

## Highlighting of a protective effect of a *Fagopyrum esculentum* seeds extract against environmental aggressions

Aïna Queiroz<sup>1</sup>, Carine Quenel<sup>1</sup>, Francine Joly<sup>2</sup>, Elodie Bombard<sup>2</sup>, Alexia Forestier<sup>1</sup>

<sup>1</sup> Société ID bio, ESTER Technopole - 6 allée Skylab - 87068 LIMOGES Cedex - FRANCE

<sup>2</sup> SEPhRA, 87, rue Voltaire, 92800 Puteaux - France

### Keywords

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### Abstract

This is a fact that our skin is exposed every day to a growing stress resulting from air pollution, cigarette smoke or ultraviolet rays. Consequences on the skin are numerous: a production of pro-inflammatory molecules as well as a significant loss of skin homeostasis illustrated by the deterioration of the skin components such as epidermal proteins and lipids. The skin starts losing its natural barrier and looks tired and asphyxiated.

ID bio has decided to focus on the natural protective properties of buckwheat seeds, highly rich in vitamins, minerals and phenolic compounds<sup>1</sup>. The purpose was to design a natural substance able to fight against environmental aggressions while protecting the extracellular matrix architectural organization.

The aim of the study carried out is to demonstrate the protective effectiveness of this buckwheat extract against various effects due to pollution and UV exposure. The R&D Department had previously demonstrated antioxidant properties for this extract (ORAC and H<sub>2</sub>DCF-DA) as well as the action against lipoperoxidation (MDA assay).

New studies on skin explants also highlight effects linked to pollution or UV exposure on:

- Inflammatory pathway (measurement of IL-8 release)
- Tight junctions oxidation (claudins-1 and -4 immunostaining)
- Detoxification mechanism (glyoxalase I level).

This enzyme has not been studied in the cosmetic field until now and is particularly involved in the elimination of compounds such as ketoaldehydes, stemming from glycolysis or other pathways<sup>2</sup>

Thus, this extract developed from buckwheat provides a cellular shield on the epidermis while protecting against tissue damages linked to harmful environmental situations. For those reasons, this extract can be an interesting cosmetic ingredient preventing the effects of time on skin and protect sensitive or urban skins, frequently exposed to high levels of pollution.

### Introduction

#### **Air pollution and its effects on the skin**

Pollution is the discharging of noxious substances in soil, water, or atmosphere. Air pollution is the fact of introducing particulates, biological molecules, or other harmful materials into the Earth's atmosphere.

Continuous exposure to air pollutants contributes to weaken the skin by increasing signs of aging and cutaneous inflammation. Epidermal homeostasis is ensured by interactive system of cells, proteins and lipids, all crucial to maintain skin protective role against external aggression. By impacting the quality of the lipids and proteins like claudins, air pollutants can lead to skin disorders. Pollution also stimulates the production of pro-inflammatory molecules, including TNF-alpha, IL-6 and IL-8<sup>3</sup>.

### **A targeted pollutant in the atmosphere**

Even if pollution is a global phenomenon, from one city to another the “cocktail” of pollutants can be distinct in ambient air. In order to offer precise results, based on a pharmacological model, the team decided to focus on a unique worldwide pollutant in this study: benzo[a]pyrene or BaP.

It is the major tracer of urban pollution due to PAHs (Polycyclic Aromatic Hydrocarbons), highly carcinogenic and mutagenic PM (particulate matter)<sup>4</sup>. This molecule that belongs to the VOCs (Volatile Organic Compounds) is quantified during pollution peaks as it is highly representative of air quality. Tobacco cigarette produces 18 to 50 ng of BaP.

### **UV, oxidative stress and effects on the skin**

Free radicals are naturally generated by physiological mechanisms inside the cells: respiration at the mitochondrial level, detoxification at the peroxisomal level, defense reaction by the phagocytes, etc. In parallel, the action of external factors such as UV radiations and other ionizing rays, tobacco, pollution<sup>5</sup> and other chemical compounds also cause an increase of ROS amount and, by extension, potential imbalances.

Oxidants when uncontrolled can result in a degradation of proteins, lipids, DNA deterioration and also in a metabolic enzymes inactivation. Those changes can lead to the alteration of membrane structures that disintegrate cells, disturb cell communication and increase water loss. Cell renewal and enzymatic mechanisms can also be damaged and result in chain modifications whose major consequence is the speeding up of skin aging.

### **Buckwheat, source of "shield molecules" against environmental aggressions**

Buckwheat (*Fagopyrum esculentum*), also called ‘beech wheat’, is a pseudocereal that belongs to the Polygonaceae family. Native of Central Asia, this plant has been introduced to Europe during the medieval Crusades and offers a willowy stem that wears dark green heart-shaped leaves as well as white flowers gathered in clusters.

ID bio R&D department, specialized in botanical extraction, managed to extract active compounds like vitamins, minerals and phenolic molecules thanks to an innovative and green process. The active fraction of buckwheat extract has been identified thanks to a TLC bioautography guided isolation of antioxidant compounds (results presented at the IFSCC 2014 Congress).

## **Materials and methods**

### **Biological material**

The human skin explants used in this study (diameter of 8 mm) are provided by the company Biopredic International (Rennes) and come from mammary plastic surgery (Caucasian origin 43 year-old woman). The pollutant is benzo[a]pyrene or BaP (Sigma CRM40071).

### **Human skin explants model of pollution**

The skin explants are incubated in survival culture media and treated with formulations (0%, 1% or 3% of buckwheat extract). This formulations are topically applied as a pre-treatment during 24h, then the skin explants are polluted or not with BaP (Sigma CRM40071, 20µM

systemic) for another 24h, while formulations are applied again (topic). Then medium (or supernatants) is removed for IL-8 dosage and explants are preserved for analysis (CLDN1 and 4 immunostainings and histological control).

Each non-polluted condition is tested in triplicates (3 explants: 2 for immunostainings and 1 for histology), each polluted condition is tested in quadruplicates (4 explants: 3 for immunostainings and 1 for histology). Dosages in supernatants (removed for each condition, for the 3 non-polluted explants and the 4 polluted explants) are done in duplicates.

### IL-8 dosage

The aim of this test is to evaluate the effect of buckwheat extract on inflammation, formulated at two concentrations (1 and 3%), versus placebo, in a human skin explants model of pollution, in which explants are polluted or not with benzo(a)pyrene (or BaP). The proinflammatory cytokine IL-8 was measured in the culture supernatants thanks to an IL-8 ELISA Assay kit (R&D System Europe, Lille, France).

### Claudin-1 and Claudin-4 immunostainings

Expression of claudins -1 and -4, epidermis tight junctions proteins, are studied by immunostainings. Skin explants, polluted or not with benzo(a)pyrene (or BaP), treated or not with formulations (1% or 3% of buckwheat extract), are cut and fixed in acetone. Immunolabeling is performed using anti-claudin-1 and anti-claudin-4 (SantaCruz, USA).

## Results

### Protection of sensitive skins against the inflammatory process

IL-8 is an inflammatory cytokine released in case of harmful microbiological or chemical attacks inflicted to the skin. This autocrine mediator is particularly responsive to pollutants and is over-released if skin is attacked. At 1% and 3%, the buckwheat extract developed by ID bio enables to significantly reduce IL-8 release by -11,2 % and -26,2 % respectively compared to the placebo (figure 1).

% of IL-8 release /  
polluted placebo

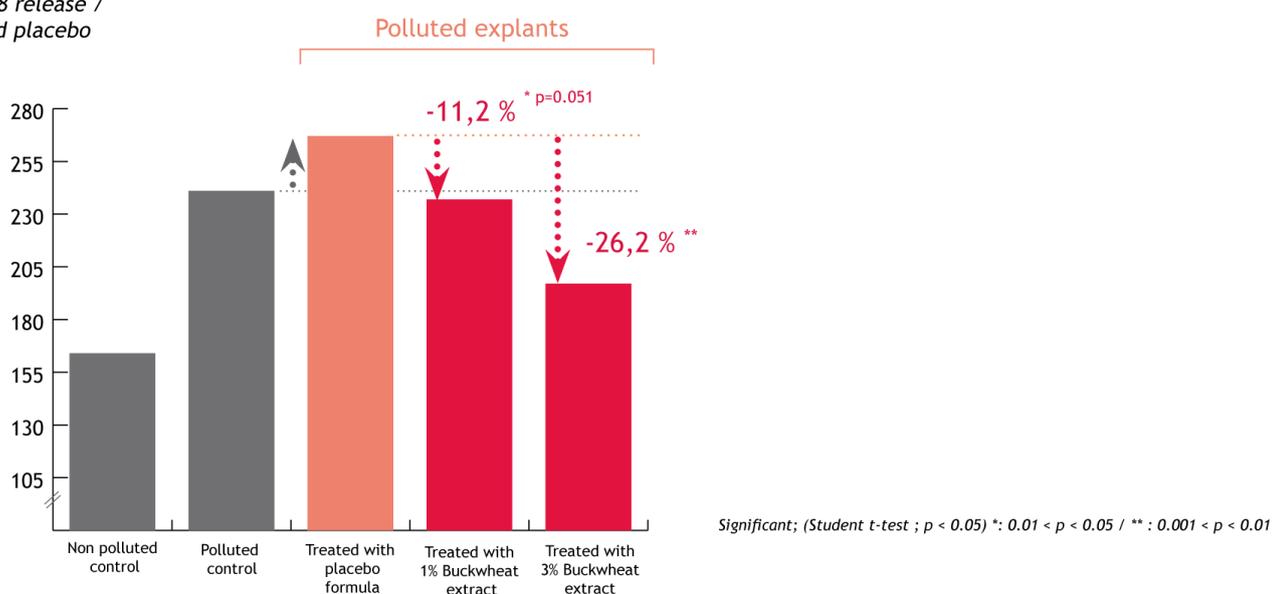


Figure 1: Inhibition of proinflammatory answer induced by BaP against placebo ex vivo (NHK)

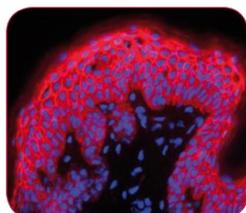
Cutaneous hyperreactivity and diffuse redness are reduced in case of aggressions in urban area. Discomfort feeling (tautness, tingling, heating up, etc) is soothed. Thanks to the beneficial and significant action on IL-8, skin is less sensible and sensitive to pollution.

## Preservation of premature aging

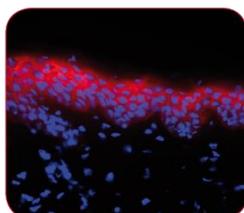
Claudins-1 and -4 are key proteins that actively participate to skin barrier protection by preventing small molecules entry and by keeping the epidermis integrity. In case of pollutants aggressions, homeostasis is disrupted and this barrier is damaged. Calcium flux is increased and skin architectural network is tumbled out which increases risks of premature aging.

In polluted condition, without the buckwheat extract, the network of tight junctions is modified, less structured and dense (slight fluorescence) whereas in presence of the active ingredient, the architectural network is re-established and tightened (high fluorescence) compared to polluted control (figure 2).

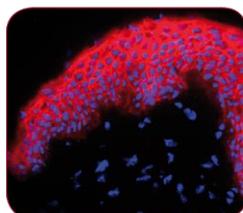
### > Claudin-1



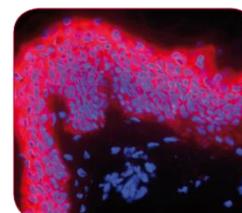
Non polluted explant



Polluted explant

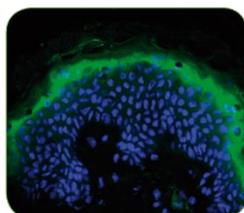


Polluted explant treated with  
1% Buckwheat extract

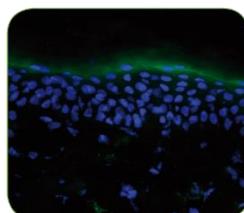


Polluted explant treated with  
3% Buckwheat extract

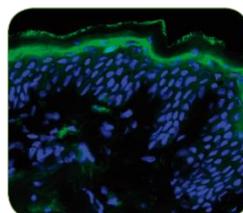
### > Claudin-4



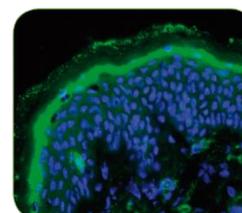
Non polluted explant



Polluted explant



Polluted explant treated with  
1% Buckwheat extract



Polluted explant treated with  
3% Buckwheat extract

Figure 2 : Protection of CLDN1 and 4 against BaP – ex vivo (immunostaining on NHK)

The product enables to increase cells cohesion of superficial epidermal layers and to reinforce tissue barrier function. Skin is more resistant to environmental attacks and premature aging signs are kept away.

## Discussion & Conclusion

These new results in polluted conditions can therefore be added to those obtained previously under UV conditions: protection against lipid peroxidation, oxidation of key proteins and the activation of a biological detoxification pathway (glyoxalase I).

The combination of all the results allows to make assumptions on the biological action of the buckwheat extract. Firstly, pollution leads to increased levels of interleukin 8 via the AhR pathway and therefore inflammation. The buckwheat extract thus would inhibit the inflammatory process through this way.

The AhR pathway is also interacting with the Nrf2 pathway that activates different detoxification enzymes such as GST, GPX, etc (Figure 3).

Pollution, as UV rays, will also generate an increase in the level of reactive oxygen species (ROS) which involves several consequences: damage to DNA, lipids and proteins of the skin. The Nrf2 pathway is thus activated, which increases the level of expression of cellular detoxification enzymes *via* the ARE (Figure 3).

Therefore, the buckwheat extract developed by ID bio has an effect on inflammation and premature aging of the skin through AhR and Nrf2 pathways: it could constitute an interesting solution to help fighting against environmental aggression and inflamm'aging, the upregulation of the inflammatory response that occurs with advancing age.

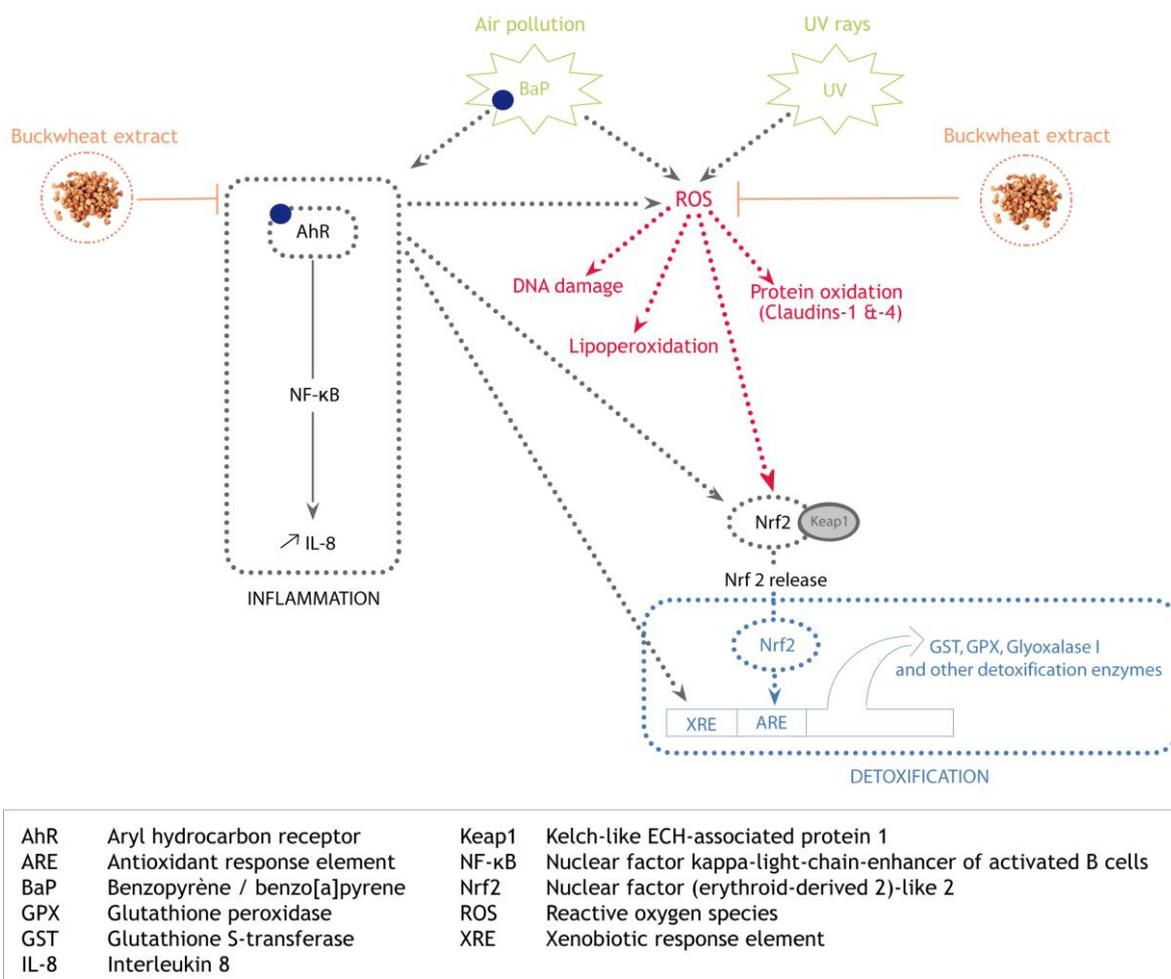


Figure 3: Potential action mechanisms of buckwheat extract via AhR and Nrf2 pathways

## References

- <sup>1</sup> Pomeranz Y.; **Buckwheat: structure, composition, and utilization.** *Crit Rev Food Sci Nutr.* 1983; 19(3):213-58.
- <sup>2</sup> Sousa Silva M, Gomes RA, Ferreira AE, Ponces Freire A, Cordeiro C.; **The glyoxalase pathway: the first hundred years... and beyond.** *Biochem J.* 2013; 453(1):1-15
- <sup>3</sup> Ushio H, Nohara K, Fujimaki H.; **Effect of environmental pollutants on the production of pro-inflammatory cytokines by normal human dermal keratinocytes.** *Toxicol Lett.*; 1999; 105(1):17-24.
- <sup>4</sup> Jakovljević I1, Zužul S.; **Polycyclic aromatic hydrocarbons in air.** *Arh Hig Rada Toksikol* 2011; 62(4):357-70
- <sup>5</sup> Møller P, Danielsen PH, Karottki DG, Jantzen K, Roursgaard M, Klingberg H, Jensen DM, Christophersen DV, Hemmingsen JG, Cao Y, Loft S.; **Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles.** *Mutat Res Rev.* 2014; 762:133-66.