POSTER COMMUNICATIONS

Study of the relationship between gluten-free foods and microbiota in celiac subjects

Alessandro Annunziato (alessandro.annunziato@uniba.it)

Dept. of Food and Soil Science, University of “Aldo Moro”, BARI, Italy

Tutor: Prof. Ruggiero Francavilla

Co-Tutor: Dott. Francesco Maria Calabrese

The activities carried out in the second year of PhD project are below described. The present work is aimed at characterizing a gluten-free laboratory bread (B) using rice flour supplemented with an artichoke leaf powder extract (AE). The AE has been added to the gluten-free experimental bread. Four different batches of bread were prepared: two mixtures (SB and SB-AE) with the addition of a gluten-free type II mother yeast (tII-SD) and two control mixtures (YB and YB-AE) not containing tII-SD. The purpose of this study is to modify a traditional food product consumed daily worldwide to support celiac patients.

**Studio della relazione tra alimenti gluten-free e microbiota dei soggetti celiaci**

Le attività svolte nel secondo anno di dottorato sono descritte. Il presente lavoro mira a caratterizzare un pane da laboratorio senza glutine (B) utilizzando farina di riso integrata con un estratto di foglie di carciofo in polvere (AE). L'AE è stato aggiunto al pane sperimentale senza glutine. Sono stati preparati quattro diversi batch di pane: due miscele (SB e SB-AE) con l'aggiunta di un lievito madre di tipo II senza glutine (tII-SD) e due miscele di controllo (YB e YB-AE) senza tII-SD. Lo scopo dello studio è modificare un alimento tradizionale consumato quotidianamente nel mondo per supportare i pazienti celiaci.

**Key words**: *celiac disease, artichoke, antioxidant, nutrigenomics.*

# **1. Introduction**

Gluten-free products have gained significant attention due to the increasing prevalence of gluten-related disorders and the growing consumer demand for gluten-free alternatives. Artichoke (Cynara scolymus) leaf extract has been reported to possess various bioactive compounds with potential health benefits, including anti-inflammatory properties. This study aimed to evaluate the nutritional profile, impact on gut microbiota, and anti-inflammatory response of a gluten-free bread formulation enriched with artichoke leaf powder extract.

# **2. Materials and Methods**

In the study, Type-II sourdough (tII-SD) with a dough yield (DY) of 200 was prepared using commercial rice flour and a single-strain inoculum of Leuconostoc pseudomesenteroides DSM 20193. Six batches of tII-SDs were prepared by varying incubation temperatures (20, 25, and 30 °C) and starter cell densities (6 or 7 log CFU/mL). Gluten-free bread batches were manufactured at the pilot plant of the Department of Soil, Plant, and Food Science of the University of Bari. Four batches of bread were used in the study: (i) baker's yeast gluten-free bread without sourdough and artichoke extract (YB), (ii) baker's yeast gluten-free bread with artichoke extract (YB-AE), (iii) tII-SD gluten-free bread (SB), and (iv) tII-SD gluten-free bread with artichoke extract (SB-AE). An artichoke leaf powder extract characterized by 5% titratable chlorogenic acid stabilized in maltodextrins was provided by Farmalabor S.r.l. To simulate *in vitro* digestion, standardized procedures involving oral, gastric, and intestinal phases were followed. The bread samples underwent simulated in vitro digestion, and during this process, an aliquot of fermented fecal batch was used to analyze the volatile organic compound (VOC) profiles. The VOC analysis was performed using solid-phase microextraction (SPME) with a divinylbenzene/Carboxen/polydimethylsiloxane fiber, and the volatile compounds were analyzed using gas chromatography. Two different human cell lines, Caco-2 ICLC HTL97023 and human keratinocyte NCTC 2544, were used in the experiments. These cell lines were obtained from the National Institute for Cancer Research of Genoa. The anti-inflammatory properties of the digested bread samples were assessed by measuring the expression levels of tumor necrosis factor-alpha (TNF-α) and interleukin 1-β in Caco-2 cells.

# **3. Results and Discussion**

**3.1. Fecal Volatile Organic Compounds in Fecal Batches**

The study conducted metabolic profile characterization of fecal batches after 20 hours and 42 hours of incubation using qualitative and quantitative analysis of volatile organic compounds (VOCs). A total of 59 volatile metabolites were identified and classified into different chemical classes, including alcohols, aldehydes, esters, hydrocarbons, indoles, ketones, organic acids, phenols, and terpenes. Additionally, four compounds that did not belong to these classes were also identified. The presence of specific metabolites with potential health benefits was evaluated in the residual from fecal microbiota fermentation. After 20 hours of fermentation, the profiles of VOCs distinguished the tII-SD gluten-free bread (SB) samples from the baker's yeast gluten-free bread (YB) samples. Aldehydes and hydrocarbons were more prevalent in the YB samples, while their presence was not significantly influenced by the addition of artichoke extract (AE). However, high scores of hydrocinnamic acid were specifically observed in SB-AE-T20. After an additional 22 hours of incubation (42 hours in total), a wider range of organic acids, phenols, and indoles were detected in the samples containing artichoke extract (SB-AE). Statistically significative differences in the above-mentioned compounds were found as a consequence of the artichoke extract addition. Interestingly, although the artichoke extract was also added to the baker's yeast gluten-free bread (YB-AE) samples, hydrocinnamic acid and cyclohexanecarboxylic acid were not observed to the same extent as in SB-AE. This led us to hypothesize a positive interaction between the microbiota of tII-SD and the metabolism of artichoke extract. These differences in the metabolic profiles of the samples may explain the variations in anti-inflammatory activity observed when Caco-2 cells were exposed to lipopolysaccharide (LPS). The presence of specific metabolites, such as hydrocinnamic acid, in the SB-AE samples could potentially contribute to enhanced anti-inflammatory effects.

**3.2. Anti-Inflammatory Effects**

The results of the study indicate that the supernatants from colonic fermented bread samples, specifically SB-AE (T20 and T42), exhibited significant anti-inflammatory activity in Caco-2 cells. The expressions of pro-inflammatory cytokines, TNF-α and IL-1β, were measured to assess the anti-inflammatory effectiveness of the supernatants. When compared to the positive control (LPS from E. coli), SB-T42, SB-AE-T20, and SB-AE-T42 demonstrated a significant decrease in TNF-α expression. Notably, SB-AE-T42 showed no significant difference from the negative control (cells not exposed to LPS), indicating its potential in suppressing TNF-α production even in the presence of a pro-inflammatory trigger. In terms of IL-1β expression, both supernatants from SB-AE (T20 and T42) exhibited a significant reduction compared to the positive control. Furthermore, these two supernatants did not significantly differ from the negative control, suggesting their ability to effectively inhibit IL-1β expression even in the presence of LPS. Overall, the highest anti-inflammatory effectiveness was observed in the supernatants from SB-AE samples. These samples exhibited a significant reduction in both TNF-α and IL-1β expressions under all tested conditions in Caco-2 cells exposed to LPS. This indicates that the colonic fermentation of bread, along with the incorporation of artichoke leaf powder extract (AE), enhanced the anti-inflammatory properties of the bread samples. These findings highlight the potential of colonic fermented bread supplemented with artichoke leaf powder extract in reducing pro-inflammatory cytokine expressions, specifically TNF-α and IL-1β, which are associated with inflammatory processes.

# **4. Conclusion**

Based on these results, the study supports the application of artichoke leaf extract as a functional ingredient in the development of gluten-free products with improved biological properties. The use of artichoke extract in the formulation of gluten-free products could potentially contribute to reducing inflammation and oxidative stress in individuals with celiac disease. Furthermore, the study suggests that research focused on artichoke extract could pave the way for innovative and customized therapies in the nutritional management of celiac disease. The data collected from this research can be integrated into the broader field of research exploring the combination of various dietary components to design functional and clean label products. Overall, the study highlights the potential of artichoke leaf extract as a dietary supplement for individuals with celiac disease, providing evidence of its positive effects on oxidative stress and proinflammatory cytokine expression. Further research is warranted to elucidate the underlying mechanisms and evaluate the impact of these bread samples on inflammatory disorders in vivo.

# **4. References**

1. De Angelis M, Siragusa S, Vacca M, Di Cagno R, Cristofori F, Schwarm M, Pelzer S, Flügel M, Speckmann B, Francavilla R, Gobbetti M. (2021) Selection of gut-resistant bacteria and construction of microbial consortia for improving gluten digestion under simulated gastrointestinal conditions. *Nutrients* 13, 992.