



INTERNATIONAL
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26th World Congress & Exhibition
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Processing of wheat non-starch polysaccharides for the optimization of their functional and nutritional characteristics

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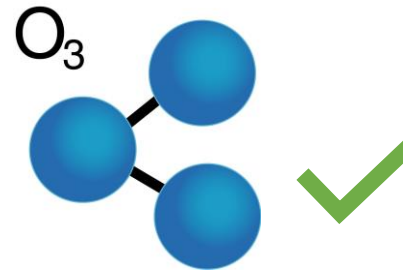
Ozone and Advanced Oxidation
Leading-edge science and technologies





Context - Background of Ozone application on food

- Ozone is classified as « Generally Recognized As Safe » (G.R.A.S) 




No Residues





Context - Background of Ozone application on grain processing

- O₃ treatment of wheat grain :
 - Microbial reduction (bacteria, fungi, viruses)
 - Food storage (Insects elimination)
 - Grain processing (Processing aid) ANSES 



Context - Background of Ozone application on grain processing

Treatment conditions (O_3 concentration, time)

Change in starch and protein structures

Rheological and technical properties of flour and dough

- Flour brightness improvement
- Dough strength enhancement
- Increase peak viscosity of flour



Context - Background of Ozone application on grain processing

Ozone treatment on wheat grain

Changes in molecular structure

- Introduction of carbonyl, carboxyl groups into starch (\searrow pH)
- Creation of S-S bounds in proteins
- Change in protein secondary structure
- Lipid oxidation
- Polyphenols content reduction
- Endogenous enzymes inactivation



Context - Background of Ozone application on grain processing

What about other wheat grain components?



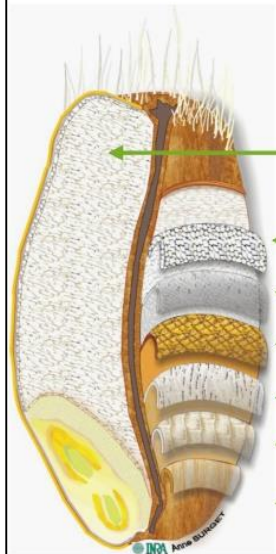
Context - Wheat grain fiber

Lipids: 2 - 2.5% of dry kernel weight

Non-polar lipids (**NL**): mainly triglycerides / Polar lipids: Glycolipids (**GL**) & Phospholipids (**PL**)

NSP: 12-18% of dry kernel weight

ArabinoXylans (**AX**) / Mixed Linked Glucans (**MLG**) / HeteroXylans (**HX**) / Cellulose



Starchy endosperm

80-83 % in weight of grain
Starch & Proteins

NL: 40% / GL: 30% / PL: 30%
+ starch associated lipids mainly PL
AX: 70% / MLG: 20%
AX are partly water-soluble

Aleurone layer

6-7 % in weight of grain

NSP (25% of total NSP) & Proteins

NL: 80% / GL: 5% / PL: 15%
AX: 65% / MLG: 30%

Outer layers

6-8 % in weight of grain

NSP (50% of total NSP)

NL: 90% / GL: 5% / PL: 5%
HX:60% / Cellulose:35% / lignin: 5%

Germ

3 % in weight of grain

Protein and Lipids

NL: 85% / GL: 0% / PL: 15%

Wheat bran composition

- **43-60% NSP**
- 11-24% Starch
- 14-20% Proteins
- 3-4% Lipids
- 3-8% Minerals



Context - Wheat grain fiber

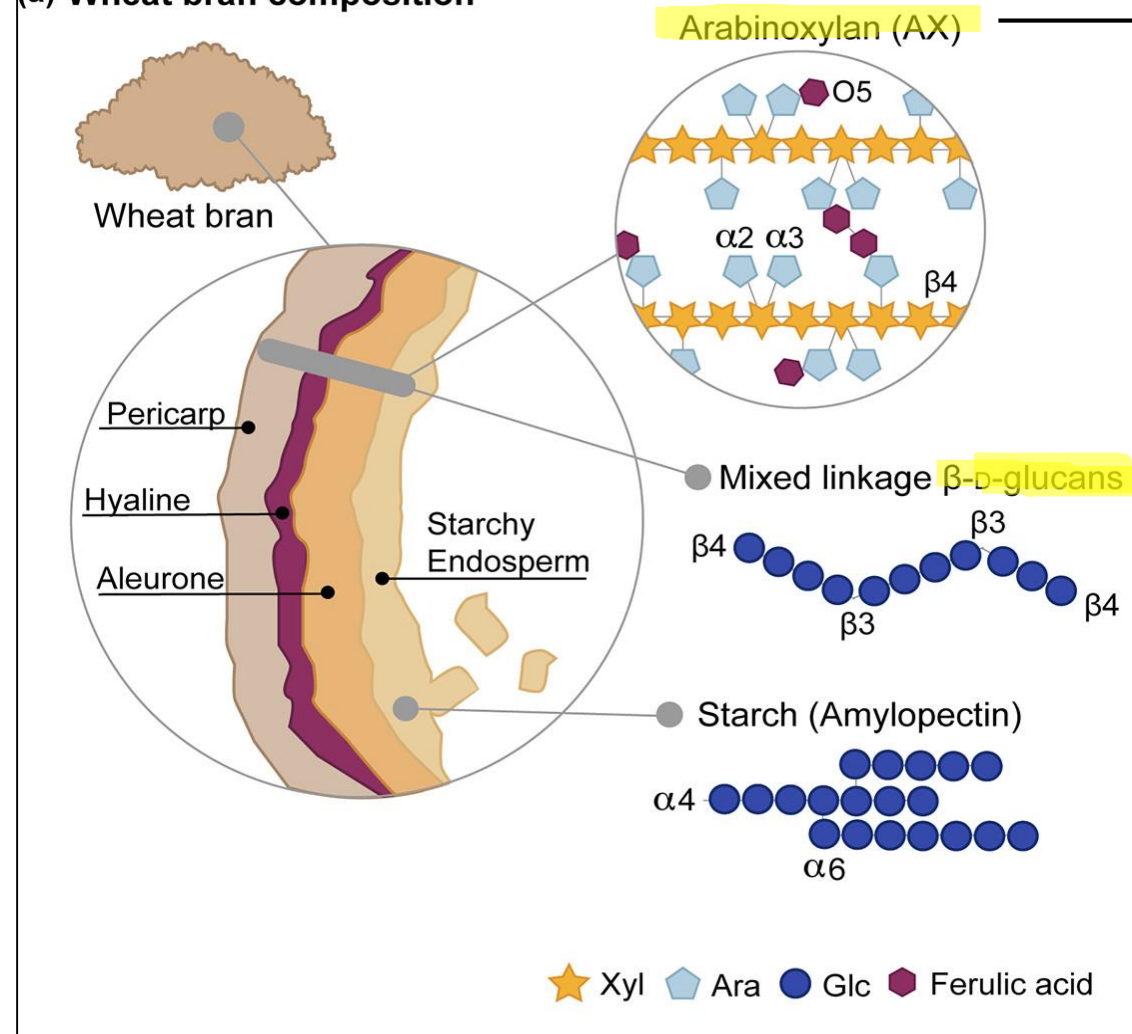
Dietary fiber (DF):

NSP linked with lignin and other non glucuronic compounds

(ex: polyphenols).

(AFSSA, 2002)

(a) Wheat bran composition



70% of the WB NSP
(Insoluble + Hydrosoluble)

6% of the WB NSP
(Hydrosoluble)



Context - Fiber physico-chemical properties

Functional

- **Solubility (H₂O)**
 - Mw, DP, molecular structure
 - > 90% of fiber are insoluble
- **Viscosity**
 - Mw, substitution degree, Mw distribution, concentration
 - β-glucans have more impact on viscosity than AX
- **Binding capacities**
 - Water, Oil, Cholesterol, Glucose, Sodium cholate, Cation exchange, Nitrite ion

Physico-chemical





- **Antioxidant activities**
 - Polyphenols (Bound, Conjugated, Free)



Context - Wheat bran fiber health benefits

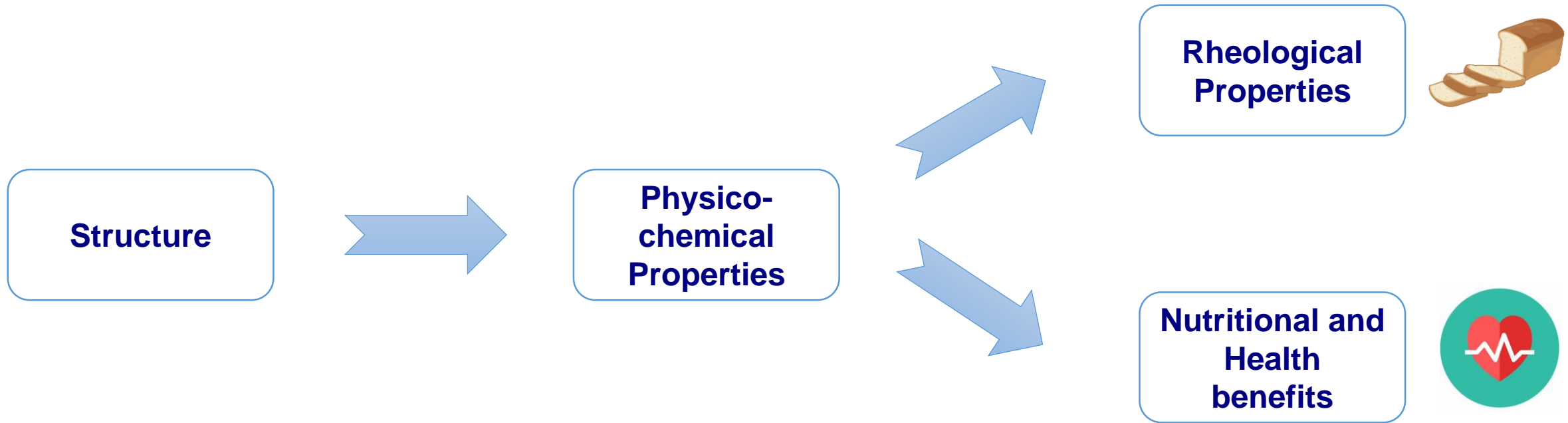
Fibers are non digestible but fermentable by gut microbiota

Fibers Health benefits

Type 2 diabetes prevention	Cardiovascular disease prevention	Colon cancer prevention	Obesity prevention
			
<ul style="list-style-type: none"> ↘ Glucose answer ↘ Hypertension 	<ul style="list-style-type: none"> ↘ Cholesterol absorption ↘ Hypertension 	<ul style="list-style-type: none"> ↗ Antioxidant effects SCFA production ↗ Intestinal transit 	<ul style="list-style-type: none"> + Microbiota ↘ Inflammation

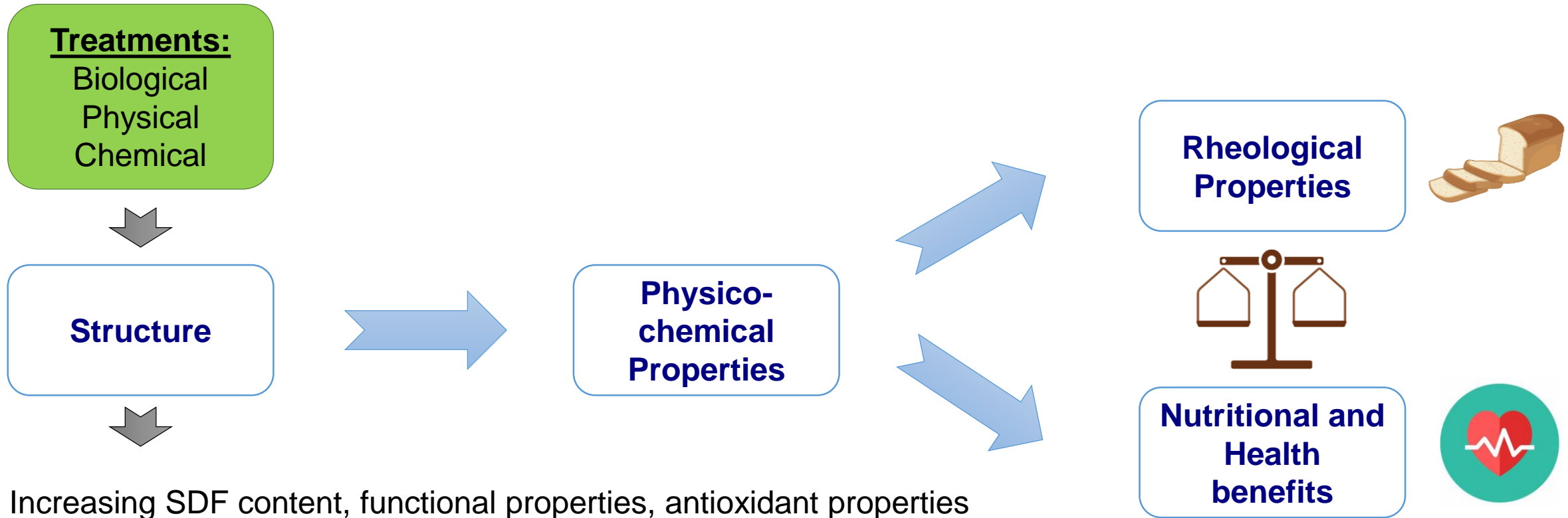


Context – Wheat bran fiber properties





Context – Wheat bran fiber properties



- + Increasing SDF content, functional properties, antioxidant properties
- Destructuring effects, modification of rheological and organoleptic properties



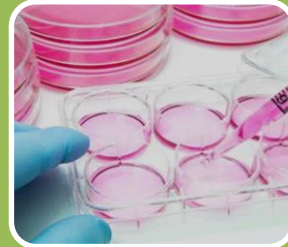
Objectives

Modification of fiber properties



Ozone treatment of the non-starch polysaccharides fraction and chemical characterization

Nutritional and health effects characterization



In vitro digestibility study of the treated-NSP



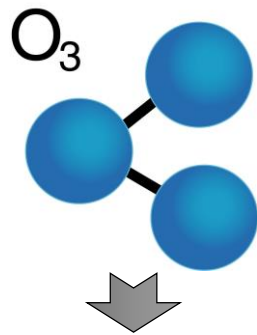
In vivo health effects associated evaluation



Context - Ozone effects on NSP

Ozone effect on NSP

- Lignin and polyphenols oxidation
 - Carbonyl, Carboxyl groups
- Increase SDF content
 - Breaking of crosslinks between NSP
- β -glucan Mw reduction
 - Glycosidic bound cleavage
- Enhanced rheological properties



Structure

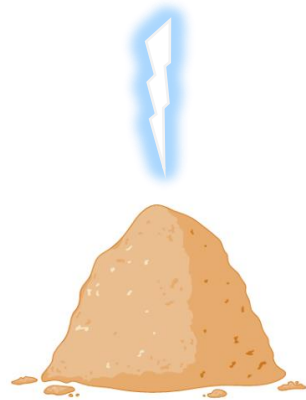
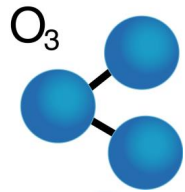
Depending of Ozone consumption



Method - Treatment

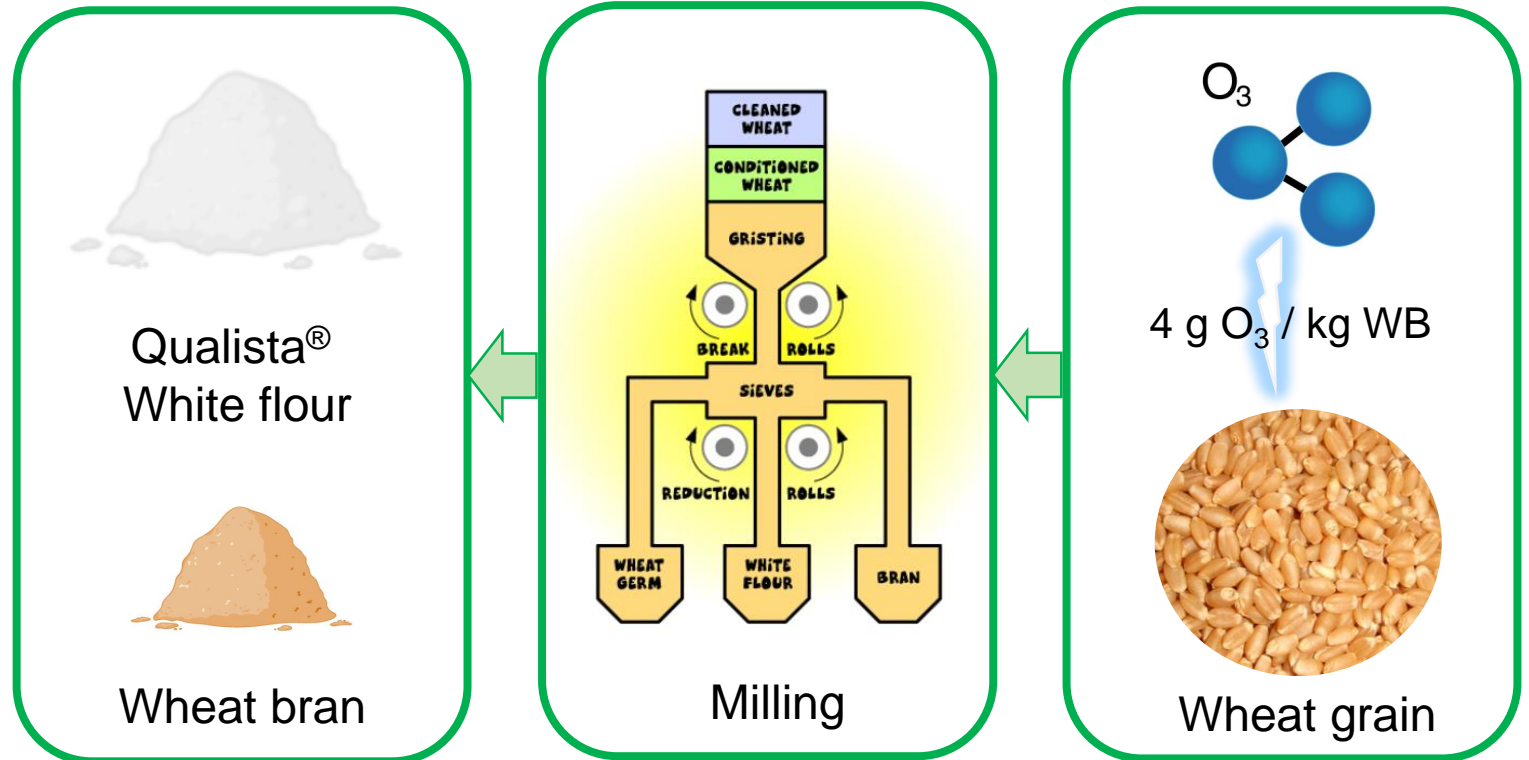
Ozone process

Gaseous O₃ treatment



Isolated Wheat Bran (WB)*

* Oxygreen process





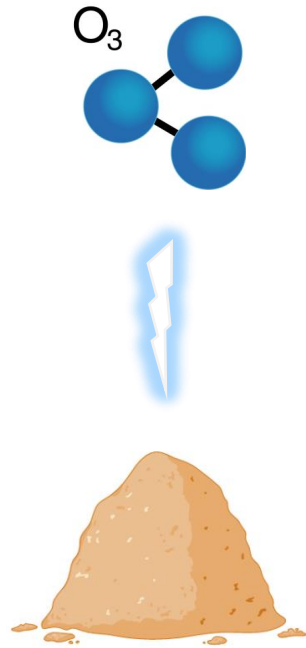
Method - Treatment

Treatment parameters

- Humidification 4%
- Batch mass: 40 kg
- Oxygen Flowrate : 5.05 m³/h
- Ozone concentration: 110 g/m³



Gaseous O₃ treatment



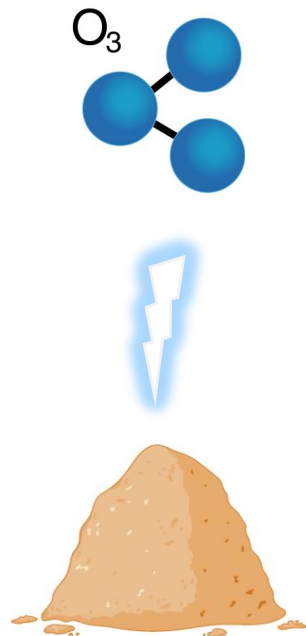
Sample identification	Ozonation reaction time (min)	Ozone consumed (g O ₃ / kg WB)
C (Control)	13.5	0.0
3G	13.5	3.2
6G	27.0	6.3
9G	40.0	9.0
12G	53.0	11.9
24G	106.0	23.4

Isolated Wheat Bran (WB)



Method - Analysis

Gaseous O₃ treatment



Isolated Wheat Bran (WB)

Physico-chemical characterization

Solubility

Functional properties

Viscosity

Particle size

NSP structure and conformation
(DP, Mw, molar ratio)

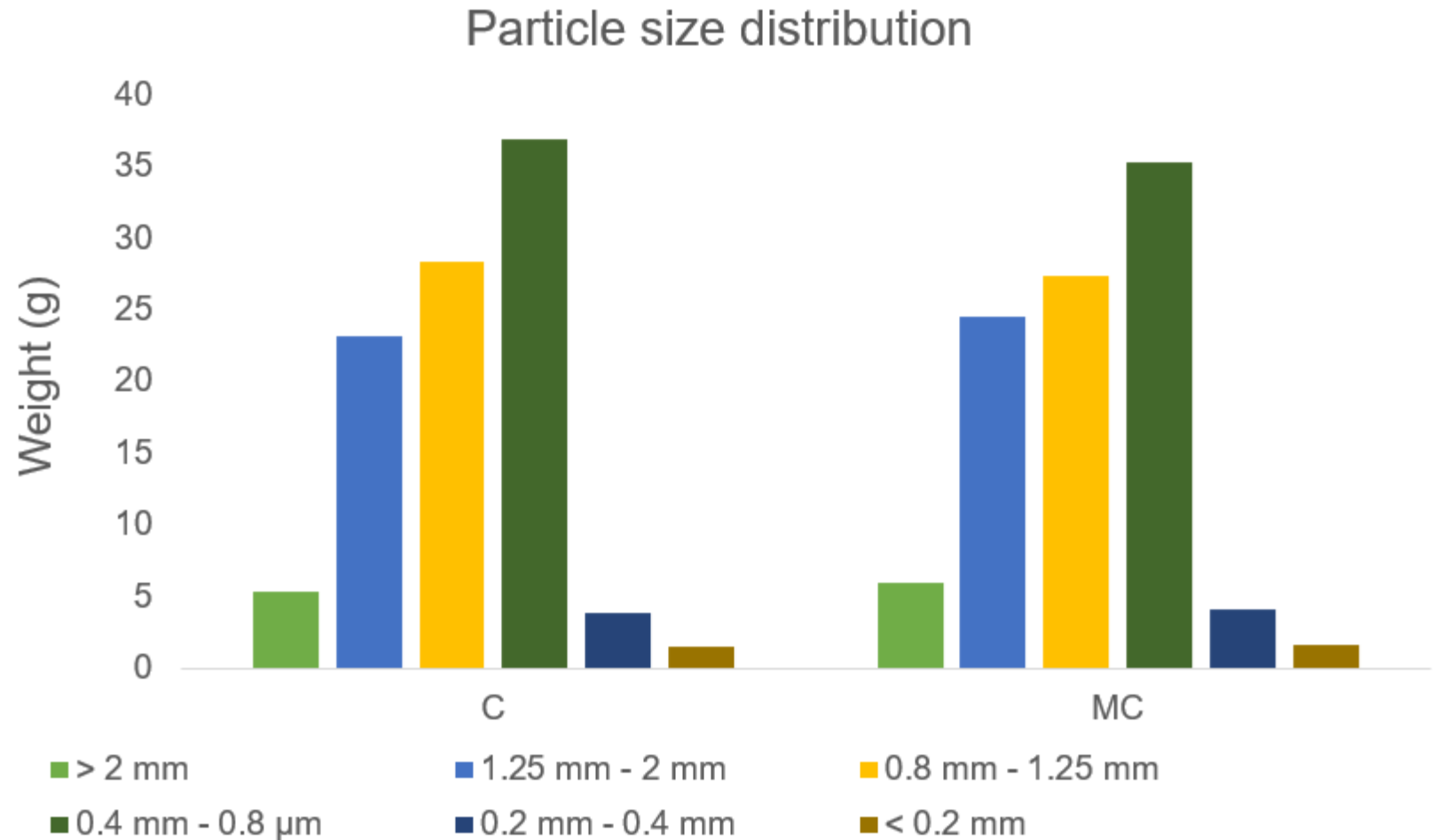
Polyphenols characterization

Antioxidant capacity analysis
(reduction, scavenging power)



Results- Particle size distribution

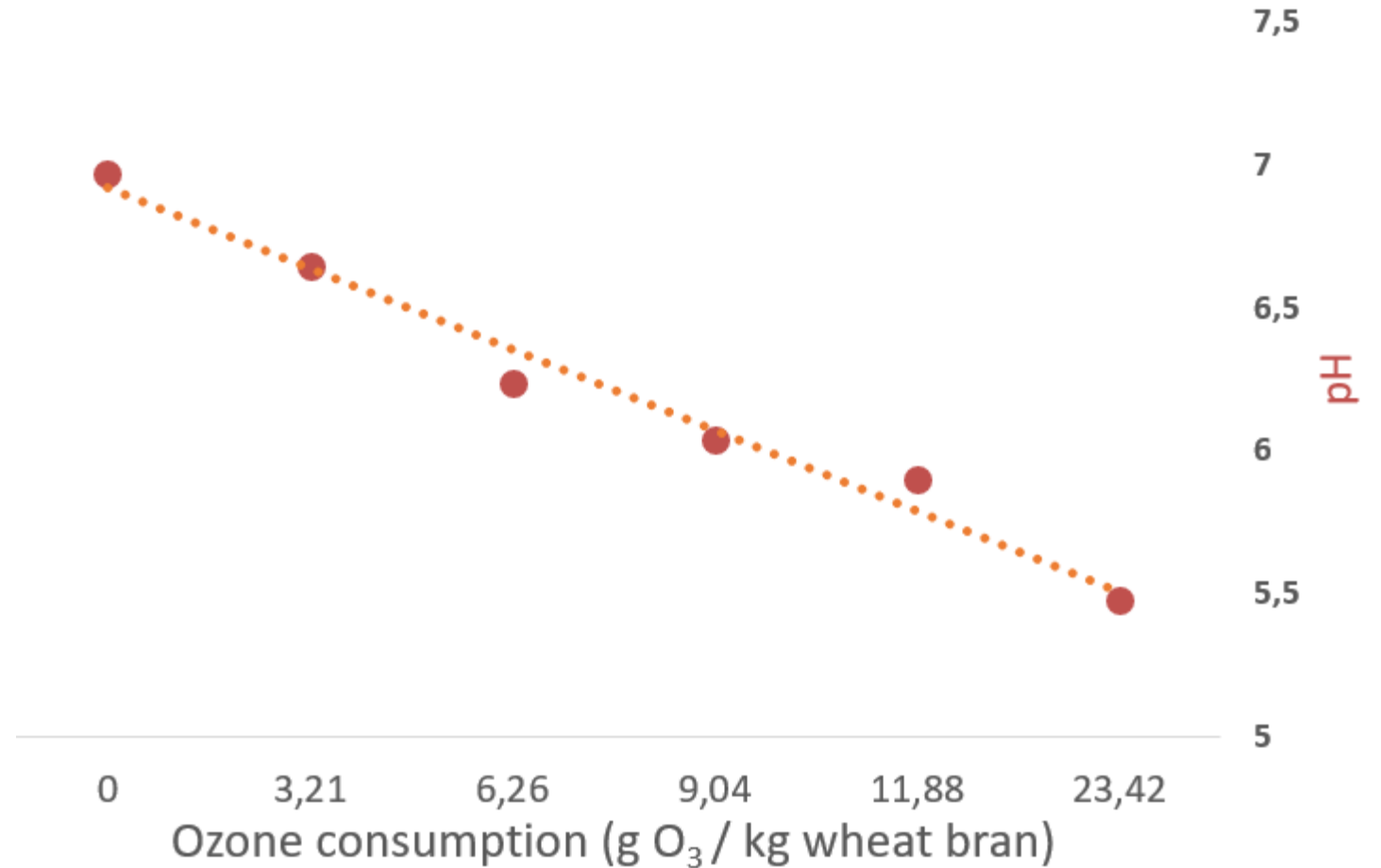
- Most important fraction = 0.4 - 0.8 mm
- D50 = 0.8 mm – 1.25 mm
- C : 13.5 min, MC (Mechanical control): 106 min
- **Mechanical process do not modify particle size distribution**





Results - pH

➤ pH reduction of 1.5 point





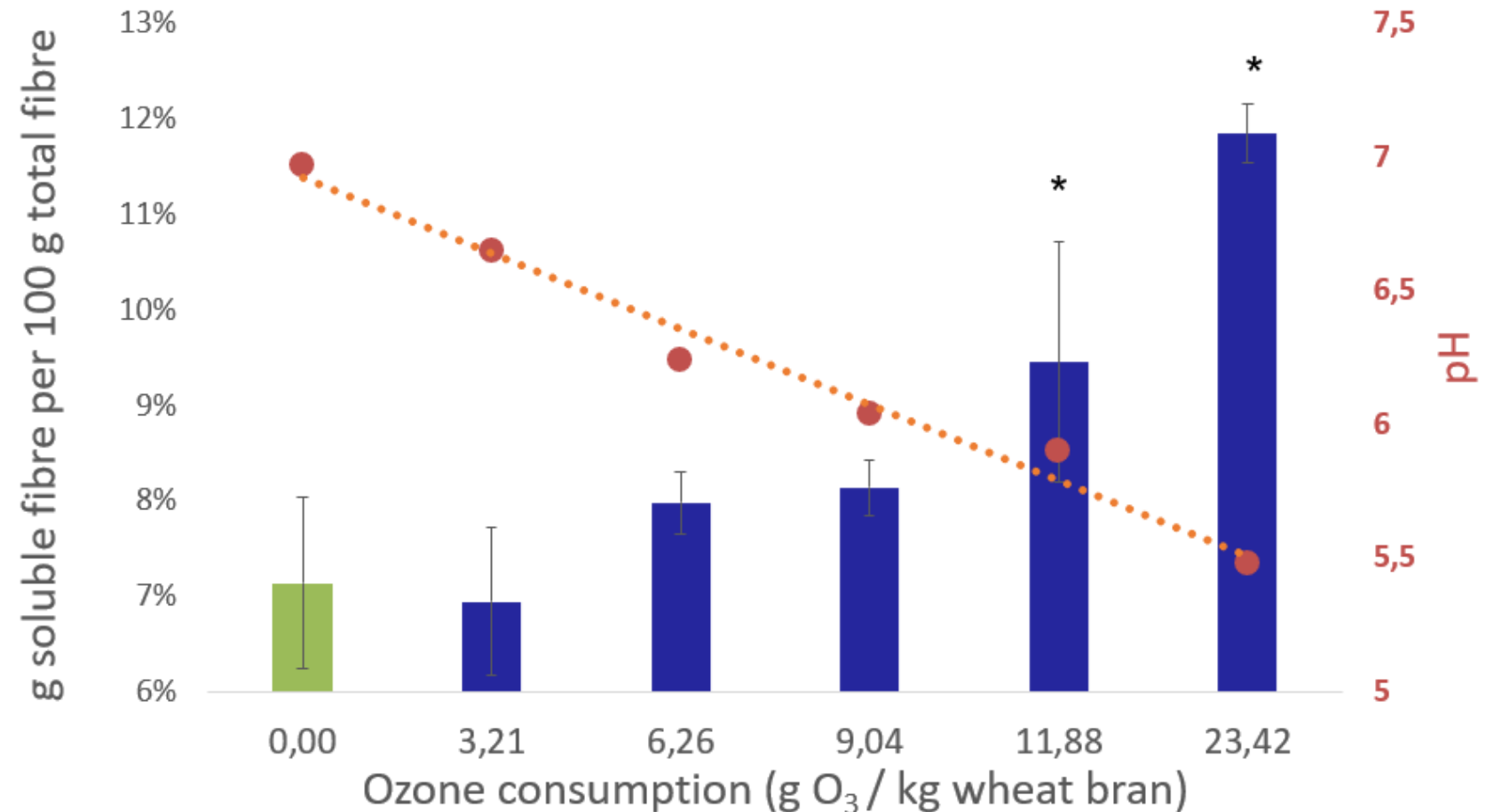
Results

- Ozone treatments do not significantly affect:
 - **Protein** content : 15.0 – 15.8 g / 100 g WB DW (Dumas Factor: x 5.7)
 - **Mineral** content : 5.4 – 5.7 g / 100 g WB DW (Ash content)
 - **Simple sugar** content : 77.6 – 84.9 mg sugar / g WB DW (HPLC-ELSD)
 - **Starch** content : 6.5 – 6.8 g starch / 100 g WB DW (Megazyme Total Starch)
 - **B-glucan** content : 5.8 – 6.2 g / 100 g WB DW (Megazyme McCleary method)
 - **Total dietary fiber** content: 41.6 – 51.0 g / 100 g WB DW (Megazyme TDF)



Results - Fiber solubility

- pH reduction of 1.5 point
- Method megazyme Total Dietary Fiber
- Solubility maximal increasing **65.9%**

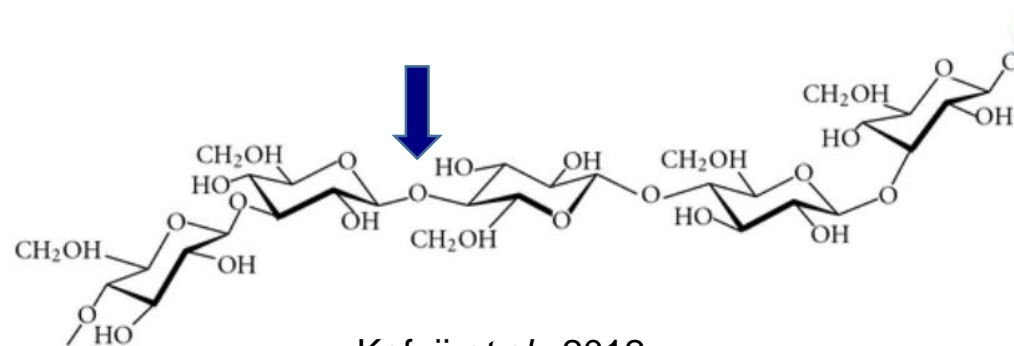




β -glucans

- Preliminary results on Asymmetrical Flow Field-Flow Fractionation (AFFFF)
- 10 fold reduction of β -glucan Mw

Ozone consumption (g O ₃ / kg WB)	Mw (x10 ³ g.mol ⁻¹)
0 (C)	23.0
2.0	31.0
4.0	2.0
6.0	1.1
7.5	2.7

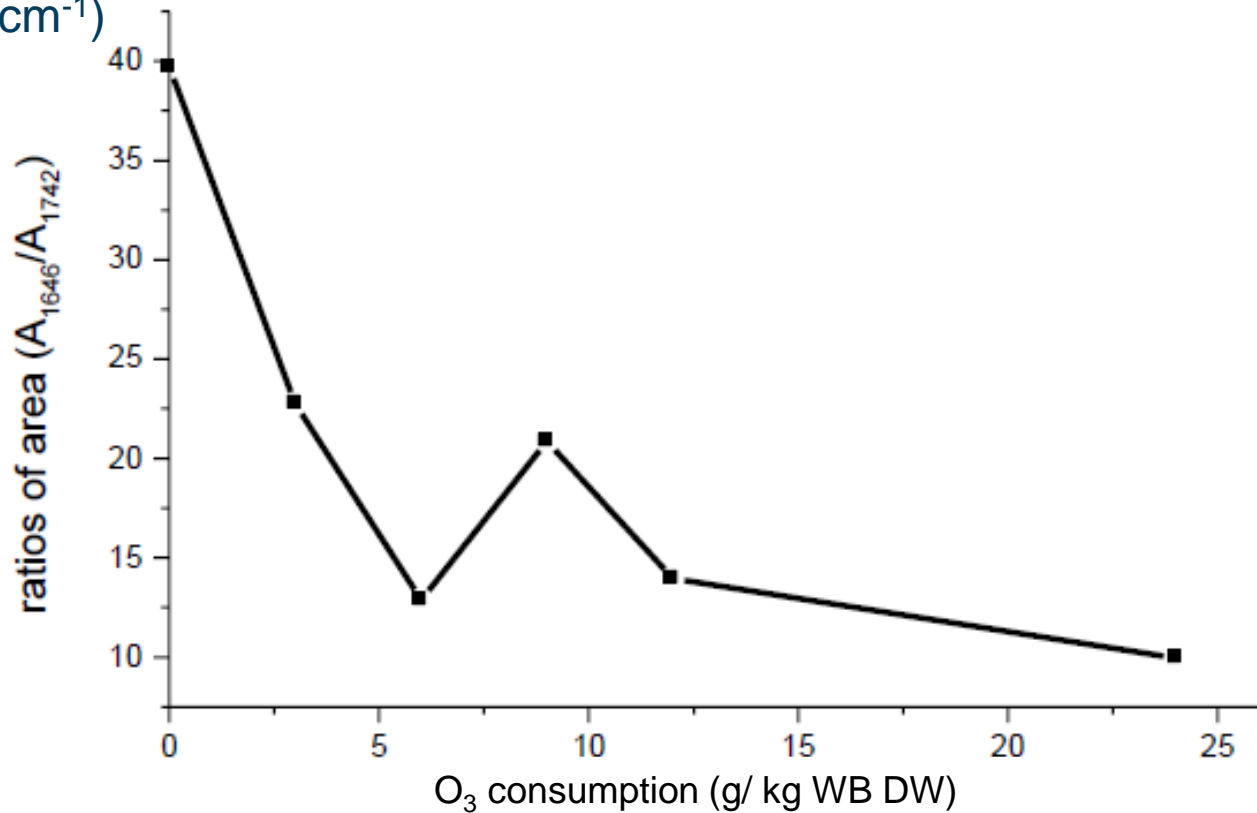


Kofuji *et al.*, 2012

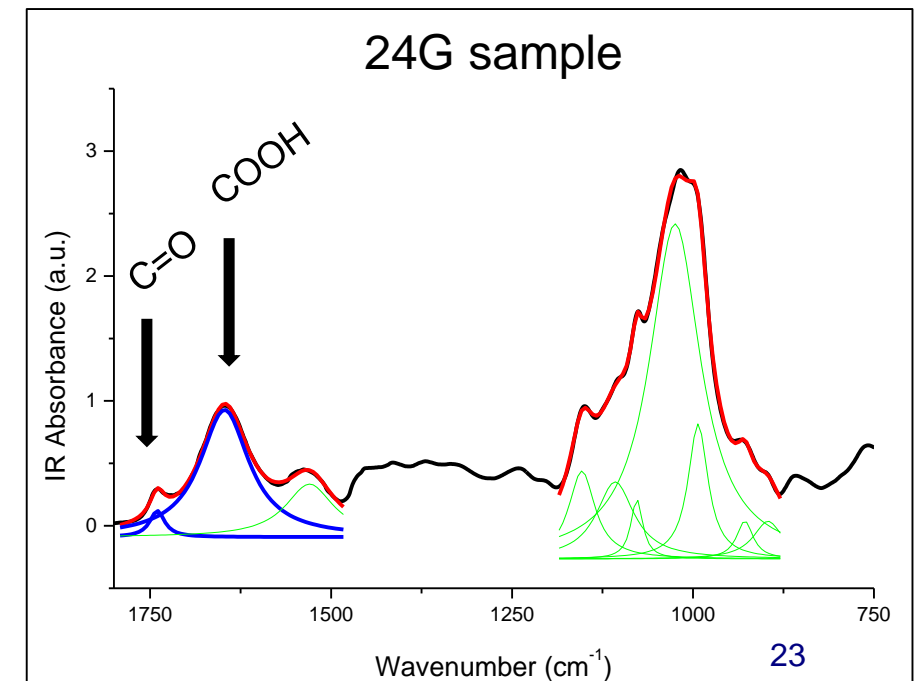
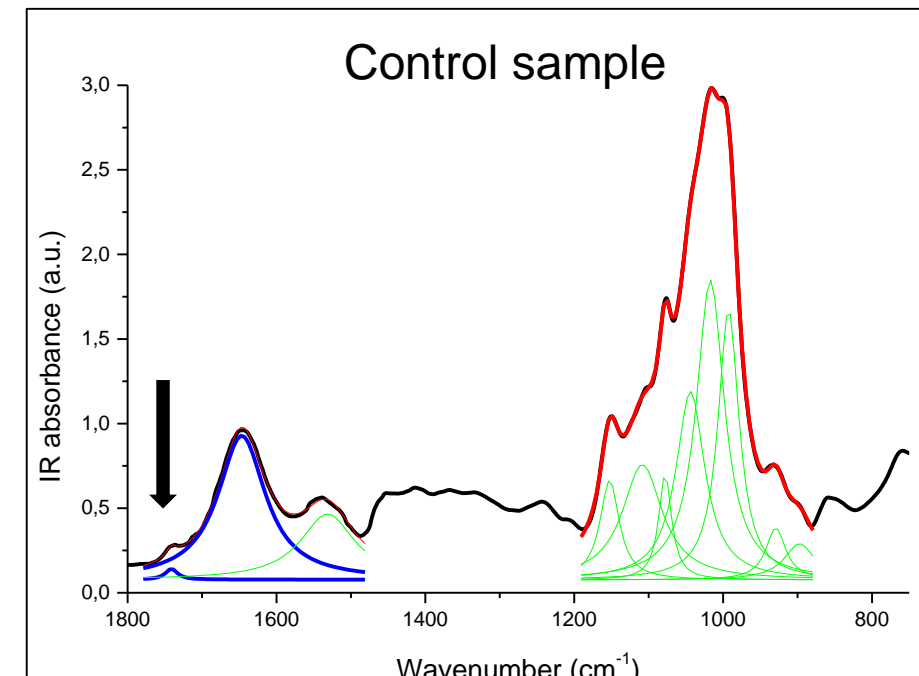


Molecular structure

- Fourier Transform InfraRed spectroscopy (FT-IR)
- Increasing of C=O contribution (1742 cm^{-1}) in comparison to COOH (1645 cm^{-1})



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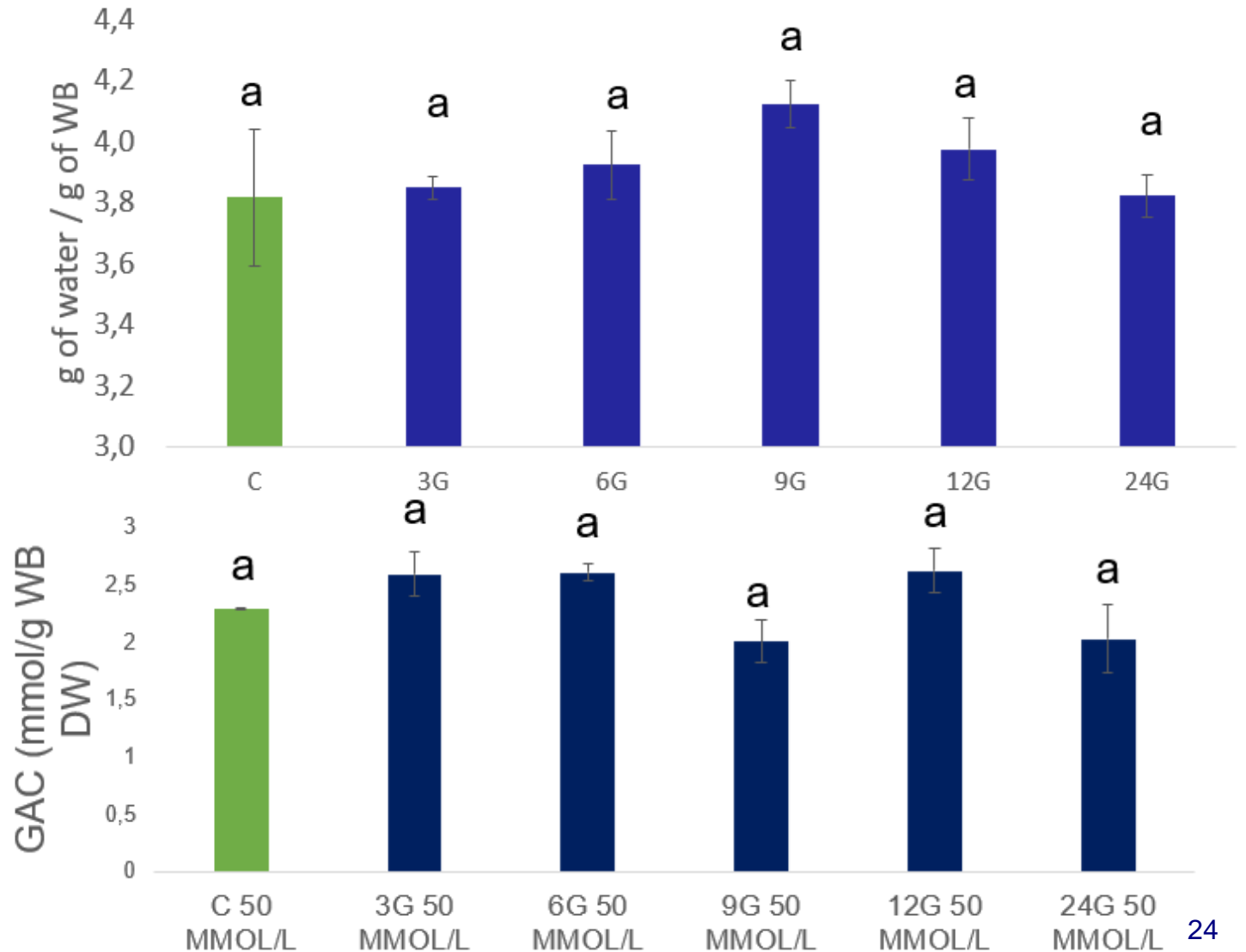
Functional properties

Water Binding Capacity (WBC)

- Range 3.7- 4.1 g of water/ g WB DW
- No significative difference

Glucose Adsorption Capacity (GAC)

- Range 2.0- 2.7 g of glucose/ g WB DW
- No significative difference at 50, 100 and 200 mmol/L glucose concentration

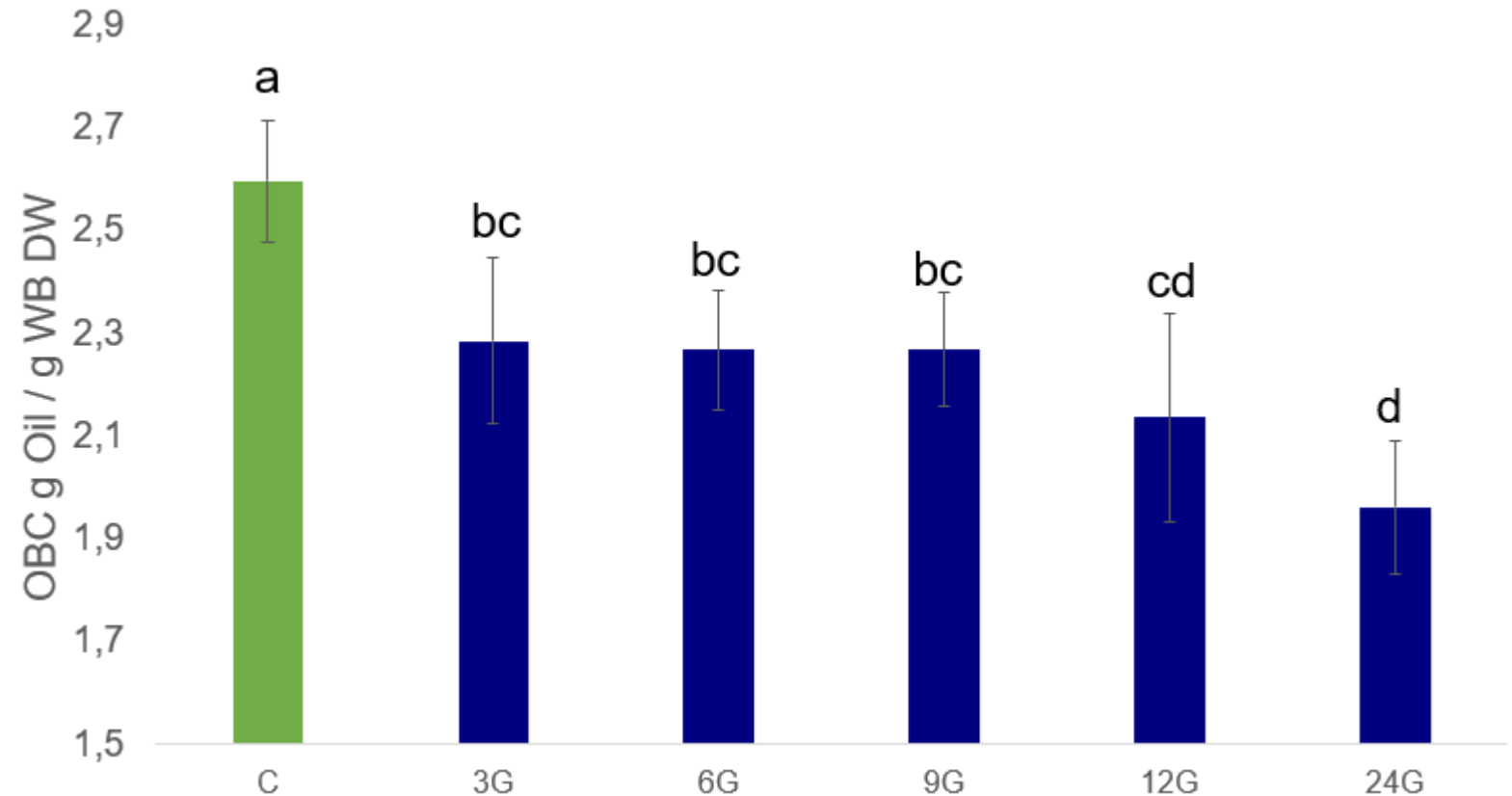




Functional properties

Oil Binding Capacity

- Range 1.9 – 2.6 g oil/ g WB DW
- Decrease of OBC with increase of ozone consumption → lipophilic sites





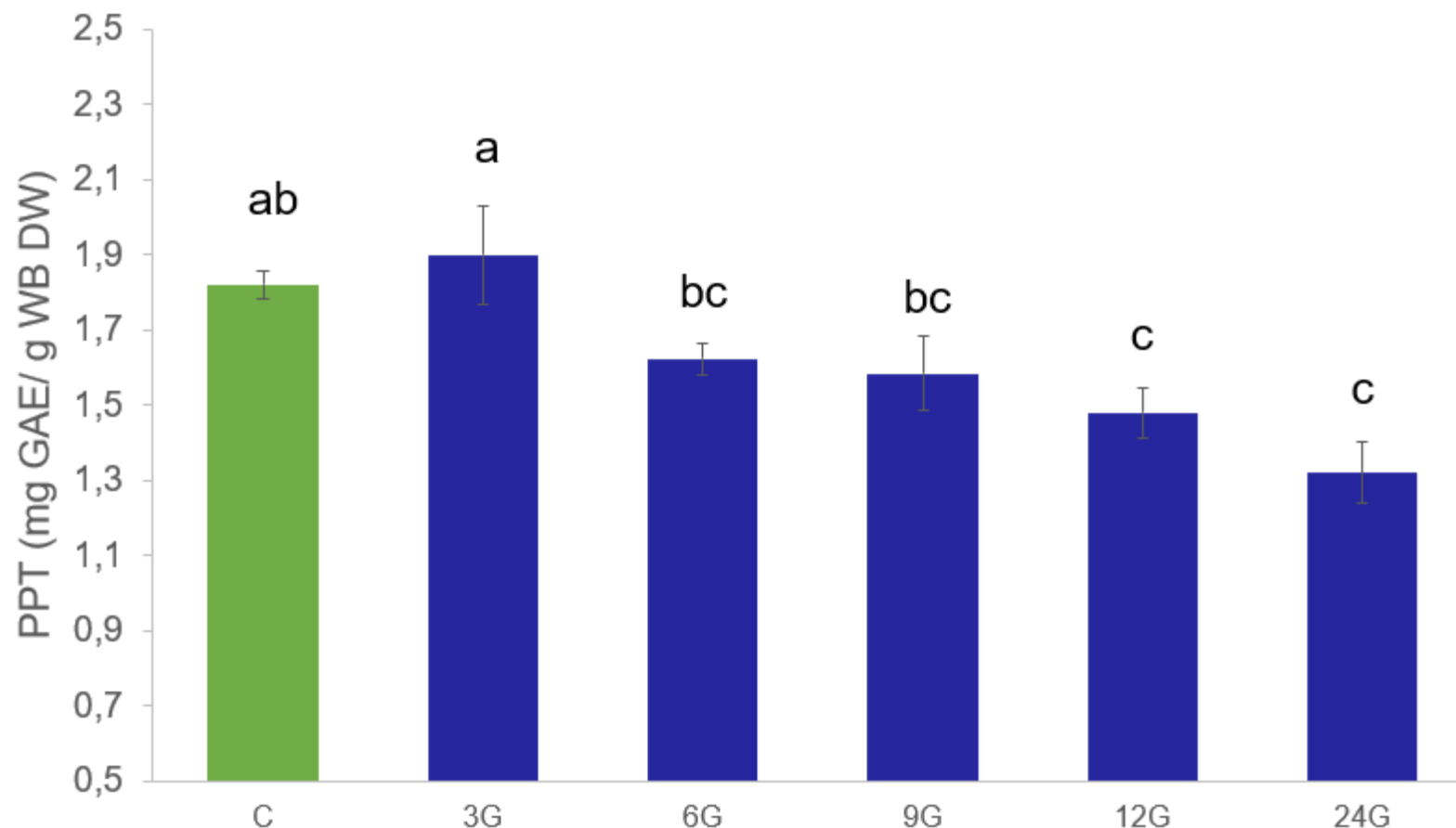
Free Total Polyphenols

- **Folin-Ciocalteu method**

Solvent: Ethanol 80% (v/v)

- Range 1.3 – 1.9 mg GAE / g WB DW

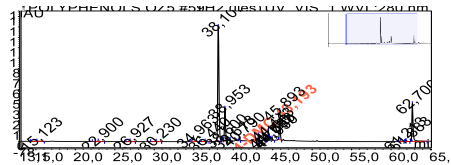
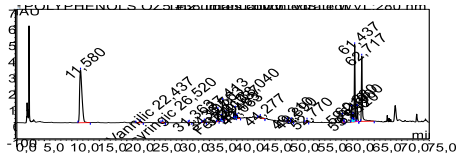
- Significant reduction of free polyphenol content



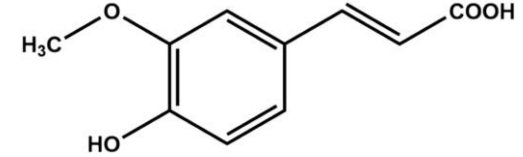


Polyphenols – Free and bound profiles

◀ Free phenolic compounds
Ethanol 80% fraction

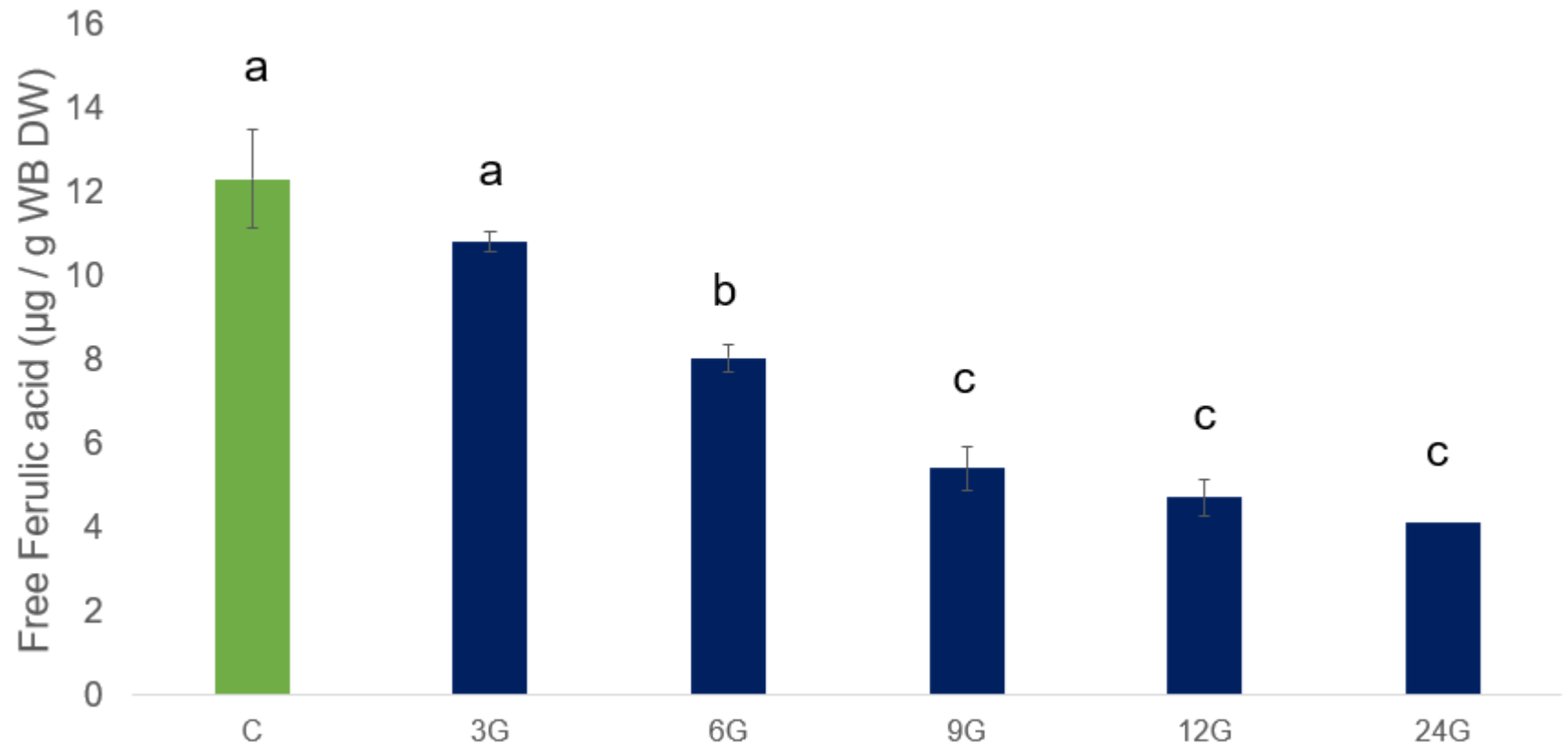


Alkali extractable phenolic compounds ▶



Phenolic acid – Free Ferulic acid HPLC

- Significant reduction of free Ferulic acid (FA)





Phenolic acid – Ferulic acid HPLC

- Bound and conjugated FA characterization

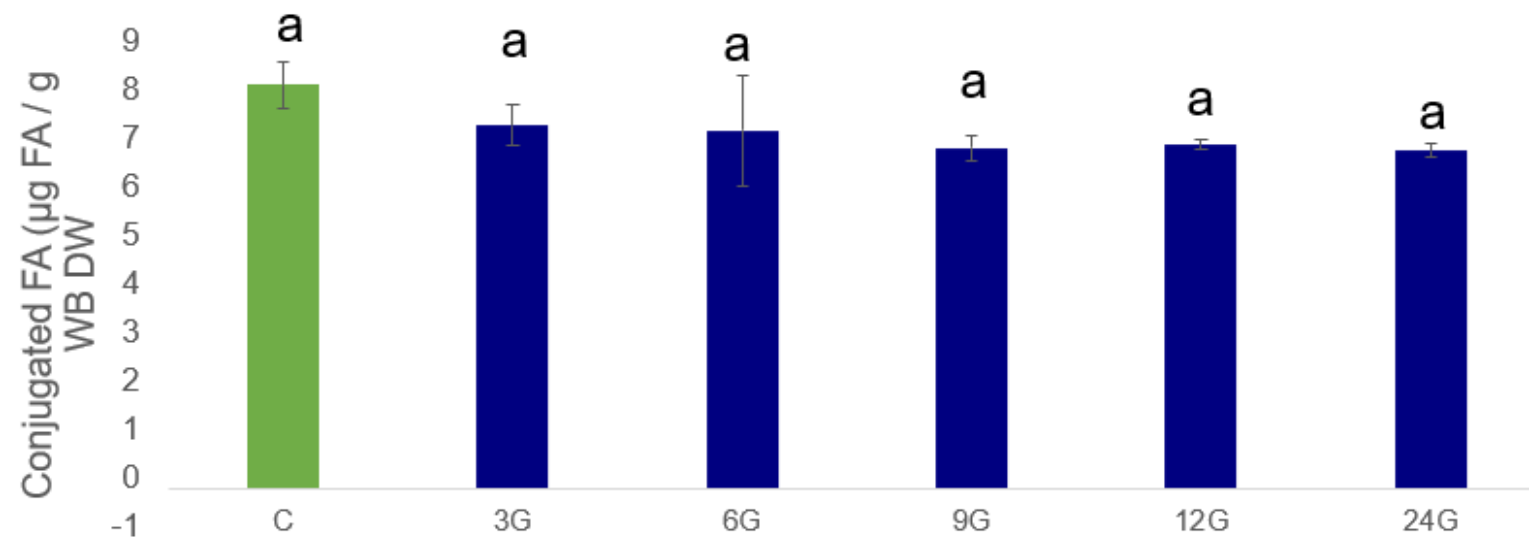
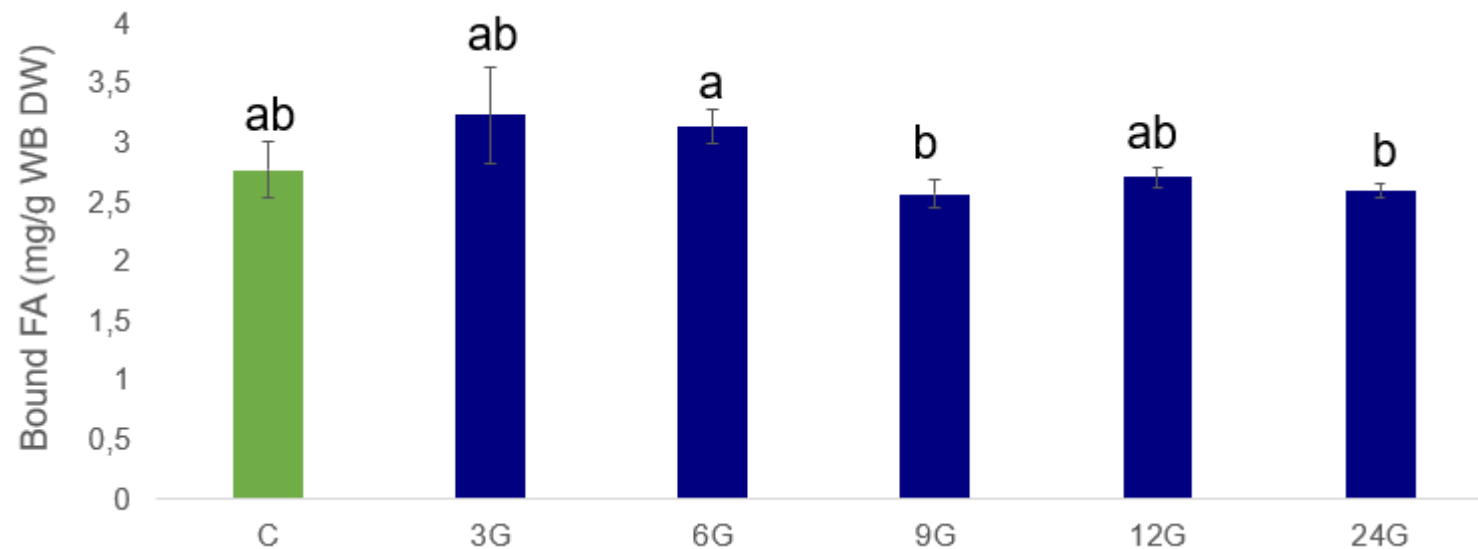
- Alkaline extractable FA

- ∇ Free FA

- = Bound FA

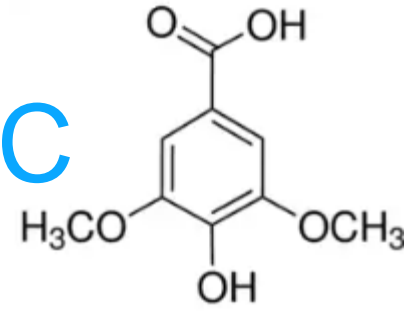
- = Conjugated FA

→ Oxidation

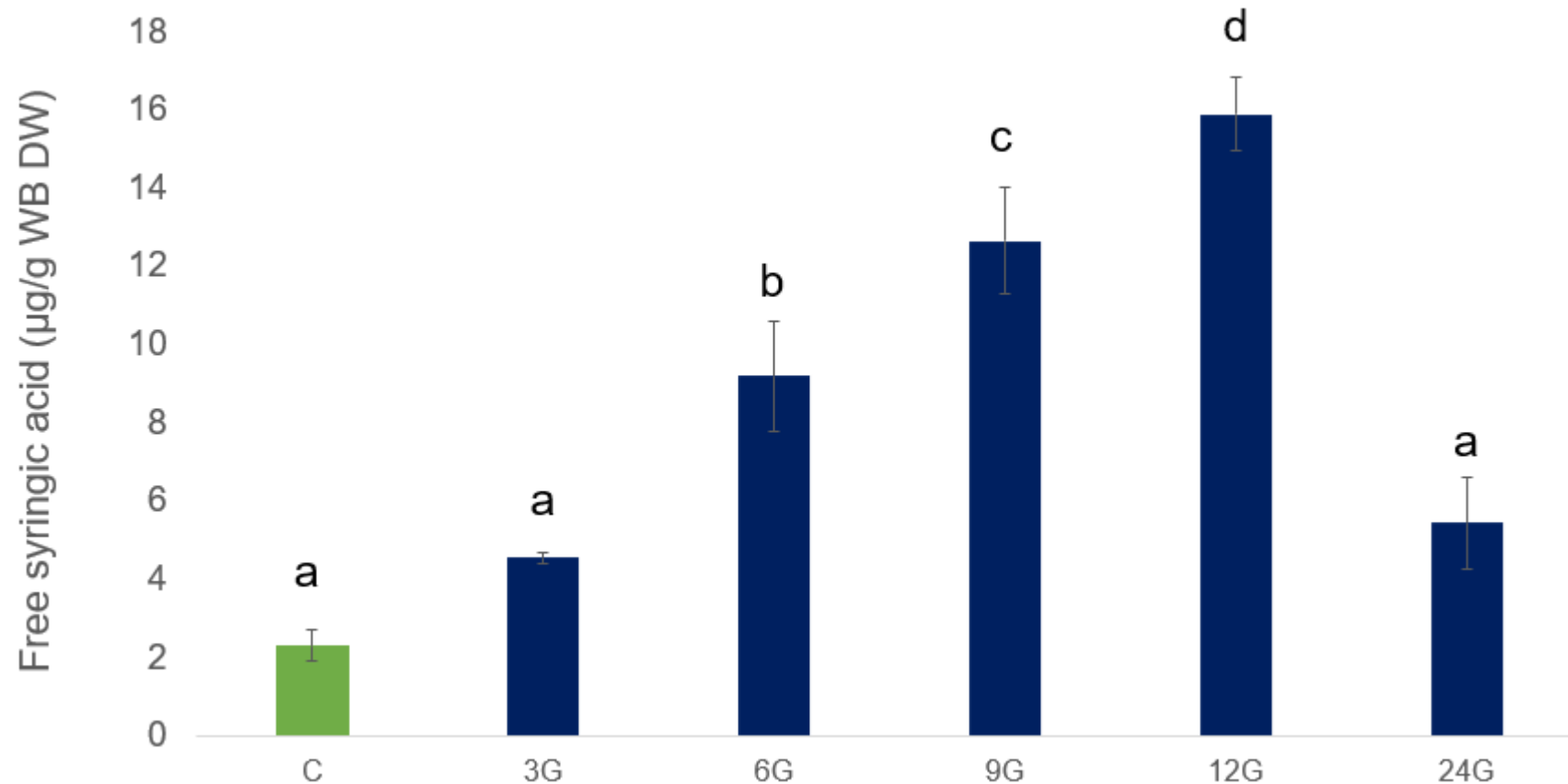




Phenolic acid – Syringic acid HPLC

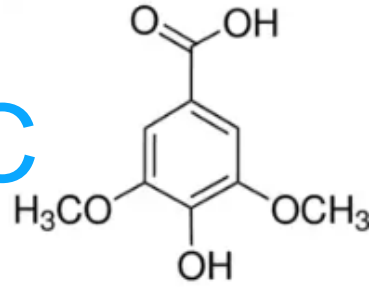


- Significant increasing of free Syringic acid (SA)

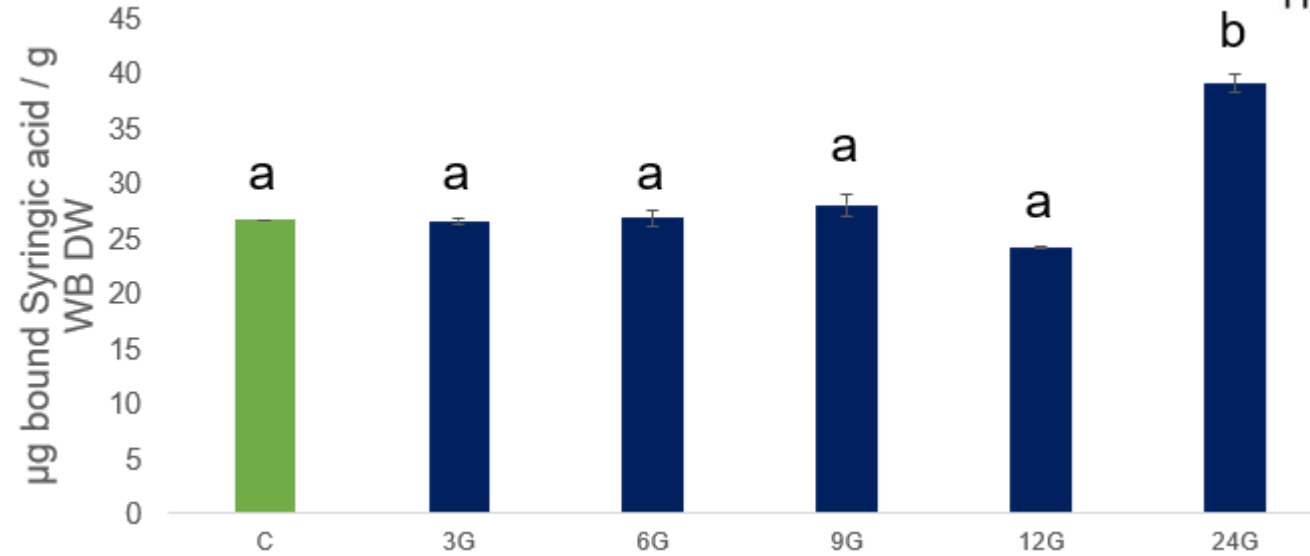




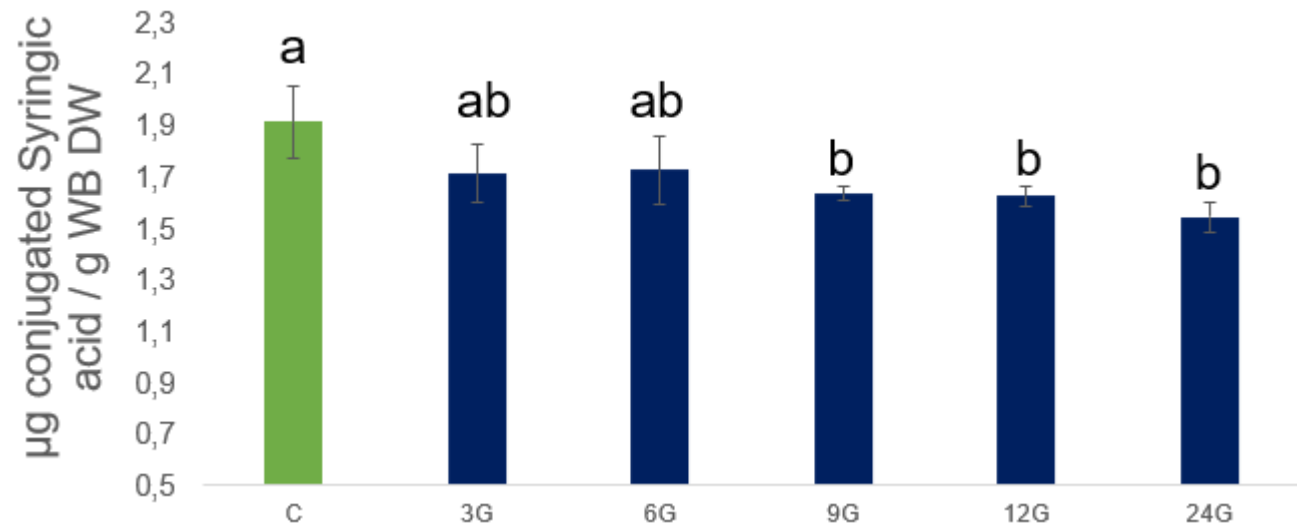
Phenolic acid – Syringic acid HPLC



- Stable bound SA



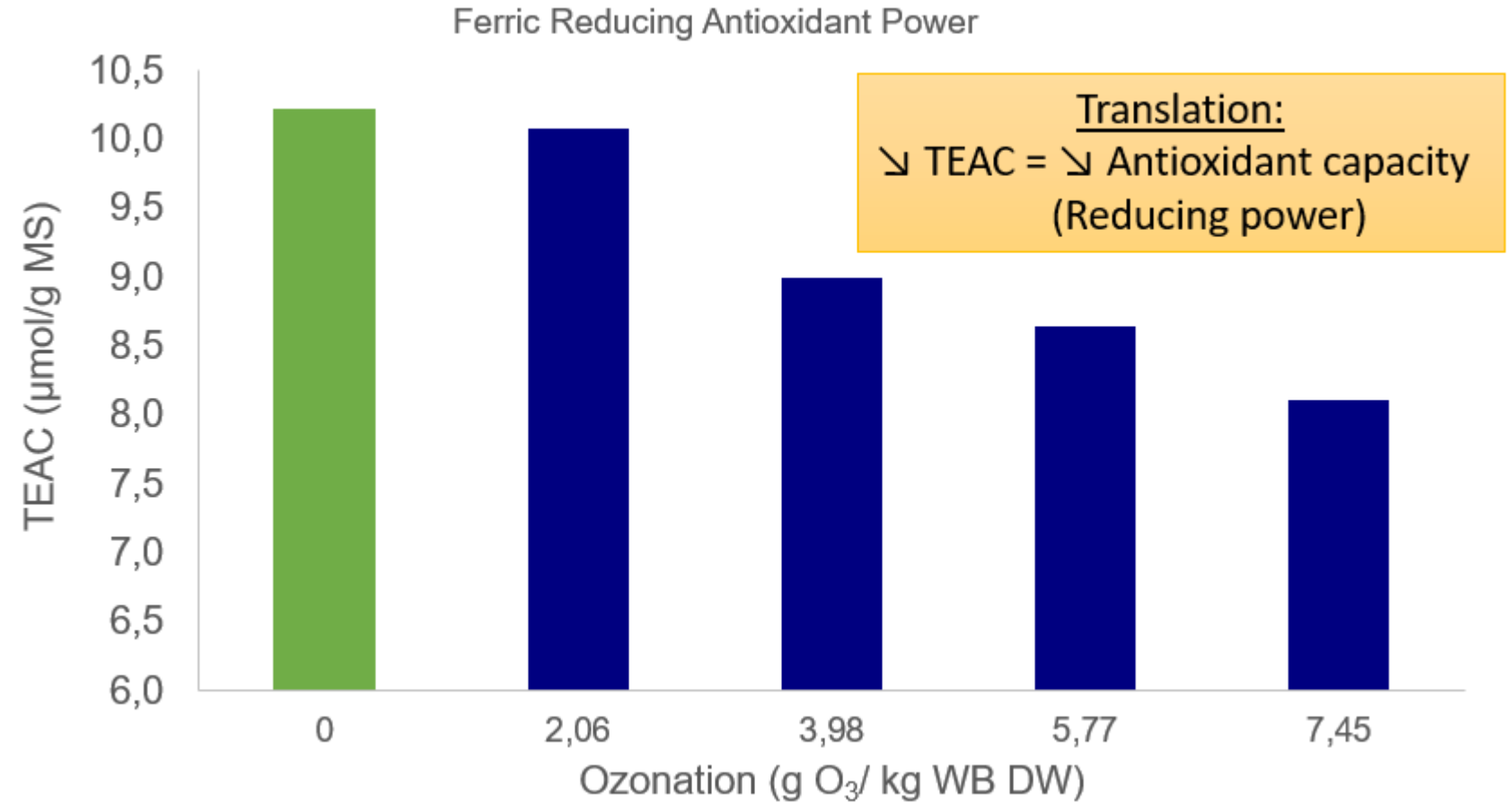
- Loss in conjugated SA





Antioxidant capacity - FRAP

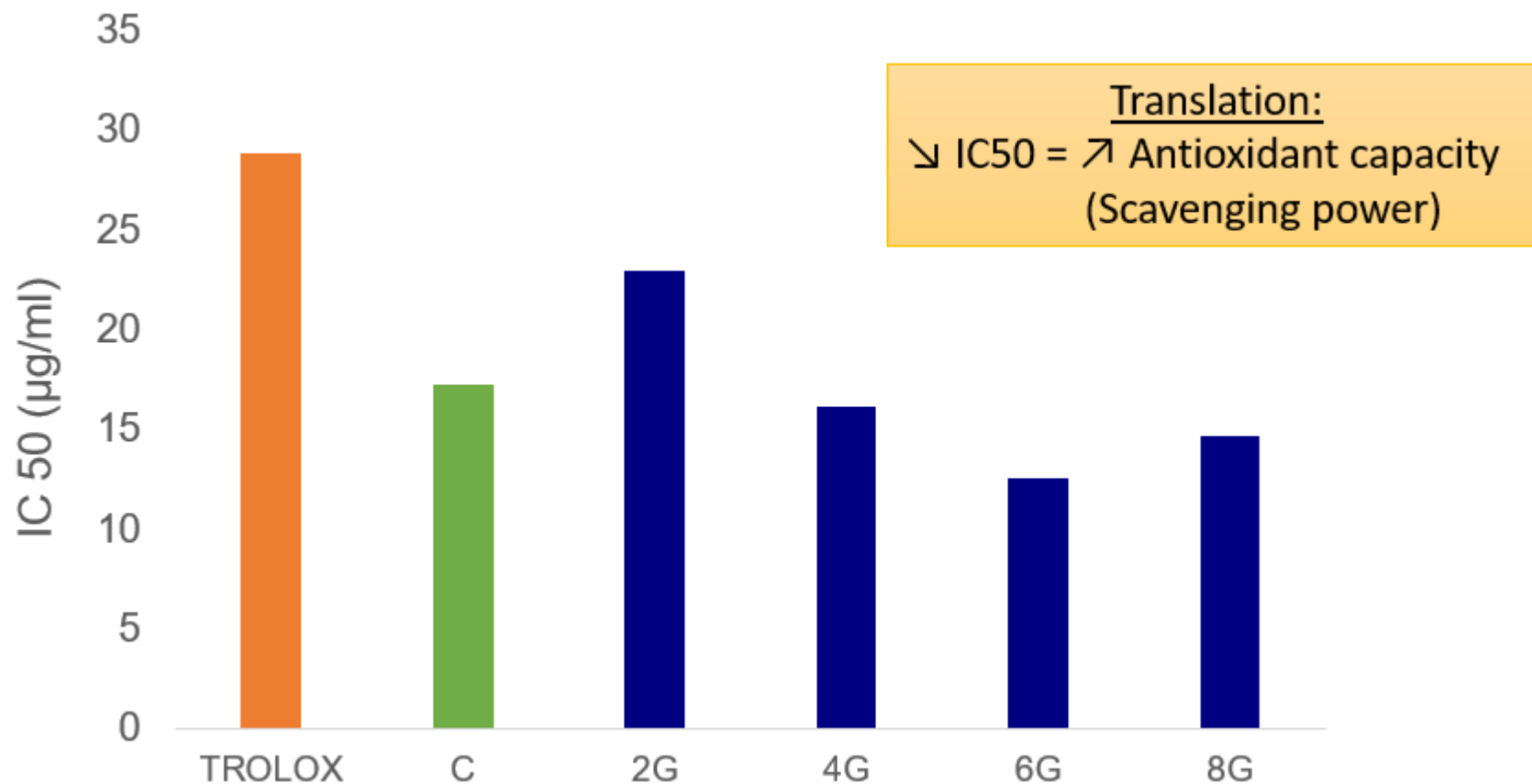
- Free polyphenols Ferric ion reducing antioxidant power
- Maximal reduction of antioxidant capacity **21%**
- TEAC: Trolox Equivalent Antioxidant Capacity





Antioxidant capacity - DPPH

- Free polyphenols antioxidant capacity
- Radical scavenging capacity by the sample
- Half Maximal Inhibitory Concentration (IC 50)





Bread rheological properties



Standard flour
Or
Qualista®
White flour

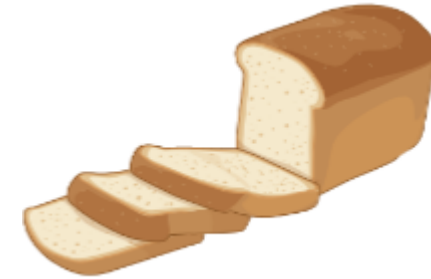
87%



Control WB
Or
Ozonated WB

13%

Bread-making
(BIPEA Method)



White and “High fibre”^{*} breads



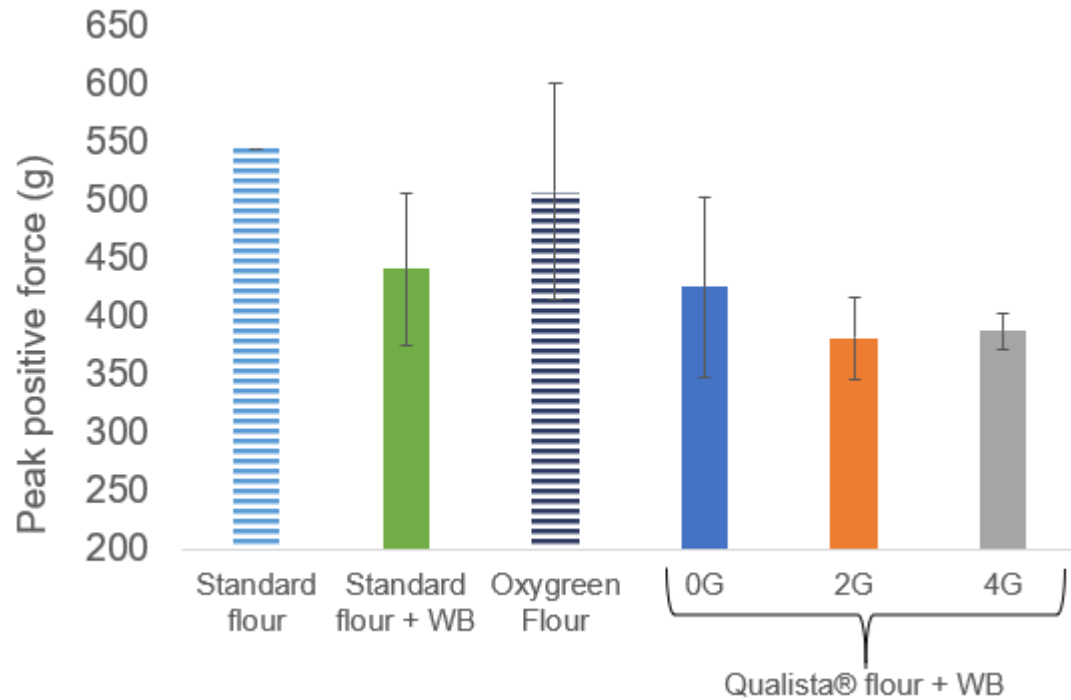
- Rheological characterization for 48h
- Elasticity, density, weight, volume, crumb Texture Profile Analysis (TPA) and crust hardness analysis.

^{*} “High fibre” Claim obtained for > 6% (w/w) fiber

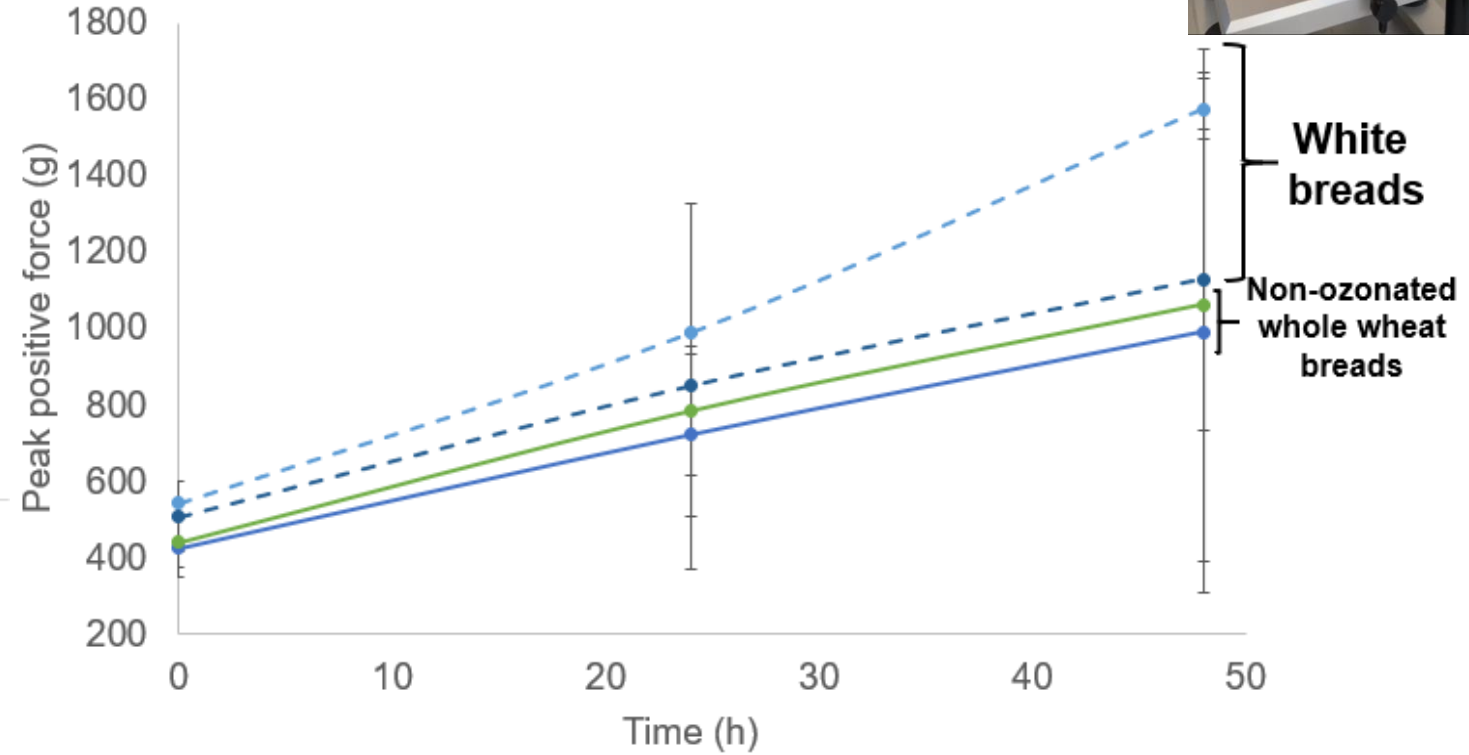


Crust hardness

Initial crust hardness



Evolution of crust hardness in 48 hours

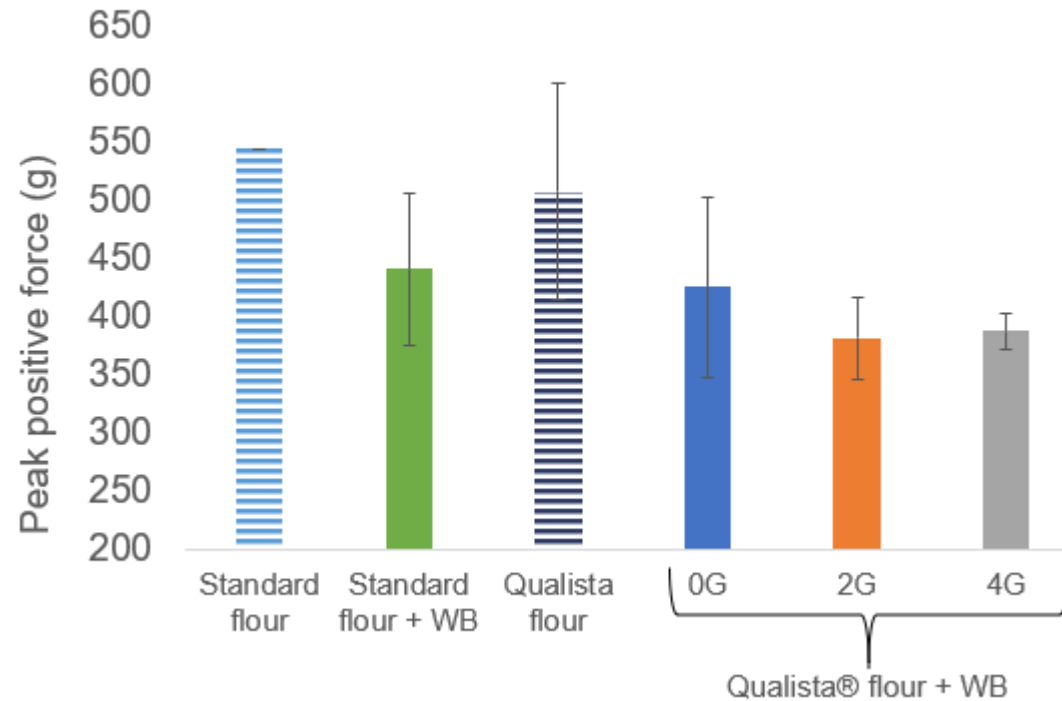


- Fiber incorporation reduces crust hardness
- Fiber ozonation reduces crust hardness and limits retrogradation (Better conservation of the rheological properties)

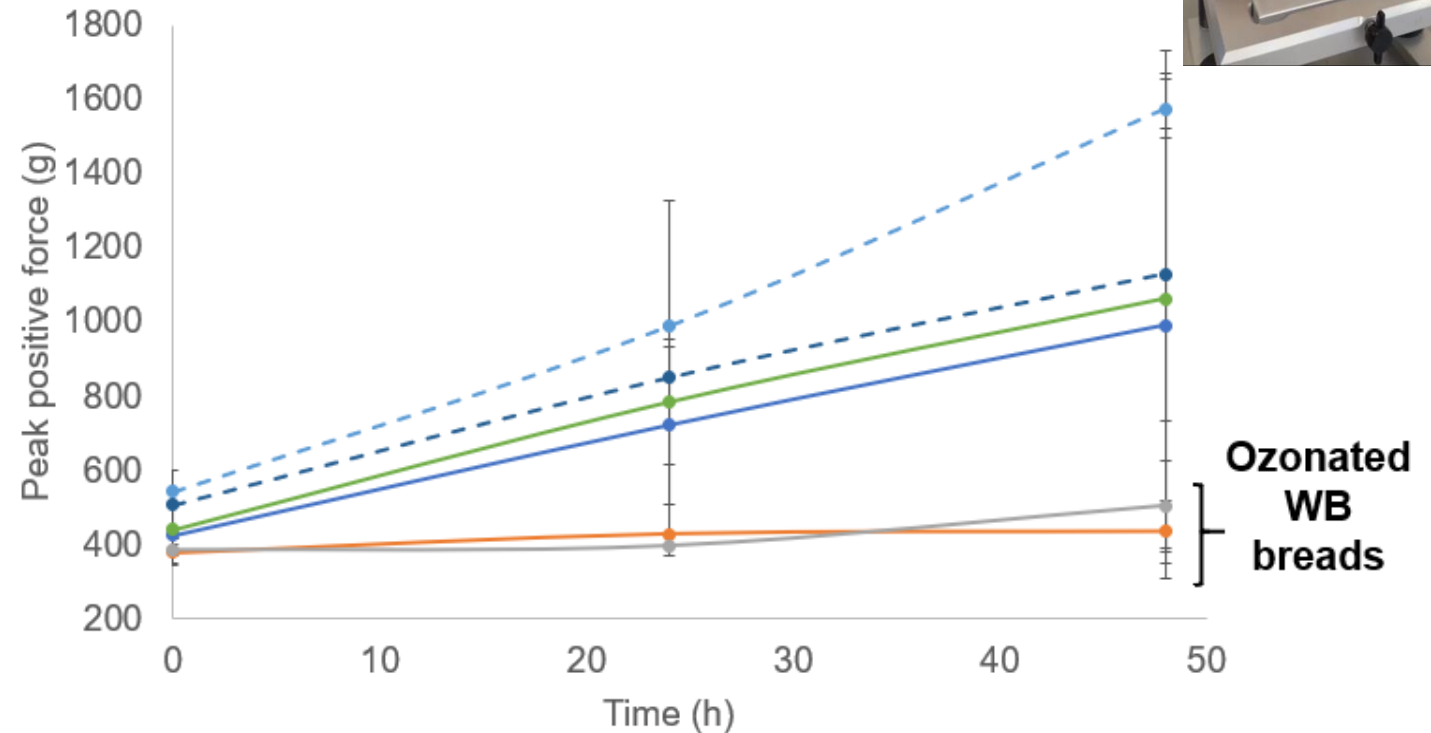


Crust hardness

Initial crust hardness



Evolution of crust hardness in 48 hours



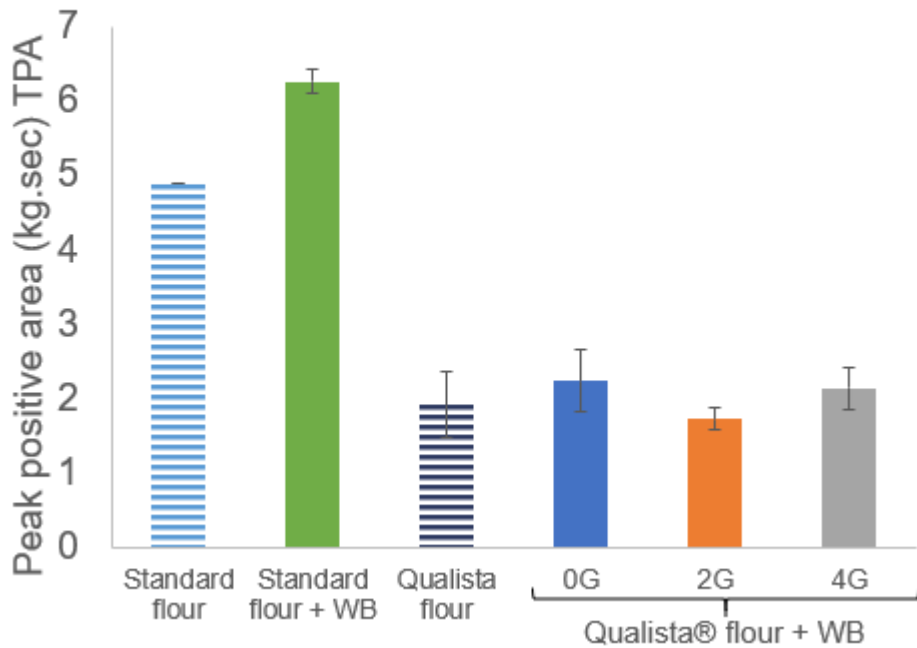
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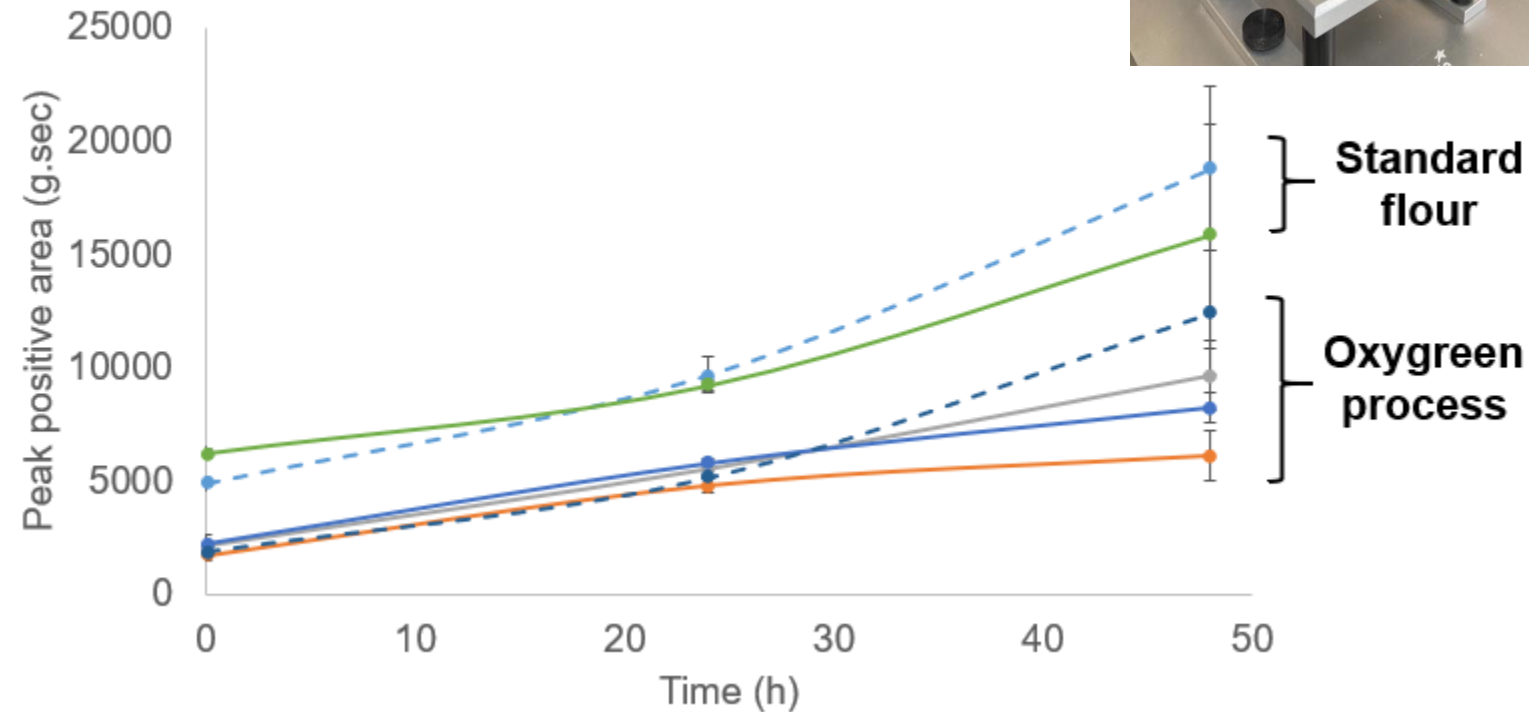
Crumb hardness



Initial crumb hardness -TPA



Evolution of crumb hardness - TPA



- Oxygen process on grain reduces crumb hardness and limits retrogradation
- WB ozonation do not present additional effect on crumb



Conclusion & Perspectives

Ozone treatment impacts functional and physico-chemical properties of wheat bran

Structure (Hypothesis)

- AX phenolic or glycosidic bounds cleavage
- B-glucans glycosidic bound cleavage: Viscosity?
- Polyphenols oxidation



Functional, physico-chemical and rheological properties (Observations)

- ↗ NSP solubility
- Modify antioxidant and binding properties
- ↗ Some Rheological parameters



Nutritional and Health properties

- Digestibility / Fermentation
- Glucose metabolism



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THANK YOU FOR YOUR ATTENTION

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Paulic Meunerie SA for providing wheat bran and flours