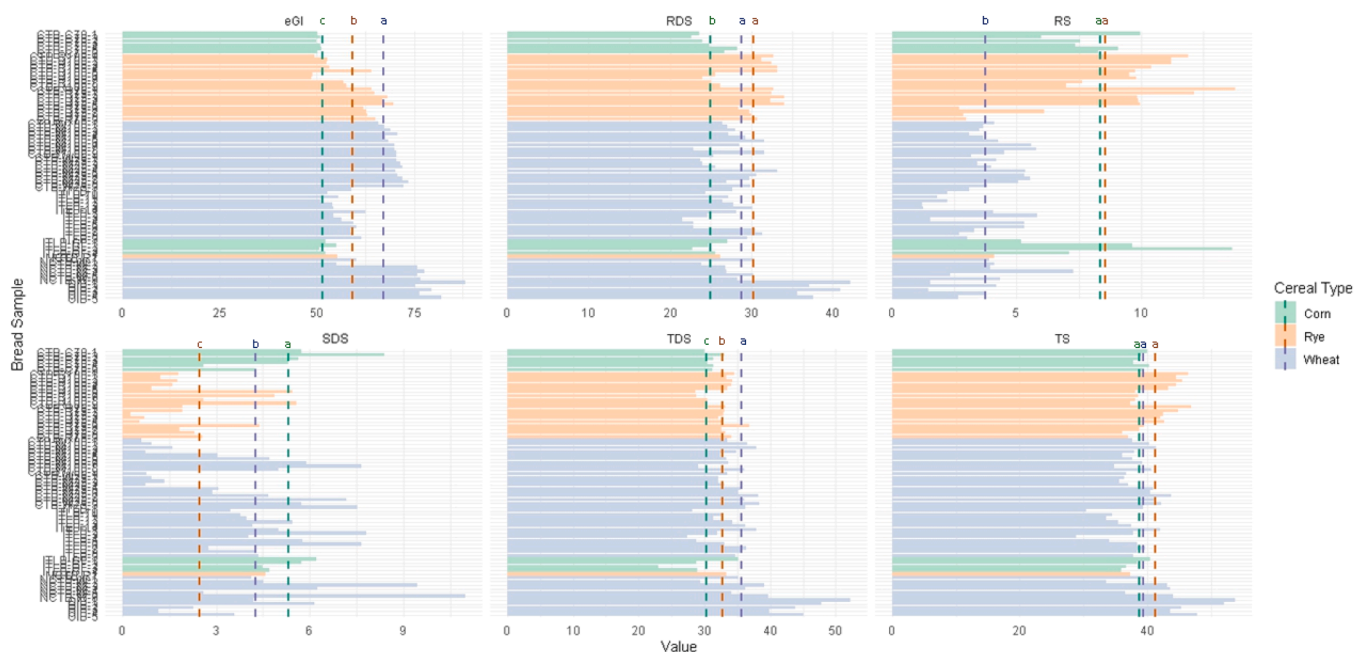
Overall, the multivariate analysis highlights that both compositional and technological factors influence starch digestibility and glycemic potential in bread.

### 3.3. Group comparison of starch digestibility and glycemic response

To assess the combined effect of cereal type and processing conditions on starch digestibility and glycemic response, bread samples were compared across defined categories. Significant differences were observed for each factor and their interaction (Table S3, Supplementary Material). Thus, cereal type had a marked influence on all parameters except TS, while processing category significantly affected all variables. Moreover, significant interactions between cereal type and processing were found for RDS, SDS, RS, HI, and eGI, indicating that the impact of processing conditions on these parameters varied depending on the cereal used. These interaction effects are further explored in the stratified analysis presented below.

#### 3.3.1. Effect of cereal type

As illustrated in Fig. 3, starch digestibility and glycemic response parameters differed significantly among cereal types. Breads made with corn exhibited the lowest eGI values (mean =  $51.32 \pm 1.90$ ), which according to international classifications (Augustin et al., 2015) places them within the low GI category ( $\leq 55$ ). Along with this low eGI, corn breads showed the highest mean levels of SDS, elevated RS levels, and the lowest TS and TDS contents. These results suggest that corn-based formulations promote slower starch digestibility and a reduced glycemic impact. This behavior can be attributed to the inherent physicochemical characteristics of maize starch, such as its higher amylose content, lower gelatinization extent, and reduced susceptibility to enzymatic hydrolysis compared to wheat starches (Arif et al., 2025).



**Fig. 3.** Mean values of estimated glycemic index (eGI) and starch fractions (Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS)) in bread samples grouped by cereal type (wheat, rye, corn). Different letters indicate significant differences between cereal groups ( $p < 0.05$ , Tukey's test). Dashed vertical lines represent group means.

Rye breads showed an intermediate profile, with mean eGI of  $59.30 \pm 6.74$ , falling within the medium GI category (56–69), and exhibiting the highest RS content among cereals. These effects can be attributed not only to the specific properties of rye starch, but also to its higher content of soluble dietary fiber, and to the acidic environment generated during sourdough fermentation. According to Ghazvini et al. (2025), these factors play a crucial role in modulating starch digestibility: increased crumb compactness, fiber–starch interactions, and organic acid content in rye-based formulations collectively reduce enzymatic access to starch granules. As a result, rye breads are associated with reduced glycemic response.

In contrast, wheat breads presented the highest average eGI (mean =  $67.21 \pm 8.90$ ), also within the medium GI category, but close to its upper threshold. These results align with those of Skřivan et al. (2024), who reported that wheat breads, particularly those made from refined flour, tend to exhibit greater starch gelatinization during baking and reduced retrogradation during storage, leading to lower levels of resistant starch. Furthermore, the lower fiber content and less compact crumb structure of many wheat breads may enhance enzyme accessibility, further contributing to the higher glycemic response observed. It is important to highlight that wheat breads showed the greatest variability in eGI values among all cereal types analyzed. This variability is consistent with the wide range of *in vivo* glycemic index values reported for wheat-based breads in the *International Tables of Glycemic Index and Glycemic Load Values* (Atkinson et al., 2008), where GI values differ substantially depending on formulation and processing, from  $75 \pm 2$  for white wheat bread and  $74 \pm 2$  for whole-wheat bread to  $70 \pm 5$  for unleavened wheat bread and as low as  $53 \pm 2$  for specialty grain breads. These reference values, together with the ranges observed in the present study, underscore the strong influence of technological and compositional factors on the glycemic behavior of wheat breads. This variability among wheat breads and its potential causes will be further discussed in the following section.

### 3.3.2. Effect of processing category

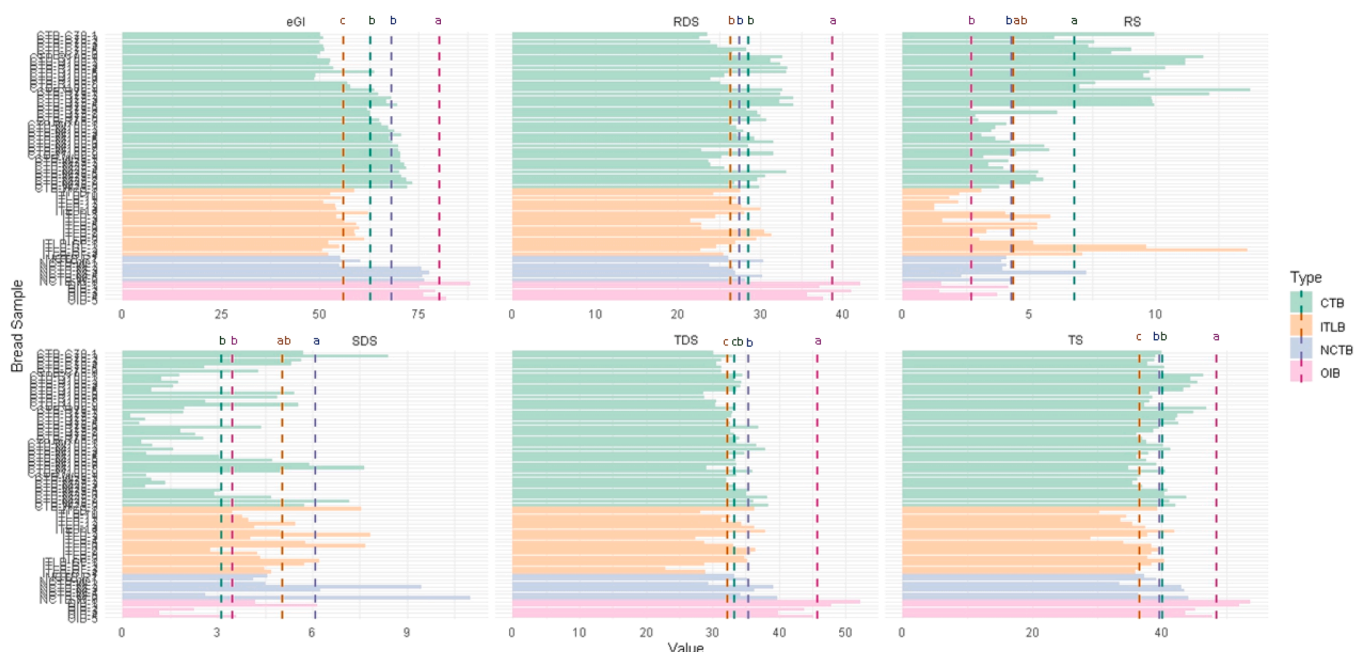
Bread samples were classified into four processing categories (CTB, NCTB, ITLB, and OIB) to evaluate the influence of formulation and processing conditions on starch digestibility and predicted glycemic

response (Fig. 4).

Among all bread categories analyzed, ITLB exhibited the lowest mean eGI values (mean =  $55.99 \pm 3.76$ ), despite their industrial origin. This result can be attributed primarily to their specific formulation, which commonly includes added fats capable of reducing starch digestibility by forming amylose–lipid complexes that delay enzymatic hydrolysis (Khatun et al., 2019; Sun et al., 2023). Additionally, these products often contain emulsifiers (E 471), which have also been shown to modify the *in vitro* starch digestibility (Skřivan et al., 2024). Furthermore, ingredient labels (Table S2, Supplementary Material) revealed the frequent incorporation of fiber-enriched flours and legume derivatives, ingredients known to lower postprandial glycemia by increasing matrix density and enhancing protein and soluble fiber content (Di Cairano et al., 2020; Pasqualoni et al., 2024). Consistent with these observations, Jiang et al. (2021) demonstrated that the incorporation of insoluble dietary fiber, such as that derived from ginseng, into bread formulations can delay glucose bioavailability and absorption, thereby contributing to a reduced postprandial glycemic response. ITLB samples also presented the lowest total starch (TS) content across all groups, which may have contributed further to their reduced glycemic potential.

Notably, four samples within this group corresponded to gluten-free formulations (ITLB-GF), typically based on corn starch. Consistently, these samples exhibited significantly higher RS contents and lower HI and eGI values than their conventional counterparts. Moreover, the inclusion of rice flour in these formulations (Table S2, Supplementary Material) may partially explain these results, since rice-based ingredients have been associated with reduced starch digestibility and lower glycemic response. (Qin et al., 2025) reported that the incorporation of black rice and osmanthus flours into wheat bread decreased starch digestibility and glycemic potential, mainly due to the interaction between polyphenols and digestive enzymes, which inhibited starch hydrolysis. Additionally, the high dietary fiber content of these ingredients was suggested to further reduce starch digestibility by entrapping starch granules or forming water-absorbing gels that slow down enzymatic access.

However, despite these favorable digestibility results, the overall nutritional quality of ITLB remains questionable. These products are



**Fig. 4.** Mean values of estimated glyceimic index (eGI) and starch fractions (Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS)) in bread samples grouped by processing category: characterized traditional breads (CTB), industrial tin loaf breads (ITLB), non-characterized traditional breads (NCTB), and other industrial breads (OIB). Different letters indicate significant differences between cereal groups ( $p < 0.05$ , Tukey's test). Dashed vertical lines represent group means.

often low in fiber and micronutrients while containing relatively high amounts of added fats and additives (Shakhman et al., 2025). Their classification as ultra-processed foods (NOVA Group 4) is of particular concern, given the consistent evidence linking frequent consumption of ultra-processed products to increased risks of type 2 diabetes, dyslipidemia, abdominal obesity, and elevated cardiovascular and all-cause mortality (Barbaresco et al., 2025; Monteiro et al., 2019).

In contrast, OIB samples had the highest eGI (mean =  $80.33 \pm 5.16$ ), falling within the high GI category ( $\geq 70$ ). They also exhibited the highest values of RDS, TS, and TDS, along with the lowest contents of RS and SDS, confirming their unfavorable digestibility profile. Their formulations typically rely on refined wheat flour, short fermentation, and limited structural complexity, which increase starch bioaccessibility and enzymatic hydrolysis rates (Borcak et al., 2018).

On the other hand, CTB samples showed higher TS content than ITLB but still ranked second in terms of glyceimic control, with low RDS and TDS values, and elevated RS content. These profiles reflect the beneficial effects of traditional breadmaking practices such as the use of long fermentation and sourdough starters, which are known to improve starch resistance and reduce postprandial glycemia (Costantini et al., 2022; Temkov et al., 2024; Terrazas-Avila et al., 2024).

Within the CTB group, the proportion of local flour used in the formulation influenced the glyceimic response of wheat- and rye-based breads. For wheat breads (CTB-W) samples, no significant differences were observed across most starch digestibility parameters. However, the percentage of local wheat flour had a significant effect on HI and eGI

(Table 5). Breads formulated with 100 % local wheat flour showed significantly lower HI and eGI values compared to those with only 25 % local flour, suggesting improved glyceimic control. These results align with prior reports indicating that Galician local wheat cultivars may provide higher levels of protein, complex carbohydrates, lipids, and dietary fiber (Espana-Fariñas et al., 2025; Fernández-Canto et al., 2024), which can modulate starch digestibility and postprandial glycemia.

In the case of rye breads (CTB-R), formulation with 100 % local rye flour exhibited significantly lower RDS, TDS, HI, and eGI compared to those containing only 75 % rye local flour blended with commercial wheat. Additionally, trends toward higher RS and SDS contents were observed in the 100 % local rye group, although these differences did not reach statistical significance ( $p = 0.062$  and  $p = 0.063$ , respectively). These findings suggest that the inclusion of commercial wheat flour may compromise the beneficial glyceimic profile of traditional rye breads by increasing the digestibility of starch.

Finally, NCTB samples (Fig. 4) displayed substantial heterogeneity in starch digestibility metrics. Some resembled CTB in their RS or SDS content, while others were closer to OIB in their high RDS and eGI values. This inconsistency likely results from the diverse processing methods used in artisanal local bakeries. According to the information summarized in Table S1 (Supplementary material), the NCTB-R1, NCTB-W1, and NCTB-W2 samples, those prepared with sourdough, showed lower eGI values (55.24, 60.23, and 54.91, respectively) compared with the remaining NCTB breads produced without sourdough, which ranged between 75.93 and 77.82. This trend is consistent with previous

**Table 5**  
Mean values ( $\pm$  standard deviation) of starch fractions and glyceimic parameters in traditional breads according to the percentage of local flour.

Cereal	%Local Flour	eGI	HI	RDS	SDS	TDS	RS	TS
Wheat	100 %	$68.81 \pm 1.81^a$	$70.32 \pm 2.10^a$	$28.06 \pm 4.27^b$	$3.35 \pm 2.57^a$	$34.15 \pm 2.69^a$	$4.25 \pm 1.10^b$	$38.40 \pm 2.23^b$
	25 %	$71.44 \pm 1.64^a$	$73.36 \pm 1.90^a$	$27.65 \pm 3.99^b$	$3.03 \pm 2.33^a$	$34.92 \pm 2.36^a$	$4.43 \pm 0.95^b$	$39.35 \pm 2.90^{ab}$
Rye	100 %	$53.82 \pm 4.96^c$	$52.92 \pm 5.76^c$	$29.30 \pm 3.84^{ab}$	$2.87 \pm 1.85^a$	$32.02 \pm 2.37^b$	$9.83 \pm 1.67^a$	$41.84 \pm 3.58^a$
	75 %	$65.14 \pm 3.07^b$	$66.06 \pm 3.56^b$	$31.59 \pm 2.09^a$	$1.82 \pm 1.38^a$	$33.41 \pm 1.41^{ab}$	$7.80 \pm 4.13^a$	$41.16 \pm 3.49^a$

eGI: Estimated Glyceimic Index; HI: Hydrolysis Index; RDS: Rapidly Digestible Starch; RS: Resistant Starch; SDS: Slowly Digestible Starch; TDS: Total Digestible Starch; TS: Total Starch. Bold values denote statistically significant differences ( $p < 0.05$ ). Different letters indicate statistically significant differences according to a post hoc Tukey's test.

evidence indicating that organic acid production and enzymatic modifications during sourdough fermentation can slow starch hydrolysis and lower the glycemic impact of bread (Costantini et al., 2022; Temkov et al., 2024; Terrazas-Avila et al., 2024). In addition, NCTB-W1 was made with local wheat cultivar ‘Caaveiro’, while NCTB-W2 combined the use of a local cultivar with exclusively whole-wheat flour. These characteristics may have further contributed to their reduced eGI due to the higher fiber content and limited starch gelatinization associated with less refined flour (Koksel et al., 2023). Altogether, these results suggest that both the use of sourdough and the choice of flour type can modulate the eGI of artisanal breads. This highlights the need for further studies encompassing a broader range of traditional products with fully characterized processing conditions; in order to better understand how artisanal practices influence starch digestibility and glycemic response.

It should also be noted that these results are based on *in vitro* estimations of glycemic response, which, although standardized and reproducible, cannot fully reproduce human digestive physiology (Li & Hu, 2022). Therefore, the eGI values reported here should be interpreted as predictive indicators rather than absolute measures, and future research should include *in vivo* studies to validate these findings.

Additionally, one limitation of this study is the exclusive evaluation of breads in their fresh state. Although this approach reflects the conditions under which these products are typically consumed, it does not account for potential changes in starch digestibility associated with storage or ageing. Future research should therefore address how storage time and conditions may influence the glycemic response of different bread types.

#### 4. Conclusion

This study provides a comprehensive assessment of starch digestibility and eGI in breads from the Galician market, highlighting the influence of cereal type and processing practices on their nutritional quality. Breads made with corn and rye flour, especially those produced using traditional techniques and local flours, consistently exhibited lower eGI values and higher contents of SDS and RS, respectively, indicating a more favorable glycemic profile. Conversely, many industrial breads, particularly those with refined wheat flour and short fermentation, showed higher levels of rapidly digestible starch and higher glycemic responses. These results underscore the potential of traditional breads formulated with local cereals, sourdough, and extended fermentation to provide a more favorable glycemic profile.

Further research should validate these *in vitro* results with *in vivo* studies, while also exploring the impact of storage conditions on the glycemic response of breads.

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#### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used AI-assisted technologies in order to enhance the language readability of the manuscript. No AI tools were involved in data analysis, interpretation, or content generation. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

#### CRedit authorship contribution statement

**M Pilar España-Fariñas:** Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Esperanza M Méndez-Sánchez:** Methodology, Investigation, Conceptualization. **Santiago Pereira-Lorenzo:** Writing – review & editing, Supervision, Project administration. **María Ángeles Romero-Rodríguez:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101483.

#### Data availability

Data will be made available on request.

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