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Oat and lentil-based muffins as a functional food: Enhanced glycaemia control, anti-inflammatory effects, and intestinal epithelium integrity

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ABSTRACT

The increased interest in functional foods has driven research into health-promoting properties. This study evaluated muffins made with 100 % oatmeal (OM) or a 50:50 lentil/oatmeal flour mix (LM) using the INFOGEST *in vitro* model. Analysis included starch composition, glycaemic response, cytotoxicity, and immunomodulatory effects in Caco-2 cells under basal and stimulated conditions. Digestion revealed limited hydrolysis in oral/gastric phases, with increase in intestinal phase. OM exhibited 8.9 % higher starch and greater hydrolysis. In contrast, LM presented 9.3 % lower estimated glycaemic index. Both muffins were non-toxic. Under stimulation, LM showed stronger anti-inflammatory effects, decreasing interleukin-6/8 mRNA and cytokine levels, while upregulating *Transforming Growth Factor-β* under basal conditions. LM enhanced barrier integrity, reduced *Acetyl-Coenzyme A acetyltransferase 2*, and upregulated *Peroxisome proliferator-activated receptor-γ* and *Sirtuin 1*, indicating improved metabolic regulation. These findings highlight lentil/oatmeal muffins as functional options that may support glycaemic control and intestinal health, aiding strategies against diabetes and cardiovascular disease.

1. Introduction

Lentils (*Lens culinaris*), a widely consumed legume grain, are recognised for their high nutritional value and associated health benefits (Li et al., 2024). As a functional food, lentils are rich in protein, dietary fibre, and essential micronutrients, and recent epidemiological studies have suggested potential benefits against cardiometabolic diseases, particularly cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (Dhull, Kinabo, & Uebersax, 2023; Ferreira, Vasconcelos, Gil, & Pinto, 2021; Kaale, Siddiq, & Hooper, 2023). These effects are partially attributed to their low glycaemic index (GI), which helps avoid postprandial blood glucose peaks, improving metabolic control (Clarke et al., 2022; Ferreira et al., 2021). A contributing factor is that part of the lentil starch remains encapsulated within cotyledon cell walls, reducing enzymatic accessibility and slowing digestion (Li, Zhang, & Dhital, 2019). This structural feature contributes to a reduced glycaemic response, although the benefit may be reduced when pulses are mechanically disrupted or incorporated as raw flours (Duijsens et al., 2023). Furthermore, lentils are rich in bioactive compounds, such as phenolic acids and flavonoids, which exhibit anti-inflammatory and antioxidant properties (Mustafa et al., 2022).

CVD and T2DM share common pathophysiological features, including chronic low-grade inflammation, oxidative stress, defined as an imbalance between the presence of pro-oxidative species and antioxidative defences, and often impaired blood glucose homeostasis (Lima, Moreira, & Sakamoto-Hojo, 2022; Sharif et al., 2021; Steven et al., 2019). As such, dietary interventions capable of regulating glycaemic fluctuations and inflammatory responses are crucial in the prevention and management of both diseases (Senesi, Ferrulli, Luzi, & Terruzzi, 2021). Among pulses, lentils and lentil foods are promising candidates in this context due to their low glycaemic index and, therefore, ability to modulate postprandial glycaemia (Chamberlin, Wilson, Gaston, Kuo, & Miles, 2024). Similarly, oats (*Avena sativa*) and oat-based products have been extensively studied for their glycaemic benefits, attributed to their high content of β-glucans, a type of soluble fibre that slows glucose absorption and improves insulin sensitivity (Wolever

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et al., 2019). Together, these ingredients may offer complementary benefits when incorporated into the same food matrix. The glycaemic impact of foods is closely related to their starch compositions, typically classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). A higher proportion of SDS and RS contributes to a slower digestion rate, leading to attenuated glucose responses and improved glycaemic control (Breyton et al., 2021; Wang, Zhou, Xiang, & Miao, 2023). Furthermore, the fibre and protein content in lentils and oats may further influence satiety, glucose homeostasis, and inflammatory markers, reinforcing their therapeutic potential (Ganesan & Xu, 2017; Martinez, Meyer, & Duca, 2021). While GI estimates the quality of carbohydrate digestion by measuring postprandial blood glucose response, it does not account for the quantity of carbohydrates consumed (Vlachos, Malisova, Lindberg, & Karaniki, 2020). Thus, glycaemic load (GL), which combines both GI and carbohydrate content per serving, provides a more comprehensive indicator of a food's overall impact on blood glucose (Lambadiari, Korakas, & Tsimihodimos, 2020; Vlachos et al., 2020). Notably, a food with high GI may still have a low GL if its carbohydrate content per serving is minimal. Therefore, evaluating both metrics along with starch fraction composition (RDS, SDS, and RS) offers a more comprehensive understanding of carbohydrate digestion and metabolic effects (Dhull et al., 2023; Ramdath et al., 2020; Wang, Zhou, Xiang, & Miao, 2023). To estimate these parameters, *in vitro* digestion models, such as the INFOGEST protocol, have become widely used (Brodkorb et al., 2019). This method mimics the enzymatic and physiological conditions of the human gastrointestinal tract. It has been widely adopted for evaluating the glycaemic potential of foods (Fernandes, Madalena, Pinheiro, & Vicente, 2020; Peng et al., 2023), offering a reproducible and ethical alternative to human or animal studies. However, its application to complex, real-world food matrices with pulses remains limited, with only a few studies available (Duijsens et al., 2023), and almost none focusing on bakery products with pulses or oats. Additionally, this model has proven useful for evaluating the release and bioavailability of bioactive compounds (Ketnawa, Reginio Jr, Thuengtung, & Ogawa, 2022; Wojtunik-Kulesza et al., 2020), including polyphenols, whose anti-inflammatory properties are particularly relevant in metabolic diseases such as obesity and T2DM (Rohm, Meier, Olefsky, & Donath, 2022).

The growing demand for gluten-free alternatives, driven by both medical needs and consumer trends, has also fostered the development of cereal- and pulse-based bakery products (Gómez, 2022). Lentils and oats, naturally gluten-free, represent ideal ingredients for the formulation of innovative bakery products targeting the gluten-free market, which still faces challenges in offering nutritionally balanced options with proven metabolic benefits (Geraldo et al., 2024; Stantiall & Serventi, 2018). Despite the promising properties of these ingredients, direct comparisons of their functional effects in equivalent food formats are scarce. Moreover, while each ingredient has been individually associated with improved glycaemic responses and anti-inflammatory effects (Guo et al., 2023; Paudel, Dhungana, Caffe, & Krishnan, 2021), the potential synergistic benefits of combining lentil and oat flours in a single matrix are still not well characterised. Additionally, data on the impact of *in vitro* digestion products from these composite foods on inflammatory and metabolic responses are still scarce, warranting further investigation.

Considering this, the purpose of this work was to investigate the digestion and metabolic impact of a lentil-based muffin (LM) compared to a classical oatmeal muffin (OM). Although both products are composed of familiar and widely consumed ingredients, their combination into a new matrix may alter the bioavailability of starches and bioactive compounds. Given the increasing consumer demand for functional foods that actively promote health, it is essential to evaluate both their glycaemic potential and impact on key cellular pathways relevant to metabolic diseases. Therefore, beyond assessing estimated GI and GL, and starch fractions using the INFOGEST protocol, the cytotoxicity, immunomodulatory activity and effects of the digested products in an *in vitro* model of intestinal epithelial cells were explored. These provide a more comprehensive evaluation of functional properties and biological safety of novel legume-based food matrices.

2. Material and methods

2.1. Products

The products used in this work were formulated based on the recipe described previously (Geraldo et al., 2024). The LM and OM muffins were prepared using 50 % lentil flour (green lentil Kermit, University of Saskatchewan, Canada) mixed with 50 % oatmeal flour (Seara, Produtos Naturais, Lda, Portugal) and 100 % oatmeal flour, respectively, as described. Both flours were combined with xylitol, baking powder, baking soda, vanilla essence, olive oil, and water. Batters were mixed, portioned into silicone moulds, and baked in a convention oven at 180 °C (15 min for LM; 25 min for OM). Both formulations were designed to offer gluten-free alternatives with improved nutritional value and a lower environmental footprint, aligning with current trends in sustainable and functional food development.

2.2. Total starch determination

The total starch content of LM and OM samples was determined using the Total Starch Assay Kit (AA/AMG) from Megazyme (K-TSTA, Ireland). The protocol was followed according to the manufacturer's instructions, with a final volume adjustment to allow absorbance measurement in a 96-well microplate using a Multidetection microplate reader (Synergy H1, BioTek, Vermont, USA). Analyses were performed in triplicate.

2.3. In vitro digestion

The standardised static digestion model, INFOGEST 2.0 protocol (Brodkorb et al., 2019) was employed to simulate the gastrointestinal digestion. This procedure encompasses the oral, gastric and intestinal phases, using corresponding fluids replicating the *in vivo* conditions. In the oral phase, samples were diluted 1:1 (*w*/w) with simulated salivary fluid containing salivary α-amylase from human saliva (A1031-5KU, Sigma-Aldrich, Missouri, USA) and incubated at 37 °C for 2 min. For the gastric phase, the oral bolus were diluted 1:1 (*v*/v) with simulated gastric fluid and gastric enzymes, including pepsin and lipase from gastric rabbit extract (RGE 15, Lipolytech, Marseille, France). The pH of the mixtures was adjusted to 3.0 and then the samples were incubated for 2 h at 37 °C with agitation in an orbital shaker (Stuart SI500, Keison Products, United Kingdom) at 120 rpm. In the intestinal phase, the resulting gastric chymes were further diluted 1:1 (v/v) with simulated intestinal fluid, bile salts (B8631, Sigma-Aldrich, Missouri, USA) and pancreatin from porcine pancreas (P7545 Sigma-Aldrich, Missouri, USA), providing 100 U/mL of pancreatic α-amylase activity in the final mixture. The mixtures were adjusted to a pH of 7.0 and incubated for 2 h at 37 °C in the orbital shaker. Three replicates were conducted for each sample (3 g fresh weight/sample). Blank was performed using distilled water.

2.4. Evaluation of starch hydrolysis

For the evaluation of starch hydrolysis, aliquots were collected from the *in vitro* digestion process at the end of the oral phase and every 30 min thereafter. The collected samples were heat-shocked for 5 min at 100 °C to stop the enzymes' activities. The samples were then centrifuged at 1800 *g* for 10 min and the supernatant was used for glucose quantification with GOPOD reagent. Samples were incubated at 50 °C for 20 min, and absorbance was measured at 510 nm using a Multidetection microplate reader (Synergy H1, Biotek, Vermont, USA). The D-glucose percentages were converted to starch percentages.

2.5. Determination of estimated glycaemic index, glycaemic load and starch fractions

Estimated GI (eGI) and starch fractions were determined following (Fernandes et al., 2020). Briefly, starch percentages determined during *in vitro* digestion (0–240 min) were used to calculate the area under the curve (AUC), hydrolysis index (HI) and GI. The reference food used was potato starch (Panreac, Spain). Starch fractions were derived from the hydrolysis profile, using the approach described in the same protocol. The estimated GL (eGL) was calculated according to (Eq.1):

eGL = eGI/100 x (Digestible carbohydrates per serving/2) (1).

The digestible carbohydrate content of OM and LM was obtained from the previous study (Geraldo et al., 2024) and adjusted to a serving size of 35 g.

2.6. Cell line

In this study, one cell line was used: Human Caucasian colon carcinoma epithelial cells, Caco-2 (ECACC 86010202) obtained from the European Collection of Authenticated Cell Cultures. Cells were cultured in DMEM (Gibco, Thermo Scientific, USA) supplemented with 10 % (*v/v*) foetal bovine serum (FBS, Biowest, France), 1 % (v/v) of Penicillin-Streptomycin-Fungizone (Lonza, Belgium) and 1 % (v/v) non-essential amino acids (NEAA, Gibco, Thermo Scientific, USA). They were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2 and 95 % air.

2.7. Cytotoxicity assay

Cytotoxicity assays were adapted from ISO 10993-5:2009 guidelines. Briefly, Caco-2 cells were seeded at a density of 1.0×10^4 cells per well in 96-well tissue culture plates (Nunclon Delta, Thermo Scientific, Waltham, MA, USA) and incubated for 24 h. Subsequently, the culture medium was replaced with 90 μL of either LM or OM digested samples at different dilutions in medium, plain medium (negative control), or medium containing 30 % v/v DMSO (positive control). Cells were incubated for an additional 24 h. After treatment, 10 μL of Presto Blue (Thermofisher, Waltham, MA, USA) was added to each well. The plates were incubated for 1 h at 37 °C, and fluorescence was measured (excitation: 560 nm; emission: 590 nm) using a microplate reader (Synergy H1, Biotek Instruments, USA). All assays were performed in quadruplicate.

2.8. Caco-2 monolayer immunomodulation

For the immunomodulation assay, Caco-2 cells were seeded at a density of 1.3×10^6 cells per flask in T25 flasks and incubated for 24 h. The culture medium was then replaced with medium supplemented with OM or LM-digested samples (diluted in culture medium at a ratio of 1:75), or culture medium (basal control). To evaluate anti-inflammatory activity, a pro-inflammatory stimulus of 10 ng/mL of IL-1β (Invitrogen, Waltham, MA, USA) was added to all conditions. After 24 h exposure, both the supernatants and cell pellets were collected and stored at −80 °C until analyses. All assays were conducted using five independent replicates.

Inflammatory interleukin (IL) levels were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits following the manufacturers' instructions: the Legend Max Human ELISA Kit IL-6 and IL-8 (BioLegend, San Diego, California, USA). Results were expressed as fold change relative to the basal control group.

2.9. Real-time PCR

RNA was extracted from Caco-2 cells exposed to the experimental conditions using the TripleXtractor directRNA kit (GRISP, Porto, Portugal), following the manufacturer's instructions. Total RNA concentration and purity were evaluated by measuring absorbance (A) at 260 nm and calculating the A260/A280 and A260/A230 ratios, respectively, using a Nanodrop spectrophotometer (Thermofisher Scientific, Wilmington, DE). Complementary DNA (cDNA) was synthesised from 1 μg of total RNA using the NZY First-Strand cDNA Synthesis kit (NZYTech, Lisboa, Portugal). Quantitative real-time PCR (RT-qPCR) was performed with SYBR® Green PCR Master Mix (Applied Biosystems, Life Technologies) on the CFX Opus 384 Real-Time PCR System (Bio-Rad). Primer sequences are listed in Table 1. All reactions followed the conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All reactions were run in duplicate and gene expression levels were normalised to housekeeping gene *hypoxanthine phosphoribosyltransferase* (*HPRT)* (Piana et al., 2008).

2.10. Statistical analyses

All data were analysed using GraphPad Prism (v.9.0.0, for Windows, GraphPad Software, San Diego, California, USA). Student's *t*-test was applied to compare the starch fractions, the eGI and eGL between OM and LM. For immunomodulatory and gene expression assays, differences among groups were assessed using one-way ANOVA. Data are presented as mean ± standard deviation of the means (SEM), and statistical significance was set at $p < 0.05$.

3. Results

3.1. In vitro digestion and starch hydrolysis

The starch hydrolysis profile of OM and LM was influenced by their total starch content, with OM presenting a significantly higher amount (35.9 %) than LM (32.7 %), as observed in Table 2. Fig. 1 represents the starch hydrolysis for OM and LM during *in vitro* digestions. During the oral phase (0–2 min), α-amylase activity resulted in the hydrolysis of less than 3.5 % of starch in OM and approximately 1 % in LM (Fig. 1). In the gastric phase (2–120 min), both samples exhibited minimal starch breakdown, reaching around 4 % in OM and 1.5 % in LM. A substantial increase in hydrolysis occurred during the intestinal phase (120–240 min), with OM reaching 40 % and LM 35 % (Fig. 1).

Table 1
Primer sequence used for RT-qPCR.*

Primer	Sequence (5′ → 3′)
IL-6	F: AAGCCAGAGCTGTGCAGATG R: CTGGCATTTGTGGTTGGGTC
IL-8	F: GTTTTTGAAGAGGGCTGAGAATTC R: ATGAAGTGTTGAAGTAGATTTGCTTG
IL-1β	F: TGGCAATGAGGATGACTTGTTC R: CTGTAGTGGTGGTCGGAGATT
TGFβ	F: ATTCCTGGCGTTACCTTGG R: AGCCCTGTATTCCGTCTCCT
GLUT2	F: CAGGACTATATTGTGGGCTAA R: CTGATGAAAAGTGCCAAGT
ACAT2	F: CGCGGACCATCATAGGTTCCTT R: TTAGGCCTGACCCACAGATCA
PPARγ	F: AGGCGAGGGCGATCTTGACAG R: GATGCGGATGGCCACCTCTTT
SIRT1	F: GCGATTGGGTACCGAGATAAC R: GGCCTTGGAGTCCAGTCACTA
Occludin	F: CGACACACCACACCTACACT R: TTCAGGATGAGCAATGCCCT
CDH1	F: AAGGAGGCGGAGAAGAGGAC R: CGTCGTTACGAGTCACTTCAGG
HO-1	F: CCAGCAACAAGGTGCAAGATT R: CACATGGCATAAAGCCCTACA

* Abbreviations: ACAT2, Acetyl-Coenzyme A acetyltransferase 2; CDH1, Cadherin-1; F, Forward; GLUT2, Glucose transporter 2; HO-1, Heme oxygenase 1; IL, Interleukin; PPARγ, Peroxisome proliferator-activated receptor-γ; R, Reverse; SIRT1, Sirtuin 1; TGFβ, Transforming Growth Factor-β.

Table 2
Total starch, rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), estimated glycaemic index (eGI), and estimated glycaemic load (eGL) of LM and OM. Each value represents the mean ± SEM. Different letters indicate significant differences ($p < 0.05$).

	LM	OM
Total starch (%)	32.7 ± 0.9^{b}	35.9 ± 0.1^{a}
RDS (%)	0.96 ± 0.07^{b}	3.50 ± 0.16^{a}
SDS (%)	32.1 ± 0.4^{a}	27.0 ± 0.6^{b}
RS (%)	66.1 ± 2.5	61.0 ± 2.0
eGI	64.2 ± 0.1^{b}	70.8 ± 0.1^{a}
eGL	3.99 ± 0.01^{b}	5.75 ± 0.00^{a}

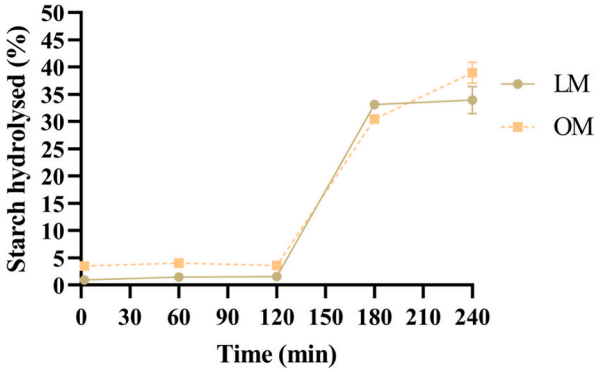

Fig. 1. Starch hydrolysed during *in vitro* digestion of oatmeal (OM) and lentil (LM) muffins. The digestion process includes the oral phase, (0–2 min), gastric phase (2–120 min) and intestinal phase (120–240). LM are represented by circles and OM by squares. No significant differences were observed. Data are presented as mean ± SEM ($n = 3$).

3.2. Total starch and its fractions, estimated glycaemic index and load

Table 2 summarises the total starch content, starch fractions (RDS, SDS, RS) and eGI/eGL values of OM and LM. OM also exhibited a significantly higher proportion of RDS (3.50 %) than LM (0.96 %), whereas LM contained a significantly superior proportion of SDS (32.1 %) compared to OM (27.0 %). RS content was similar between the two samples. The eGI and eGL values were significantly lower in LM.

3.3. Cytotoxicity and immunomodulation

The cytotoxicity of *in vitro*-digested OM and LM samples was assessed in Caco-2 cells, as seen in Fig. 2a. These results guided the selection of an appropriate dilution for subsequent analysis of biological properties. Neither OM nor LM exhibited cytotoxicity effects at dilutions equal to or below 1:75 (Fig. 2a), as metabolic inhibition remained below the 30 % cytotoxicity threshold established by the ISO 10993-5 standard (ISO). This dilution was selected for the next assays.

The immunomodulatory effects of OM and LM were evaluated under normal physiological (basal) conditions (left side of graphs in Fig. 2b) and an inflammatory state induced by IL-1β stimulation (right side of graphs in Fig. 2b). Under basal conditions, neither OM nor LM significantly altered IL-6 or IL-8 secretion. In IL-1β-stimulated cells, both OM and LM reduced IL-6 and IL-8 secretion. The reduction in IL-6 was only significant for LM, which lowered approximately 21 %, while OM and LM decreased IL-8 levels about 22 % and 25 %, respectively, compared to the IL-1β-stimulated control.

3.4. Quantification of gene expression by RT-qPCR

RT-qPCR results for the quantification of gene expression related to inflammation (*IL-6, IL-8, IL-1β, TGFβ*), metabolic regulation [*Glucose transporter 2* (*GLUT2*), *Acetyl-Coenzyme A acetyltransferase 2* (*ACAT2*), *Peroxisome proliferator-activated receptor-γ* (*PPARγ*), *Sirtuin 1* (*SIRT1*)], intestinal barrier integrity [*Occludin*, *Cadherin-1* (*CDH1*)], and oxidative stress [*Heme oxygenase 1* (*HO-1*)] are shown in Fig. 3. Under basal conditions, OM significantly upregulates *IL-8* (3.7-fold) and *IL-1β* (1.5-fold) relative to the control, with a non-significant increase in *IL-6* (Fig. 3a-c), indicating a mild pro-inflammatory effect. LM did not significantly alter inflammatory gene expression (Fig. 3a-c). Under IL-1β stimulation, both OM and LM significantly downregulated *IL-8* and *IL-6,* although no significant differences were observed for *IL-1β* (Fig. 3a-c), suggesting an anti-inflammatory response. *TGFβ* expression was upregulated by both muffins under basal conditions (3.4- and 2.7-fold for OM and LM, respectively), but was not significantly affected during inflammation (Fig. 3d). Regarding barrier integrity, (Fig. 3i-j) *Occludin* and *CDH1* were upregulated by both muffins under basal conditions. *Occludin* showed a 2.3-fold increase for both samples relative to basal control, while *CDH1* increased 1.5-fold with OM and 1.8-fold with LM, though the *CDH1* increase in OM was not significant. Only LM reversed the inflammatory-induced decrease in *CDH1* under IL-1β stimulation (2.1-fold increase relative to IL-1β-stimulated control) (Fig. 3i-j), suggesting protection of barrier integrity during inflammatory stress. For metabolic markers, OM increased *GLUT2* not significantly under basal conditions (Fig. 3e), while LM downregulated *ACAT2* (Fig. 3f). Under inflammatory stimulus, both samples significantly reduced *GLUT2* and *ACAT2* expression (Fig. 3e-f), consistent with a potential reduction in glucose uptake and cholesterol esterification. *SIRT1* and *PPARγ* were upregulated by both OM and LM under basal conditions (*SIRT1*: 1.9- and 2-fold increase; *PPARγ*: 1.4- and 1.5-fold increase for OM and LM, respectively, relative to basal control), though this increase was not significant for *PPARγ* (Fig. 3g-h). Under inflammatory stimulus, LM significantly increased the expression of both genes (*SIRT1*: 1.9-fold; *PPARγ*: 2.4-fold increase relative to IL-1β-stimulated control), whereas OM significantly upregulated only *PPARγ* (1.7-fold increase relative to IL-1β-stimulated control) (Fig. 3g-h), showing modulation of key metabolic regulators. *HO1* expression remained unchanged under basal conditions but was significantly upregulated by LM under IL-1β stimulation (2.2-fold increase relative to IL-1β-stimulated control) (Fig. 3k), indicating an enhanced antioxidant response.

4. Discussion

4.1. Starch digestion and glycaemic response

The present study evaluated the impact of *in vitro* digestion of an environmentally positive previously developed OM and LM (Geraldo et al., 2024) on glycaemic response and physiological responses of intestinal epithelial cells. These muffins were developed not only with nutritional and functional goals in mind but also as environmentally positive alternatives, given the sustainability profile of ingredients. OM was formulated with 100 % oat flour, while LM combined 50 % oat flour with 50 % lentil flour. Both formulations also included standard ingredients such as baking soda and powder, vanilla essence, olive oil, and xylitol, the latter used as a sugar substitute due to its minimal impact on blood glucose levels (Geraldo et al., 2024). However, such environmental benefits are only relevant if the final product is safe and promotes health. Therefore, the present study aimed to confirm the health-related properties of these muffins. While studies have explored *in vitro* digestion properties and glycaemic potential of various baked products (Faubel et al., 2022; Johnston, Ying Leong, Teape, Liesaputra, & Oey, 2023; Peng et al., 2023), few have simultaneously assessed digestion kinetics alongside effects in an *in vitro* intestinal model. This integrated approach provides added value and novelty, as it allows for a broader functional characterisation beyond carbohydrate digestion alone. OM are often consumed for their fibre content, gluten-free nature, and associated health benefits, while LM incorporates legume-based ingredients that may enhance metabolic and anti-inflammatory responses

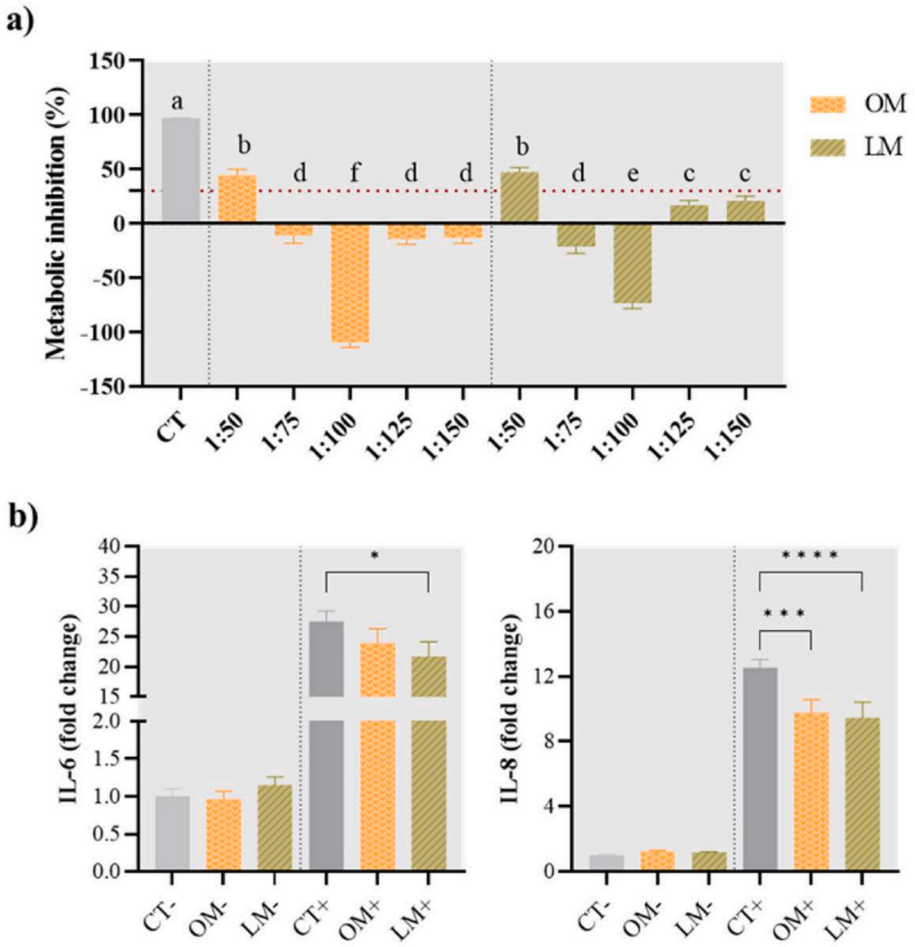

Fig. 2. Cytotoxicity (a) and modulation of inflammatory response (b) in Caco-2 cells exposed to *in vitro* digested oatmeal (OM) and lentil (LM) muffins samples. a) Post-digestion samples at different dilutions. CT is the positive control (30 % DMSO). The dotted line represents the 30 % cytotoxicity limit (ISO 10993-5:2009). Different letters indicate statistically significant differences (one-way ANOVA, $p < 0.05$). b) Post-digestion samples at 1:75 dilution. The left side of the graphs corresponds to the non-stimulated cells' response (−), and the right side to the cells subjected to the inflammatory effect following IL-1β treatment (+). Statistical analysis was performed using one-way ANOVA. P* < 0.05 *** $P < 0.001$; ****$P < 0.0001$.

through bioactive compounds such as phenolic acids, flavonoids, and bioactive peptides. While these compounds are abundant in lentils, they are also present in other legumes, suggesting that the observed effects may reflect broader legume-associated benefits. The findings provide valuable insights into the nutritional and functional characteristics of these products.

The rate and extent of starch digestion are critical factors influencing postprandial glycaemic responses (Freitas et al., 2025). In this study, OM and LM exhibited minimal starch hydrolysis during the oral and gastric phases, followed by a marked increase during the intestinal phase, where pancreatic amylase activity is predominant. The oral phase of digestion is brief (only 2 min), limiting enzymatic activity, while the acidic environment of the gastric phase further suppresses enzymatic function. Total starch content was higher in OM, which explains the greater starch hydrolysis observed in OM, as starch is the primary substrate for enzymatic hydrolysis (Freitas et al., 2025). However, factors beyond total starch content, such as starch structure, matrix effects from baking, higher fibre and protein in LM, and the presence of non-nutrient or polyphenol compounds, also influence digestibility (Geraldo et al., 2024; Soong, Tan, Leong, & Henry, 2014; Yang, Zhang, Wu, & Ouyang, 2023). These processing- and matrix-related factors likely explain both the modest differences observed between OM and LM and the overall limited starch hydrolysis compared to values reported for raw flours. Besides, oats typically contain more readily accessible starch granules and less fibre-bound starch than lentils, making them more susceptible to enzymatic hydrolysis (Ren, Hu, & Ma, 2023; Zhu, 2017). In line, we verified that starch fractions varied between muffins: LM, comparatively with OM, exhibited higher SDS and lower RDS content, reflecting the lentil starch's compact granule structure and higher amylose content (Varghese et al., 2023; Wang, Ou, Al-Maqtari, He, & Othman, 2024; Wani et al., 2016). SDS contributes to a more gradual glucose release and lower glycaemic responses, which were confirmed by the significantly lower eGI and eGL values in LM, compared to OM. This aligns with prior research showing that foods rich in SDS and RS elicit lower glycaemic responses (Wang, Zhou, Xiang, & Miao, 2023). Since RS content was similar in both muffins, the differences in eGI and eGL are primarily attributed to variations in RDS and SDS. According to eGI values, OM is classified as medium/high range, while LM is classified as medium. Regarding the eGL, both muffins are considered low. These results suggest that while both products may support blood glucose management, LM presents a more favourable glycaemic profile due to its lower eGI. Although the INFOGEST *in vitro* model provides a standardised and reproducible method to assess starch digestion and estimate glycaemic responses, it does not fully replicate the complexity of human digestion, including dynamic changes in pH, enzyme concentration and motility, hormonal signalling, nutrient absorption kinetics, and microbiota interactions (Brodkorb et al., 2019). As such, the eGI and eGL values presented should be interpreted with caution. Further validation *in vivo* is needed to confirm these findings and better understand the physiological impact of lentil-oat formulations.

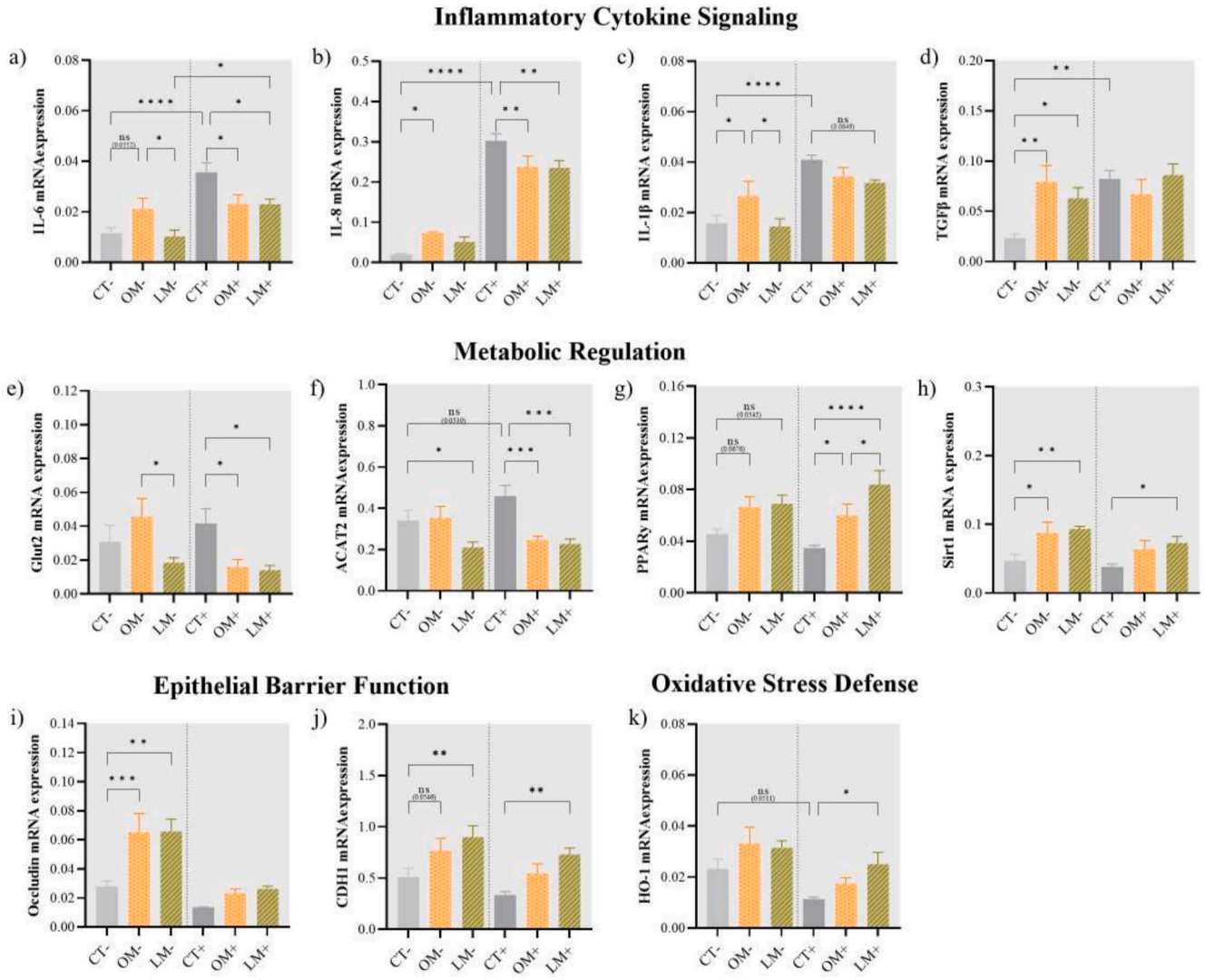

Fig. 3. Relative expression levels of genes related to inflammation, metabolic regulation, intestinal barrier integrity and oxidative stress in Caco-2 cells exposed to *in vitro* digested oatmeal (OM) and lentil (LM) muffins samples assessed by RT-qPCR. *IL-6* (a), *IL-8* (b), *IL-1β* (c), *TGFβ* (d), *GLUT2* (e), *ACAT2* (f), *PPARγ* (g), *SIRT1* (h), *Occludin* (i), *CDH1* (j) and *HO-1* (k). The left side of each graph corresponds to the non-stimulated cell's response (−), and the right side to the cells subjected to the inflammatory effect following IL-1β (+). Statistical analysis was performed using one-way ANOVA. P* < 0.05, P** < 0.01, *** P < 0.001; ****P < 0.0001.

4.2. Cytotoxicity and immune response

Cytotoxicity testing is essential to confirm the safety of novel food products. While Caco-2 cells are widely used to assess the direct effects of digested food samples on intestinal epithelial function (Machado et al., 2023; Martínez-Sánchez, Calvo, Fontecha, & Pérez-Gálvez, 2024), it is important to note that, physiologically, the gut barrier primarily interacts with nutrients and metabolites after absorption or fermentation. Thus, this *in vitro* approach provides preliminary insights but does not fully replicate *in vivo* conditions. Both OM and LM showed no cytotoxic effects on Caco-2 cells at dilutions of 1:75 or lower, confirming their suitability for further biological evaluations. Immunomodulatory effects were examined by measuring the secretion of pro-inflammatory cytokines IL-6 and IL-8 under basal and IL-1β-stimulated conditions. This stimulation mimics acute epithelial inflammation, providing a useful proxy for studying intestinal barrier responses relevant to food science. This is particularly important given the role of gut inflammation in the pathogenesis of chronic diseases. In the context of food science, such inflammatory conditions refer to intestinal environments characterised by elevated cytokine levels and impaired barrier function. These are linked to diseases such as inflammatory bowel disease and systemic metabolic disorders, including obesity, type 2 diabetes, and cardiovascular disease, all of which are influenced by dietary patterns and gut health. Under basal conditions, neither muffin significantly affected cytokine secretion. However, under stimulation, both OM and LM reduced IL-6 and IL-8 secretion, with LM showing a more pronounced effect. This indicates anti-inflammatory activity, particularly in LM, which is consistent with the reported effects of lentil-derived bioactives such as polyphenols, especially flavonoids, and saponins (Al-Khayri et al., 2022; Geraldo, Santos, Pinto, & Vasconcelos, 2022).

These anti-inflammatory properties were supported by analysis of the expression of genes involved in Caco-2 cells' response to inflammation. In fact, when under IL-1β stimulation, both muffins downregulated *IL-8* and *IL-6* gene expression. On the other hand, OM upregulated pro-inflammatory genes *IL-8* and *IL-1β* under basal conditions, while LM had no significant effect. Although oats are commonly associated with anti-inflammatory properties, mainly through β-glucans and avenanthramides (Paudel et al., 2021), this observation may reflect a transient epithelial immune response, potentially triggered by OM's higher carbohydrate content (Geraldo et al., 2024). This could result in oxidative stress and NF-κB activation, leading to elevated expression of inflammatory markers (Morresi et al., 2019). As no differences were

found between LM and non-treated cells under basal conditions, LM appeared to mitigate this activation, which may reflect synergistic effects of its phenolic profile and fibre composition. Interestingly, *TGFβ* was upregulated under basal conditions by both muffins, supporting its role in maintaining immune homeostasis (Biancheri et al., 2014). However, considering that OM simultaneously increased the expression of *IL-8* and *IL-1β*, a dual response seems evident: mild pro-inflammatory activation counterbalanced by immunoregulatory signalling. In contrast, LM increased *TGFβ* without elevating inflammatory markers, suggesting a more favourable immunomodulatory profile.

Besides promoting regulatory T cell differentiation, TGFβ increments epithelial barrier maintenance and function (Massagué & Sheppard, 2023), which was supported by the upregulation of *Occludin* that codifies a tight junction protein occludin and *CDH1* that codifies cadherin, a cell adhesion protein. Alike *TGFβ,* both genes presented an increased expression under basal conditions after treatment with both OM and LM. Lentils are known to promote tight junction integrity *via* polyphenols (Guo et al., 2023), although their release during digestion was not directly assessed in this study. In addition, starch fractions such as RS and SDS, which are more abundant in LM, may also contribute to its protective effects by attenuating epithelial stress and supporting barrier function (Chen et al., 2024). Under inflammatory stimulus, only LM was able to counteract the IL-1β-induced non-significant decrease in *CDH1*, highlighting its superior protective effect relative to OM. Although not statistically significant in our findings, oat extract was demonstrated to upregulate tight junction protein (TJP)1, TJP2, occludin, claudin-1 and claudin-3 mRNA levels in Caco-2 cells under inflammatory stimulus (Lee et al., 2024). Maintaining barrier integrity is vital for preventing intestinal inflammation and related metabolic disorders such as diabetes and CVD (Zhang et al., 2023).

4.3. Metabolic regulation

In terms of glucose transport and metabolism, OM, but not LM, upregulated *GLUT2* under basal conditions, which correlates with increased glucose absorption and potentially contributes to postprandial hyperglycaemia (Morresi et al., 2019; Röder et al., 2014; Sun, Chen, Xue, Li, & Fu, 2023). This effect is consistent with previous findings showing that oat peptides stimulate *GLUT2* protein expression (Wang et al., 2018). Conversely, both muffins downregulated *GLUT2* under inflammatory stimulus, which may help attenuate glucose absorption and support glycaemic control (Gromova, Fetissov, & Gruzdkov, 2021). LM also reduced *ACAT2* expression under both conditions, suggesting decreased cholesterol esterification activity (Wang et al., 2017) and a likely protective effect against lipid dysregulation. *ACAT2* plays a central role in cholesterol esterification and atherogenic lipoprotein formation, and its inhibition has been proposed as a strategy to reduce cardiovascular risk (Alger et al., 2010). The downregulation of *ACAT2* by LM is consistent with legumes' hypocholesterolemic effects, which have been attributed to interactions of fibre, RS and polyphenols with lipid metabolism (Arnoldi, Chiara, Carmen, & Boschin, 2015; Islam, Ahmed, Ahsan, & Lee, 2021).

In contrast, OM only decreased *ACAT2* expression under inflammatory stimulus, suggesting a less consistent modulatory potential. Interestingly, both muffins upregulated *PPARγ* under inflammatory stimulus. This nuclear receptor regulates genes involved in lipid metabolism, glucose homeostasis, and inflammation (Bougarne et al., 2018) and is known to repress *ACAT2* expression, which aligns with our findings (Colin et al., 2013). PPARγ activation is linked to improved insulin sensitivity, supporting its relevance in metabolic disease management (Choi, Park, & Choi, 2014). As expected, LM upregulated *SIRT1* under inflammatory stimulus. SIRT1 is an NAD + -dependent deacetylase that, among other functions, modulates transcription factors, including PPARγ, whose activation promotes anti-inflammatory pathways and metabolic homeostasis (Peng et al., 2022). The simultaneous upregulation of *SIRT1* and *PPARγ* by LM indicates a coordinated metabolic response impacting lipid and glucose regulation. Additionally, LM upregulated *HO-1*, a downstream target of Nrf2 that provides antioxidant, anti-inflammatory, and cytoprotective effects (Hahn, Shin, & Bae, 2020). HO-1 induction, often triggered by polyphenols, represents a critical cellular defence mechanism (Iftikhar, Iftikhar, Zhang, Gong, & Wang, 2020), and its upregulation in LM-treated cells under inflammatory stimulus indicates an additional protective mechanism against oxidative and inflammatory stress.

The findings of this study provide valuable insights into the functional and nutritional characteristics of two muffin formulations: one made entirely with oat (OM) and another partially substituted with lentil flour (LM). While both muffins demonstrate potential for supporting blood glucose management, LM exhibited a more favourable glycaemic profile, characterised by higher SDS content and lower eGI. Additionally, LM exhibited enhanced immunomodulatory and cytoprotective effects, particularly when under inflammatory stimulus, likely due to lentil-derived bioactive compounds. Gene expression analysis further supports the beneficial impact of LM on intestinal barrier function, glucose transport, lipid regulation, and oxidative stress. These findings suggest that LM could be a promising dietary option for improving metabolic health and managing inflammation, offering potential benefits in the prevention and management of conditions such as type 2 diabetes, CVD, and inflammatory bowel disease, all of which are influenced by chronic low-grade gut inflammation and dietary patterns. Further studies are needed to confirm these effects *in vivo* and explore the long-term health implications of regular LM consumption.

CRediT authorship contribution statement

Rafaela Geraldo: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Manuela Machado:** Writing – review & editing, Methodology. **Sara Silva:** Writing – review & editing, Methodology. **Simão Pinho:** Writing – review & editing, Methodology. **Alexandra Gouveia:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Delminda Neves:** Writing – review & editing, Supervision, Resources, Conceptualization. **Elisabete Pinto:** Writing – review & editing, Supervision. **Manuela Pintado:** Writing – review & editing. **Marta W. Vasconcelos:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Ethics statement

The present study did not involve human subjects or animal experiments, and therefore did not require ethical approval from an institutional ethics committee. All experimental procedures were conducted following relevant guidelines and regulations.

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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Data availability

The data that support the findings of this study are available from the corresponding author, [MWV], upon reasonable request.

References

Alger, H. M., Brown, J. M., Sawyer, J. K., Kelley, K. L., Shah, R., Wilson, M. D., & Rudel, L. L. (2010). Inhibition of acyl-coenzyme a:Cholesterol acyltransferase 2 (ACAT2) prevents dietary cholesterol-associated steatosis by enhancing hepatic triglyceride mobilization. *The Journal of Biological Chemistry, 285*(19), 14267–14274. https://doi.org/10.1074/jbc.M110.118422

Al-Khayri, J. M., Sahana, G. R., Nagella, P., Joseph, B. V., Alessa, F. M., & Al-Mssallem, M. Q. (2022). Flavonoids as potential anti-inflammatory molecules: A review. *Molecules, 27*(9). https://doi.org/10.3390/molecules27092901

Arnoldi, A., Chiara, Z., Carmen, L., & Boschin, G. (2015). The role of grain legumes in the prevention of hypercholesterolemia and hypertension. *Critical Reviews in Plant Sciences, 34*(1–3), 144–168. https://doi.org/10.1080/07352689.2014.897908

Biancheri, P., Giuffrida, P., Docena, G. H., MacDonald, T. T., Corazza, G. R., & Di Sabatino, A. (2014). The role of transforming growth factor (TGF)-β in modulating the immune response and fibrogenesis in the gut. *Cytokine & Growth Factor Reviews, 25*(1), 45–55. https://doi.org/10.1016/j.cytogfr.2013.11.001

Bougarne, N., Weyers, B., Desmet, S. J., Deckers, J., Ray, D. W., Staels, B., & De Bosscher, K. (2018). Molecular actions of PPARα in lipid metabolism and inflammation. *Endocrine Reviews, 39*(5), 760–802. https://doi.org/10.1210/er.2018-00064

Breyton, A.-E., Goux, A., Lambert-Porcheron, S., Meynier, A., Sothier, M., VanDenBerghe, L., & Nazare, J.-A. (2021). Starch digestibility modulation significantly improves glycemic variability in type 2 diabetic subjects: A pilot study. *Nutrition, Metabolism and Cardiovascular Diseases, 31*(1), 237–246. https://doi.org/10.1016/j.numecd.2020.08.010

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Chamberlin, M. L., Wilson, S. M. G., Gaston, M. E., Kuo, W.-Y., & Miles, M. P. (2024). Twelve weeks of daily lentil consumption improves fasting cholesterol and postprandial glucose and inflammatory responses—A randomized clinical trial. *Nutrients, 16*(3), 419. https://doi.org/10.3390/nu16030419

Chen, Z., Liang, N., Zhang, H., Li, H., Guo, J., Zhang, Y., & Shi, N. (2024). Resistant starch and the gut microbiome: Exploring beneficial interactions and dietary impacts. *Food Chem X, 21*, Article 101118. https://doi.org/10.1016/j.fochx.2024.101118

Choi, S. S., Park, J., & Choi, J. H. (2014). Revisiting PPARγ as a target for the treatment of metabolic disorders. *BMB Reports, 47*(11), 599–608. https://doi.org/10.5483/bmbrep.2014.47.11.174

Clarke, S. T., Sarfaraz, S., Qi, X., Ramdath, D. G., Fougere, G. C., & Ramdath, D. D. (2022). A review of the relationship between lentil serving and acute postprandial blood glucose response: Effects of dietary fibre. *Protein and Carbohydrates. Nutrients, 14*(4), 849. https://doi.org/10.3390/nu14040849

Colin, S., Briand, O., Touche, V., Wouters, K., Baron, M., Pattou, F., & Lestavel, S. (2013). Activation of intestinal peroxisome proliferator-activated receptor-α increases high-density lipoprotein production. *European Heart Journal, 34*(32), 2566–2574. https://doi.org/10.1093/eurheartj/ehs227

Dhull, S. B., Kinabo, J., & Uebersax, M. A. (2023). Nutrient profile and effect of processing methods on the composition and functional properties of lentils (Medik): A review. *Legume Science, 5*(1), Article e156. https://doi.org/10.1002/leg3.156

Duijsens, D., Alfie Castillo, A. I., Verkempinck, S. H. E., Pälchen, K., Hendrickx, M. E., & Grauwet, T. (2023). In vitro macronutrient digestibility and mineral bioaccessibility of lentil-based pasta: The influence of cellular intactness. *Food Chemistry, 423*, Article 136303. https://doi.org/10.1016/j.foodchem.2023.136303

Duijsens, D., Verkempinck, S. H. E., De Coster, A., Pälchen, K., Hendrickx, M., & Grauwet, T. (2023). How cooking time affects in vitro starch and protein digestibility of whole cooked lentil seeds versus isolated Cotyledon cells. *Foods, 12*(3). https://doi.org/10.3390/foods12030525

Faubel, N., Makran, M., Cilla, A., Alegría, A., Barberá, R., & Garcia-Llatas, G. (2022). Bioaccessibility of plant sterols in wholemeal Rye bread using the INFOGEST protocol: Influence of Oral phase and enzymes of lipid metabolism. *Journal of Agricultural and Food Chemistry, 70*(41), 13223–13232. https://doi.org/10.1021/acs.jafc.2c04024

Fernandes, J.-M., Madalena, D. A., Pinheiro, A. C., & Vicente, A. A. (2020). Rice in vitro digestion: Application of INFOGEST harmonized protocol for glycemic index determination and starch morphological study. *Journal of Food Science and Technology, 57*(4), 1393–1404. https://doi.org/10.1007/s13197-019-04174-x

Ferreira, H., Vasconcelos, M., Gil, A. M., & Pinto, E. (2021). Benefits of pulse consumption on metabolism and health: A systematic review of randomized controlled trials. *Critical Reviews in Food Science and Nutrition, 61*(1), 85–96. https://doi.org/10.1080/10408398.2020.1716680

Freitas, D., Lazaridou, A., Duijsens, D., Kotsiou, K., Corbin, K. R., Alongi, M., & Edwards, C. H. (2025). Starch digestion: A comprehensive update on the underlying modulation mechanisms and its in vitro assessment methodologies. *Trends in Food Science & Technology, 159*, Article 104969. https://doi.org/10.1016/j.tifs.2025.104969

Ganesan, K., & Xu, B. (2017). Polyphenol-rich lentils and their health promoting effects. *International Journal of Molecular Sciences, 18*(11), 2390. https://doi.org/10.3390/ijms18112390

Geraldo, R., Santos, C. S., Pinto, E., & Vasconcelos, M. W. (2022). Widening the perspectives for legume consumption: The case of bioactive non-nutrients. *Frontiers in Plant Science, 13*, Article 772054. https://doi.org/10.3389/fpls.2022.772054

Geraldo, R., Santos, C. S., Styles, D., Sousa, S., Pinto, E., Neves, D., & Vasconcelos, M. W. (2024). Nutritional value and environmental footprint of muffins made with green-lentil flour. *Journal of Cleaner Production, 477*, Article 143826. https://doi.org/10.1016/j.jclepro.2024.143826

Gómez, M. (2022). Gluten-free bakery products: Ingredients and processes. *Advances in Food and Nutrition Research, 99*, 189–238.

Gromova, L. V., Fetissov, S. O., & Gruzdkov, A. A. (2021). Mechanisms of glucose absorption in the small intestine in health and metabolic diseases and their role in appetite regulation. *Nutrients, 13*(7), 2474. https://doi.org/10.3390/nu13072474

Guo, F., Peng, L., Xiong, H., Tsao, R., Zhang, H., Jiang, L., & Sun, Y. (2023). Bioaccessibility and transport of lentil hull polyphenols in vitro, and their bioavailability and metabolism in rats. *Food Research International, 167*, Article 112634. https://doi.org/10.1016/j.foodres.2023.112634

Hahn, D., Shin, S. H., & Bae, J.-S. (2020). Natural antioxidant and anti-inflammatory compounds in foodstuff or medicinal herbs inducing Heme Oxygenase-1 expression. *Antioxidants, 9*(12), 1191. https://doi.org/10.3390/antiox9121191

Iftikhar, M., Iftikhar, A., Zhang, H., Gong, L., & Wang, J. (2020). Transport, metabolism and remedial potential of functional food extracts (FFEs) in Caco-2 cells monolayer: A review. *Food Research International, 136*, Article 109240. https://doi.org/10.1016/j.foodres.2020.109240

Islam, S. U., Ahmed, M. B., Ahsan, H., & Lee, Y. S. (2021). Recent molecular mechanisms and beneficial effects of phytochemicals and plant-based whole foods in reducing LDL-C and preventing cardiovascular disease. *Antioxidants, 10*(5). https://doi.org/10.3390/antiox10050784

Johnston, C., Ying Leong, S., Teape, C., Liesaputra, V., & Oey, I. (2023). In vitro digestion properties and use of automatic image analysis to assess the quality of wheat bread enriched with whole faba bean (Vicia faba L.) flour and its protein-rich fraction. *Food Research International, 174*, Article 113630. https://doi.org/10.1016/j.foodres.2023.113630

Kaale, L. D., Siddiq, M., & Hooper, S. (2023). Lentil (Medik) as nutrient-rich and versatile food legume: A review. *Legume Science, 5*(2), Article e169. https://doi.org/10.1002/leg3.169

Ketnawa, S., Reginio, F. C., Jr., Thuengtung, S., & Ogawa, Y. (2022). Changes in bioactive compounds and antioxidant activity of plant-based foods by gastrointestinal digestion: A review. *Critical Reviews in Food Science and Nutrition, 62*(17), 4684–4705. https://doi.org/10.1080/10408398.2021.1878100

Lambadiari, V., Korakas, E., & Tsimihodimos, V. (2020). The impact of dietary Glycemic index and Glycemic load on postprandial lipid kinetics. *Dyslipidemia and Cardiovascular Risk. Nutrients, 12*(8), 2204. https://doi.org/10.3390/nu12082204

Lee, H. J., Kim, K. J., Park, S.-Y., Choi, K., Pyee, J., Yoo, S., & Kim, J. Y. (2024). Enhancing intestinal health with germinated oats: Bioinformatics and compound profiling insights into a novel approach for managing inflammatory bowel disease. *Food Bioscience, 61*, Article 104833. https://doi.org/10.1016/j.fbio.2024.104833

Li, M., Xia, M., Imran, A., de Souza, T. S. P., Barrow, C., Dunshea, F., & Suleria, H. A. R. (2024). Nutritional value, phytochemical potential, and biological activities in lentils (lens culinaris Medik.): A review. *Food Reviews International, 40*(7), 2024–2054. https://doi.org/10.1080/87559129.2023.2245073

Li, P., Zhang, B., & Dhital, S. (2019). Starch digestion in intact pulse cells depends on the processing induced permeability of cell walls. *Carbohydrate Polymers, 225*, Article 115204. https://doi.org/10.1016/j.carbpol.2019.115204

Lima, J. E. B. F., Moreira, N. C. S., & Sakamoto-Hojo, E. T. (2022). Mechanisms underlying the pathophysiology of type 2 diabetes: From risk factors to oxidative stress, metabolic dysfunction, and hyperglycemia. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 874-875*, Article 503437. https://doi.org/10.1016/j.mrgentox.2021.503437

Machado, M., Costa, E. M., Silva, S., Rodriguez-Alcalá, L. M., Gomes, A. M., & Pintado, M. (2023). Understanding the anti-obesity potential of an avocado oil-rich cheese through an in vitro co-culture intestine cell model. *Molecules, 28*(15), 5923. https://doi.org/10.3390/molecules28155923

Martinez, T. M., Meyer, R. K., & Duca, F. A. (2021). Therapeutic potential of various plant-based Fibers to improve energy homeostasis via the gut microbiota. *Nutrients, 13*(10), 3470. https://doi.org/10.3390/nu13103470

Martínez-Sánchez, V., Calvo, M. V., Fontecha, J., & Pérez-Gálvez, A. (2024). The role of food matrices supplemented with Milk fat globule membrane in the bioaccessibility of lipid components and adaptation of cellular lipid metabolism of Caco-2 cells. *Nutrients, 16*(16). https://doi.org/10.3390/nu16162798

Massagué, J., & Sheppard, D. (2023). TGF-β signaling in health and disease. *Cell, 186*(19), 4007–4037. https://doi.org/10.1016/j.cell.2023.07.036

Morresi, C., Cianfruglia, L., Sartini, D., Cecati, M., Fumarola, S., Emanuelli, M., & Bacchetti, T. (2019). Effect of high glucose-induced oxidative stress on Paraoxonase 2 expression and activity in Caco-2 cells. *Cells, 8*(12). https://doi.org/10.3390/cells8121616

Mustafa, A. M., Abouelenein, D., Acquaticci, L., Alessandroni, L., Angeloni, S., Borsetta, G., & Vittori, S. (2022). Polyphenols, saponins and phytosterols in lentils and their health benefits: An overview. *Pharmaceuticals, 15*(10), 1225. https://doi.org/10.3390/ph15101225

Paudel, D., Dhungana, B., Caffe, M., & Krishnan, P. (2021). A review of health-beneficial properties of oats. *Foods, 10*(11), 2591. https://doi.org/10.3390/foods10112591

Peng, L., Guo, F., Pei, M., Tsao, R., Wang, X., Jiang, L., & Xiong, H. (2022). Anti-inflammatory effect of lentil hull (Lens culinaris) extract via MAPK/NF-κB signaling pathways and effects of digestive products on intestinal barrier and inflammation in Caco-2 and Raw264.7 co-culture. *Journal of Functional Foods, 92*, Article 105044. https://doi.org/10.1016/j.jff.2022.105044

Peng, X., Liu, H., Li, X., Wang, H., Zhang, K., Li, S., & Yu, W. (2023). Predicting the Glycemic index of biscuits using static in vitro digestion protocols. *Foods, 12*(2), 404. https://doi.org/10.3390/foods12020404

Piana, C., Wirth, M., Gerbes, S., Viernstein, H., Gabor, F., & Toegel, S. (2008). Validation of reference genes for qPCR studies on Caco-2 cell differentiation. *European Journal of Pharmaceutics and Biopharmaceutics, 69*(3), 1187–1192. https://doi.org/10.1016/j.ejpb.2008.03.008

Ramdath, D. D., Lu, Z. H., Maharaj, P. L., Winberg, J., Brummer, Y., & Hawke, A. (2020). Proximate analysis and nutritional evaluation of twenty Canadian lentils by principal component and cluster analyses. *Foods, 9*(2). https://doi.org/10.3390/foods9020175

Ren, N., Hu, X., & Ma, Z. (2023). Multi-scale structural insights into enzymatically hydrolyzed lentil starch concentrates prepared by in vitro method using different types of enzymes. *Foods, 12*(11), 2150. https://doi.org/10.3390/foods12112150

Röder, P. V., Geillinger, K. E., Zietek, T. S., Thorens, B., Koepsell, H., & Daniel, H. (2014). The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. *PLoS One, 9*(2), Article e89977. https://doi.org/10.1371/journal.pone.0089977

Rohm, T. V., Meier, D. T., Olefsky, J. M., & Donath, M. Y. (2022). Inflammation in obesity, diabetes, and related disorders. *Immunity, 55*(1), 31–55. https://doi.org/10.1016/j.immuni.2021.12.013

Senesi, P., Ferrulli, A., Luzi, L., & Terruzzi, I. (2021). Diabetes mellitus and cardiovascular diseases: Nutraceutical interventions related to caloric restriction. *International Journal of Molecular Sciences, 22*(15), 7772. https://doi.org/10.3390/ijms22157772

Sharif, S., Van der Graaf, Y., Cramer, M. J., Kapelle, L. J., de Borst, G. J., Visseren, F. L. J., & the, S. s. g.. (2021). Low-grade inflammation as a risk factor for cardiovascular events and all-cause mortality in patients with type 2 diabetes. *Cardiovascular Diabetology, 20*(1), 220. https://doi.org/10.1186/s12933-021-01409-0

Soong, Y. Y., Tan, S. P., Leong, L. P., & Henry, J. K. (2014). Total antioxidant capacity and starch digestibility of muffins baked with rice, wheat, oat, corn and barley flour. *Food Chemistry, 164*, 462–469. https://doi.org/10.1016/j.foodchem.2014.05.041

Stantiall, S. E., & Serventi, L. (2018). Nutritional and sensory challenges of gluten-free bakery products: A review. *International Journal of Food Sciences and Nutrition, 69*(4), 427–436. https://doi.org/10.1080/09637486.2017.1378626

Steven, S., Frenis, K., Oelze, M., Kalinovic, S., Kuntic, M., Bayo Jimenez, M. T., & Daiber, A. (2019). Vascular inflammation and oxidative stress: Major triggers for cardiovascular disease. *Oxidative Medicine and Cellular Longevity, 2019*(1), Article 7092151. https://doi.org/10.1155/2019/7092151

Sun, B., Chen, H., Xue, J., Li, P., & Fu, X. (2023). The role of GLUT2 in glucose metabolism in multiple organs and tissues. *Molecular Biology Reports, 50*(8), 6963–6974. https://doi.org/10.1007/s11033-023-08535-w

Varghese, S., Awana, M., Mondal, D., Rubiya, M. H., Melethil, K., Singh, A., & Thomas, B. (2023). Amylose–Amylopectin Ratio. In S. Thomas, A. Ar, C. Jose Chirayil, & B. Thomas (Eds.), *Handbook of biopolymers* (pp. 1305–1334). Singapore: Springer Nature Singapore.

Vlachos, D., Malisova, S., Lindberg, F. A., & Karaniki, G. (2020). Glycemic index (GI) or Glycemic load (GL) and dietary interventions for optimizing postprandial Hyperglycemia in patients with T2 diabetes: A review. *Nutrients, 12*(6), 1561. https://doi.org/10.3390/nu12061561

Wang, F., Zhang, Y., Yu, T., He, J., Cui, J., Wang, J., & Fan, J. (2018). Oat globulin peptides regulate antidiabetic drug targets and glucose transporters in Caco-2 cells. *Journal of Functional Foods, 42*, 12–20. https://doi.org/10.1016/j.jff.2017.12.061

Wang, Y., Ou, X., Al-Maqtari, Q. A., He, H.-J., & Othman, N. (2024). Evaluation of amylose content: Structural and functional properties, analytical techniques, and future prospects. *Food Chemistry: X, 24*, Article 101830. https://doi.org/10.1016/j.fochx.2024.101830

Wang, Y., Zhou, X., Xiang, X., & Miao, M. (2023). Association of Slowly Digestible Starch Intake with reduction of postprandial Glycemic response: An update Meta-analysis. *Foods, 12*(1), 89. https://doi.org/10.3390/foods12010089

Wang, Y.-J., Bian, Y., Luo, J., Lu, M., Xiong, Y., Guo, S.-Y., & Song, B.-L. (2017). Cholesterol and fatty acids regulate cysteine ubiquitylation of ACAT2 through competitive oxidation. *Nature Cell Biology, 19*(7), 808–819. https://doi.org/10.1038/ncb3551

Wani, I. A., Sogi, D. S., Hamdani, A. M., Gani, A., Bhat, N. A., & Shah, A. (2016). Isolation, composition, and physicochemical properties of starch from legumes: A review. *Starch, 68*(9–10), 834–845. https://doi.org/10.1002/star.201600007

Wojtunik-Kulesza, K., Oniszczuk, A., Oniszczuk, T., Combrzyński, M., Nowakowska, D., & Matwijczuk, A. (2020). Influence of in vitro digestion on composition, bioaccessibility and antioxidant activity of food polyphenols—A non-systematic review. *Nutrients, 12*(5), 1401. https://doi.org/10.3390/nu12051401

Wolever, T. M. S., Jones, P. J. H., Jenkins, A. L., Mollard, R. C., Wang, H., Johnston, A., & Chu, Y. (2019). Glycaemic and insulinaemic impact of oats soaked overnight in milk vs. cream of rice with and without sugar, nuts, and seeds: A randomized, controlled trial. *European Journal of Clinical Nutrition, 73*(1), 86–93. https://doi.org/10.1038/s41430-018-0329-1

Yang, Z., Zhang, Y., Wu, Y., & Ouyang, J. (2023). Factors influencing the starch digestibility of starchy foods: A review. *Food Chemistry, 406*, Article 135009. https://doi.org/10.1016/j.foodchem.2022.135009

Zhang, Y., Zhu, X., Yu, X., Novák, P., Gui, Q., & Yin, K. (2023). Enhancing intestinal barrier efficiency: A novel metabolic diseases therapy. *Frontiers in Nutrition, 10*, Article 1120168. https://doi.org/10.3389/fnut.2023.1120168

Zhu, F. (2017). Structures, properties, modifications, and uses of oat starch. *Food Chemistry, 229*, 329–340. https://doi.org/10.1016/j.foodchem.2017.02.064