# ABSTRACT OF ORIGINAL RESEARCH ON COELIAC DISEASE

# **Gluten-free pigmented cereals: chemical characterization and their role in the modulation of inflammatory status in celiac disease**

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# Introduction

The important role of oxidative stress and inflammation in celiac disease (CD) has been reported by several authors. In celiac subjects deamidated gliadin peptides activate the innate and adaptive immune response with the production of proinflammatory cytokines and auto-antibodies. In addition, some gliadin regions trigger off the oxidative stress at intestinal level1. Therefore, inflammation and oxidative stress seem to be involved in the molecular mechanisms of CD2.

Different in vivo studies have evaluated the oxidative status in celiac patients (both adults and children) observing that the oxidative stress is strongly associated with CD. Particularly, an oxidative imbalance was

#### Chart 1 –

Total Phenolic Content (A) and antioxidant capacity (B) measured in cereal extracts before and after digestion





During this project, different analytical methods and an in vitro model, suitable to study the inflammation status associated to CD, were developed. This study underlines the potential antioxidant and antiinflammatory activities of pigmented cereals at the gut level. These results could be useful to partially clarify the role of dietary phenolic compounds in the protection of intestinal mucosa in CD. Moreover, the results obtained could promote the use of pigmented cereals among celiac subjects, improving their quality of life.

#### References

observed in new diagnosed and non-responder celiac subjects. Although the gluten free diet (GFD) exerts beneficial effects on the oxidative stress at intestinal level of celiac patients, in some cases, the GFD only partially improved the physiological activity of intestinal mucosa in celiac subjects3,4.

Several dietary components possess antioxidant and antiinflammatory properties. Therefore, the consumption of foods rich in antioxidant compounds could potentially mitigate the oxidative stress characteristic of celiac patients, improving their well-being. Rice and corn are the most consumed gluten-free cereals and are often used for the formulation of cereal-based gluten-free products. The pigmented varieties of cereals are generally richer in bioactive compounds (such as anthocyanins and other flavonoids) than the usual ones and are characterized by a higher antioxidant activity. Therefore, the pigmented cereals could represent interesting ingredients for the formulation of functional cerealbased products.

In this context, Celiac Foundation funded in 2019 a project entitled "Naturally gluten free pigmented cereals to modulate the inflammatory status in celiac disease".

The aim of this project was to evaluate the possible antioxidant and antiinflammatory properties of pigmented gluten free cereals in the framework of CD. 1. Ferretti G., Bacchetti T., Masciangelo S., Saturni L. 2012. Nutrients, 4:243-257.

 Rowicka G., Czaja-Bulsa G., Chełchowska M., Riahi A., Strucińska M., Weker H., Ambroszkiewicz J. 2018. Oxid Med Cell Longev, 1324820.
Szaflarska-Popławska A., Siomek A., Czerwionka- Szaflarska M., Gackowski D., Rożalski R., Guz J., Szpila A., Zarakowska E, Oliński R. 2010. Cancer Epidemiol Biomarkers Prev, 19:1960-1965. 4 Ferretti G., Bacchetti T., Saturni L., Manzella N., Candelaresi C., Benedetti A., Di Sario A. 2012. Journal of Lipids, 587479.

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# Method

Hydro-alcoholic extracts from pigmented rice (Nerone) and corn (Scagliolo Rosso and Rosso Rostrato di Rovetta), previously selected among 19 varieties with different pigmentation, on the basis of their phenolic content, were in vitro digested and characterized in term of phenolic compounds and antioxidant activity, using both spectrophotometric and chromatographic techniques: 1) Folin-Ciocalteu's assay for the quantification of total phenolic content; 2) DPPH (1,1,- diphenyl-2picrylhydrazyl) assay for the evaluation of antioxidant capacity; 3) High Performance Thin Layer Chromatography (HPTLC) for the separation and semi-quantitative characterization of phenolic substances, assessing in parallel the associated antioxidant activity; 4) High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) for the identification and quantification of phenolic compounds.

In parallel, for the evaluation of the anti-inflammatory activity of the extracts, Caco-2 cells were treated with digested gliadin in combination with other proinflammatory stimuli, to mimic a celiac diseaserelated inflammatory status.

## Results

Extracts from pigmented cereals showed different quantitative and qualitative anthocyanins composition; the in vitro digestion determined a significant reduction in the total anthocyanins content (-23% for Scagliolo Rosso corn, -73% for Nerone rice). On the contrary, no significant difference (p>0.05) in term of phenolic compounds content was observed after digestion and an increase in term of antioxidant activity was measured (Chart 1).



GAE: equivalent of gallic acid. N: Nerone rice, SR: Scagliolo Rosso corn, RR: Rosso Rostrato di Rovetta corn, b: before in vitro digestion, a: after in vitro digestion, \* p<0.05

During the in vitro digestion, the stability of phenolic compounds and the slight increase of antioxidant activity could be explained by the transformation of anthocyanins into bioactive small compounds, such as benzoic acid derivatives.

To study the potential antiinflammatory effects of extracts at intestinal level, the direct effect of gliadin alone or in combination with typical cytokines of autoimmune diseases (IFN- $\gamma$  and IL-1 $\beta$ ) was investigated monitoring CXCL10, a chemokine overexpressed in the intestinal mucosa of CD patients and involved in the activation and recruitment of leukocytes. Gliadin, in combination with IFN-  $\gamma$  and IL-1 $\beta$  enhanced the release of CXCL10, that was inhibited by all the tested extracts (IC50s<200 µg/mL), both before and after digestion. CXCL10 is controlled by Nuclear Factor kappa B (NF- $\kappa$ B) pathway, which was partially inhibited by all the extracts at the highest concentration tested (200 µg/mL). The additional involvement of other mechanisms demands specific investigations.

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