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The *Brassica napus* (oilseed rape) seeds bioactive health effects are modulated by agronomical traits as assessed by a multi-scale omics approach in the metabolically impaired *ob*-mouse

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ABSTRACT

Beside oil, oilseed rape (*Brassica napus*) seeds contains nutritional bioactives such as polyphenols and glucosinolates. However, to date their nutritional properties have been overlooked in the new "double zero" breeds.

Seed alcoholic extracts from two *B. napus* cultivars most contrasting in their phytochemical contents as measured by mass-spectrometry were given to *ob*-mice. Biological outcomes including clinical metrics, gut and plasma metabolomes, liver transcriptome and metabolome were compared to *ob*-mice given a similar broccoli extract (*Brassica oleracea*).

One *B. napus* extract induced a reduction of the oxidative stress indicated by the decrease of plasma isoprostanoids. This was associated to the regulation of the antioxidant stress defense Nrf2 pathway, to 'omic' oxidative stress functions, metabolic and cell process regulations, and the metabolomics microbiota profile.

Extracts of *B. napus* seeds demonstrated health effects that may be improved by selecting appropriate agronomical traits, highlighting the potential benefits of better utilizing agronomy for improved human and animal nutrition

1. Introduction

Rapeseed (*Brassica napus*) is a crop that is cultivated mainly for human and animal consumption (e.g., edible oil and oilseed cake respectively), but also for non-edible industrial purposes, such as biodiesel, lubricant and, plastic.

Historically, *B. napus* seeds are known to contain high levels of antinutritional compounds such as erucic acid and glucosinolates, which were associated with toxic outcomes in farm and laboratory animals. Previous studies have shown that a high concentration of erucic acid in rapeseed oil induces cardiotoxicity (Hung, Umemura, Yamashiro, Slinger, & Holub, 1977). Glucosinolates are plant secondary metabolites which are hydrolyzed by the plant myrosinases activated by chewing or grinding, giving rise to biologically active by-products (isothiocyanates, thiocyanates, and nitriles). When present in high amounts, these active by-products can also induce multiple disorders in animals such as thyroid dysfunction and organ abnormality, reproduction, teratogenicity, growth impairment and a decreased intestinal protein intake (summarized in Scientific Panel on Contaminants in the Food Chain, 2008). Nevertheless, over recent years new breeds of *B. napus* containing trace amounts of erucic acid and up to a twelve-fold reduction of total glucosinolates have been agronomically selected. These resulting "double zero" breeds are now widely cultivated in Europe and worldwide. They are used not only for the production of high nutritious quality rapeseed oils, but also rapeseed meal as a source of protein for livestock, with both

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products containing low levels of antinutritional compounds. The importance of *B. napus* in the food industry has mainly involved primary metabolism such as its lipid or protein content. However, the nutritional value of *B. napus* could also be enhanced by considering the secondary metabolites such as phenolic compounds and paradoxically the previously cited glucosinolates (Chen, Thiyam-Hollander, Barthet, & Aachary, 2014). The seeds of *B. napus* contain large amounts of phenolics (Jiang et al., 2013) which have been associated with potential human health benefits (Bell & Wagstaff, 2017). The hydroxycinnamic acid derivatives represent the most abundant group among the phenolic compounds in *B. napus*, where sinapic, p-coumaric and ferulic acids, often found in conjunction with sugar or other hydroxycinnamic acids, are the most common (Cartea, Francisco, Soengas, & Velasco, 2011). While these compounds have been historically considered as antinutritional in animal nutrition since they can adversely influence feed palatability of rapeseed meal and protein digestibility in some circumstances (e.g. sinapic acid derivatives), they do possess potent antioxidant and anti-inflammatory properties both in vitro and *in vivo* (Vuorela, 2005; Boulghobra et al., 2020). Beside the numerous activities of sinapic acid and/or sinapine such as anti-inflammatory (Yun et al., 2008), hypoglycemic (Kanchana, Shyni, Rajadurai, & Periasamy, 2011), and cardioprotective activities (Roy & Mainzen Prince, 2013; Boulghobra et al., 2020), recent evidence also suggests that it could serve as valuable molecule for the treatment of obesity by promoting lipolysis via a PKA/p38-mediated pathway (Hossain et al., 2020). Previous studies have shown that ferulic acid could prevent the production of TNF-α, and decrease macrophage inflammatory protein-2 (MIP-2) levels in lipopolysaccharide (LPS)-stimulated RAW264.7 cells (Sakai, Ochiai, Nakajima, & Terasawa, 1997) by inhibiting the transcription nuclear factor kappa B (NF-kB) (Hole et al 2012), or by activating the anti-oxidative nuclear receptor Nrf2 signaling (Ma et al., 2011). Also, p-coumaric acid has been shown to increase the secretion and concentration of adiponectin, superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and glutathione S-transferase (GST) in TNF-α-treated 3T3-L1 adipocytes (Yen, Chen, Chang, & Hsu, 2011). In addition to phenolic compounds, the restrictive view of hydrolytic rapeseed products based on glucosinolates as antinutritionals can also be questioned (Sikorska-Zimny & Beneduce, 2020). For instance, the consumption of goitrogenic glucosinolates found in other Brassicaceae vegetables by humans does not give rise to harmful conditions (Bell & Wagstaff, 2017). Glucosinolates found in *B. napus* such as sinigrin and glucotropaeolin exert anti-inflammatory, antiatherosclerotic and antidiabetic actions (Sikorska-Zimny & Beneduce, 2020). Derivatives such as glucoraphanin found in broccoli and to a lesser extent in *B. napus* seeds elicited a wide range of beneficial health actions (Conzatti, Froes, Schweigert Perry, & Souza, 2014). In addition, several recent studies have also demonstrated the positive correlation between brassica glucosinolates content and health benefits such as *in vivo* antioxidative stress activity (Drażbo, Ognik, Zaworska, Ferenc, & Jankowski, 2018; Hannah, Subhendu, & Dipak, 2009) and those from oilseed rape provided anticancer activity (Barrett, Klopfenstein, & Leipold, 1998). Such new findings indicate the dual nature of glucosinolates and highlight that their antinutritional view may appear outdated regarding human nutrition (Björkman et al., 2011; Vuorela, 2005).

Considering these developments, it is worthwhile to revisit the *Brassica napus* health nutritional values of “double zero” breeds in both humans and animals.

We have previously established that the agro-ecosystems (including both the environment and the genetic background) can dramatically influence the content in *B. napus* bioactives (Bennouna et al., 2019), as described in other studies using other crops (Kusano et al., 2014; Röhlig, Eder, & Engel, 2009). Therefore, by comparing two contrasted *B. napus* extracts, we sought to determine if the agro-ecosystem variations could modulate health outcomes in a preclinical rodent model, as previously hypothetically suggested (Bennouna et al., 2019; Björkman et al., 2011).

The ob/ob mouse was used as this model features numerous metabolic deregulations followed by a pro-inflammatory and pro-oxidative stress outcomes (Sartori, Conti, Dias, Dos Santos, Machi, Palomino, Casarini, Rodrigues, De Angelis, & Irigoyen, 2017) that can be putatively targeted by *Brassicaceae* bioactives. We applied a non-reductionist multi-scale omic approach to investigate the biological responses to *B. napus* extracts intake.

2. Methods

2.1. Plant extracts

2.1.1. Brassica napus seeds screening

Two varieties of rapeseed were selected by screening 64 *B. napus* seeds samples using a metabolomics approach by LC-MS. The 64 samples corresponded to 8 different varieties of *B. napus* harvested in eight different regions of France by Terres-Inovia (Montpellier, France). All samples were planted during 2015 and harvested in 2016. Seeds were collected when fully ripe. The list of the samples is presented in the supplemental Table S1.

2.1.2. Brassica napus and broccoli extracts

A pilot plant extraction was carried out by the BIOGYS Center of SAS PIVERT (Venette, France) from 3 kg of rapeseed of each of the varieties chosen during the *B. napus* screening. The extraction protocol used at the bench scale (Bennouna et al., 2019) was adjusted for 3 kg of seeds for each variety. Because some of the materials used at the bench scale are not available at the pilot scale, some of the steps were modified as described in supplemental method file. The protocol used (grinding seeds in liquid nitrogen followed by a methanolic solution extraction) prevented myrosinase activation. A non myrosinase treated seed broccoli ethanol extract was supplied by FRUTAROM (Londerzeel, Belgium) and was added to the animal feed during the animal experimentation as a positive control. The information provided by the vendor, regarding the broccoli extract, are detailed in the supplemental method file.

2.2. Metabolomic LC-MS profiling of the plant extracts

The method is published in (Bennouna et al., 2019). The detailed procedure up to metabolites identification is reported in the supplemental method file.

2.3. Animal experimentation

2.3.1. Diet

All extracts were incorporated at rate of 0.1% in AIN-93G diet (1 mg extracts/1g diet) by Safe Diet (France). The 0.1% extract concentration has been estimated to be in accordance with levels in certified supplement products such as Broccoli-max® or Brocco-max® using FDA (Food and Drug Administration) recommendations to convert a human dose to a mouse dose expressed by mg/Kg body weight/-day. Translating the intake administrated to mice in the human equivalent dose, corresponds to 180 mg/kg/day, e.g. 1 g /day (Reagan-Shaw, Nihal, & Ahmad, 2008) which is within the physiological range.

2.3.2. Animal care

The care and use of mice were in accordance with the French guidelines and approved by the experimental animal ethic national committee (APAFIS#10618). Six weeks old *ob/ob* male mice were obtained from Jackson laboratory (L’Arbresle, France) and acclimatized in the experimental facility for 1 week. The mice were kept

in a temperature and humidity-controlled room and fed with water and food ad libitum. The mice were randomly divided into 4 groups matched for body weight, as follows: 2 groups made of 10 *ob*$^{-}$/*ob*$^{-}$ mice were used to test rapeseed extracts (Bonanza and Mambo variety). One group of 10 *ob*$^{-}$/*ob*$^{-}$mice was used to test broccoli extract as a reference group. One group of 10 *ob/ob* mice was used as an untreated control group. During the experiment, animal weight was monitored weekly and dietary intake was assessed daily. One week prior to sacrifice, the mice underwent an insulin tolerance test. At the end of the 6 weeks treatment period, mice were fasted overnight, and blood was collected by cardiac puncture under anesthesia (isoflurane inhalation). After sacrificing the animals by blood drawing, liver, muscle, epididymal, and perirenal adipose depots were collected then weighted and snap frozen in liquid nitrogen and stored at –80 °C until use.

2.3.3. Cytokines and chemokines quantification (adipose and liver tissue)

Tissue homogenates were prepared from 25 mg of epididymal adipose and liver tissue that were homogenized respectively in 250 ml and 1500 ml of PBS (137 mM Sodium Chloride, 10 mM phosphate, 2.7 mM Potassium Chloride; pH 7.4). The secretion of CCL2, CCL5, TNFα, IL-6 and IL-10 were quantified in the aqueous phase by ELISA, using Ready-SET-Go mouse kits (DuoSet ELISA, R&D systems, Les Ulis, France). To examine the involvement of NF-KB signaling pathway the levels of p65 (Ser) and I_KBα (Ser32/36) phosphorylation was quantified in liver tissue homogenates using the ELISA Instant One Kit according to the manufacturer's instructions (Thermo Scientific, Les Ulis, France).

2.3.4. Isoprostanoids quantification (plasma and adipose tissue)

The isoprostanoids quantification was performed on plasma and epididymal adipose tissue using the ABSciex QTrap 5500 system (SCIEX, Les Ulis, France). Twenty-six isoprostanes were quantified and are listed in the supplemental Table S2. The detailed procedure is shown in the supplemental material.

2.3.5. Transcriptomic (liver tissue)

Extraction of the mRNAs was performed by using the mirVana ™ kit (Thermo Scientific ™ mirVana ™ miRNA Isolation Kit, with phenol ref-AM1560) from 25 mg of liver tissue. The microarray transcriptome analysis of the samples was performed by the National Institute of Agricultural Research, Food and Environment (INRAE) platform of Jouy-en-Josas, France. The raw data obtained were processed by Agilent's GeneSpring GX software.

2.3.6. Metabolomics

Sample preparation is presented in the supplemental material.

2.3.6.1. LC-MS analysis. Dried polar extracts from each sample were first reconstituted with acetonitrile/water (50:50; v:v) with appropriate volumes for each tissue: the fecal/caecum samples, the plasma and the liver were reconstituted respectively with a volume of 150 µL, 75 µL and 200 µL. The samples were analyzed using a UPLC ultimate 3000 (Thermo Scientific), coupled to a high-resolution Q-Exactive Plus mass spectrometer equipped with an electrospray ionization source. The chromatographic separation was performed on a binary solvent system using a reverse phase C18 column (Hypersil Gold, Thermo Scientific, 100 mm × 2.1 mm, 1.9 µm) and a HILIC column (Merk, SeQuant® ZIC®-HILIC, 150 mm × 2.1 mm, 5 µm, 200 A). The method is detailed in the supplemental material, including data processing and metabolites identification.

2.3.6.2. Lipidomics (liver tissue). Liver samples were extracted, and the dried extracts were then solubilized in 120 µL of acetonitrile/water solution (1:1) and vortexed and centrifuged at 11,000 × g for 10 min at 4 °C. The samples were analyzed with the same LC-MS system as above. The detailed procedure including lipid extraction, data acquisition and lipid species identification can be found elsewhere (Aidoud et al., 2018) and in the supplemental material. A summary of the lipid species analyzed is displayed in the supplemental figure S1 and figure S2.

2.4. Statistical analysis

Partial least-square (PLS) analysis and 'multiblock' hierarchical PLS were performed with SIMCA P + 12 (Sartorius, Aubagne, France) as described (Martin et al., 2015). All PLS models (unit of variance transformed data) were validated using cross-validation ANOVA, permutation tests and cross-validation procedure. Transcripts significantly regulated by the dietary treatments were selected using the variable importance criteria (VIP) of a PLS-discriminant analysis (PLS-DA) performed on the control and experimental liver mouse samples. The VIP threshold value was selected from the deviation of the normal distribution of the VIP values using a normal probability plot (VIP value = 1.64). Of the 51,025 probes analyzed, 2148 representing 1642 unique genes were thus found significantly influenced by the various dietary treatment and then ascribed to biological pathways using Gene Set Enrichment Analysis (GSEA). In order to cover a wide range of meaningful biological pathways, GSEA was performed on GO, KEGG, REACTOME and BIOCARTA databases. The top 20 pathways with FDR *q*value < 0.01 were retained. Each pathway comprising the significantly regulated transcripts was scored by the hierarchical PLS SIMCA algorithm (Martin et al., 2015). Of the 1642 unique genes, 738 could be mapped into 90 different biological pathways. In order to ease interpretation, these pathways were pooled into 14 functional sets, according to the GSEA pathway definitions (supplemental Table S3 and figure S3). The corresponding gene transcript values were then used in hierarchical PLS-DA modelling, in which each functional set combining the gene transcripts can be translated into a workable composite score value for each mouse. The pathway blocks were "weighted" to take into account the number of transcripts per block. For plasma and liver metabolomics, a similar procedure was followed except that block setting did not rely on an enrichment algorithm. The spectral MS features were annotated by reference to an in-house metabolite database referencing over 800 analytes, using both the exact mass (<5 ppm) and retention time dimension in the chromatographic system to ensure identification accuracy. Each annotated metabolite was ascribed a biological role based on HMDB metabocard, PubChem description and KEGG pathways. Complementary information was found in PubMed publications whenever necessary. The annotated metabolites are reported in the supplemental Table S4 and Table S5. They were then grouped according to their functional role and analyzed using the same hierarchical PLS procedure described for the gene transcripts. For the caecal and fecal metabolites, the grouping method was different than for the plasma and liver. They mainly corresponded to microbiota metabolites, and in that instance, it is not possible to apply a metabolic role based on higher eukaryotic organisms to bacteria. To circumvent this, with used the ChemRich procedure that grouped the metabolites according to biochemical family proximity (Barupal & Fiehn, 2017) (supplemental Table S6 and Table S7). These biochemical clusters were then analyzed using the same hierarchical PLS procedure as described above. The lipidomic data were analyzed by sparse PLS, which was found superior to PLS-DA for this dataset (supplemental figure S2). Hierarchical cluster analysis with heatmap calculation, univariate ANOVA (untransformed data) and sparse PLS were performed using MetaboAnalyst, partial correlations with the R package GeneNet, network visualization using Cytoscape, and metabolite co-expression was achieved and visualized with the cytoscape plugin "MetScape".

3. Results

3.1. Brassica napus seeds selection and characterization-comparison to Brassica oleacera using LC-MS metabolomics data

From the 64 samples of *B. napus* analyzed, a total of 2442 features in both positive and negative mode were retained after the post-processing steps. Principal Component Analysis (PCA) explained 77.4% of the total variance across the samples in 14 principal components. The PCA results (supplemental figure S4) showed that the most opposed samples corresponded to ES-Mambo and Bonanza cultivated respectively in Indre and Ille-et-Vilaine regions. However, the secondary metabolite profile for both mambo and bonanza varieties were mostly driven by their genetic background (supplemental figure S5). The assay of the total polyphenols and glucosinolates showed that Bonanza was higher in these particular compounds (supplemental figure S6), but overall their content was within the range expected for double zero breeds. When re-analyzing the two selected *B. napus* varieties with the *Brassica oleracea* extract, a total of 4742 features in both positive and negative mode were retained after the post-processing steps. Overall, the results as plotted as a hierarchal cluster analysis and heatmap showed major differences in terms of primary and secondary metabolites occurring between the Broccoli and the two rapeseeds extracts (Fig. 1A) (supplemental figure S7A), although there were some pairwise overlaps. For instance, clusters 1 and 2 were similar between broccoli and mambo, clusters 3 and 4 between broccoli and bonanza, and clusters 5 and 6 between bonanza and mambo. Other clusters were more specific for either plant extracts. This heatmap obtained from the total features, was very similar to that calculated with the 84 identified compounds (Fig. 1B) (supplemental Table S8 and figure S7B). Thus, sinapoylhydroxyferuloylgentioside was higher in both broccoli and mambo whereas a hydroxycinnamic acid derivative at m/z 593.1762 was higher in both broccoli and bonanza compared to mambo. Conversely, progoitrin, 1-O-Sinapoyl-b-D-glucose, Gluconapoleiferin, p-coumaric acid, Feruloylcholinehexosides, Sinapine, Sinapoylgentiobiose, Glucobrassicanapin, Kaempferol-3-O-glucoside and 3-Methylpentyl glucosinolate were more specific to both bonanza and mambo. Overall, the results also show some specificities for each of the three extracts. Many hydroxycinnamic and sinapoyl derivatives were specific to the mambo extract, whereas bonanza was more represented by higher content in several other phenolics and glucosinolates (e.g. sinigrin, gluconapin), while the broccoli extract exhibited as expected a higher content for some glucosinolates such as glucoraphanin, glucoerucin and glucobrassicin.

3.2. Effect of the nutritional challenge on the ob-mice clinical outcomes

Sixty-five different clinical variables were measured in the challenged mice, including an insulin tolerance test, cytokine and isoprostane measurements, clinical biochemistry, and weight monitoring (supplemental Table S9 and figure S8). These clinical and biochemical measurements profiles are summarized in Fig. 2A.

Although the differences between the groups were not significant overall except for isoprostanoids (see below), it highlighted that both the broccoli and mambo diets induced closer effects compared to the bonanza and control diet for these clinical metrics. The same trend was observed with the 96 'omics' sets, (Fig. 2B) but with a highly significant effect ($P = 1.57*10^{-21}$ after PLS-DA cross-validation ANOVA).

3.3. Effect of treatments on isoprostanoids

Among all the clinical variables, only five plasma isoprostanoids were significantly decreased in the mice fed the mambo diet (Fig. 3). A partial correlation network revealed that these isoprostanoids were linked to the expression of other isoprostanoids in both plasma and adipose tissue, but also to the activation of pro-inflammatory/oxidative stress nuclear factor NFKB and to the IL6 and RANTES pro-inflammatory cytokines (Fig. 4). Although these above clinical parameters did not demonstrate a statistically significant difference across treatments, indicating a mild effect, they were correlated to the isoprostanoids that were significantly impacted

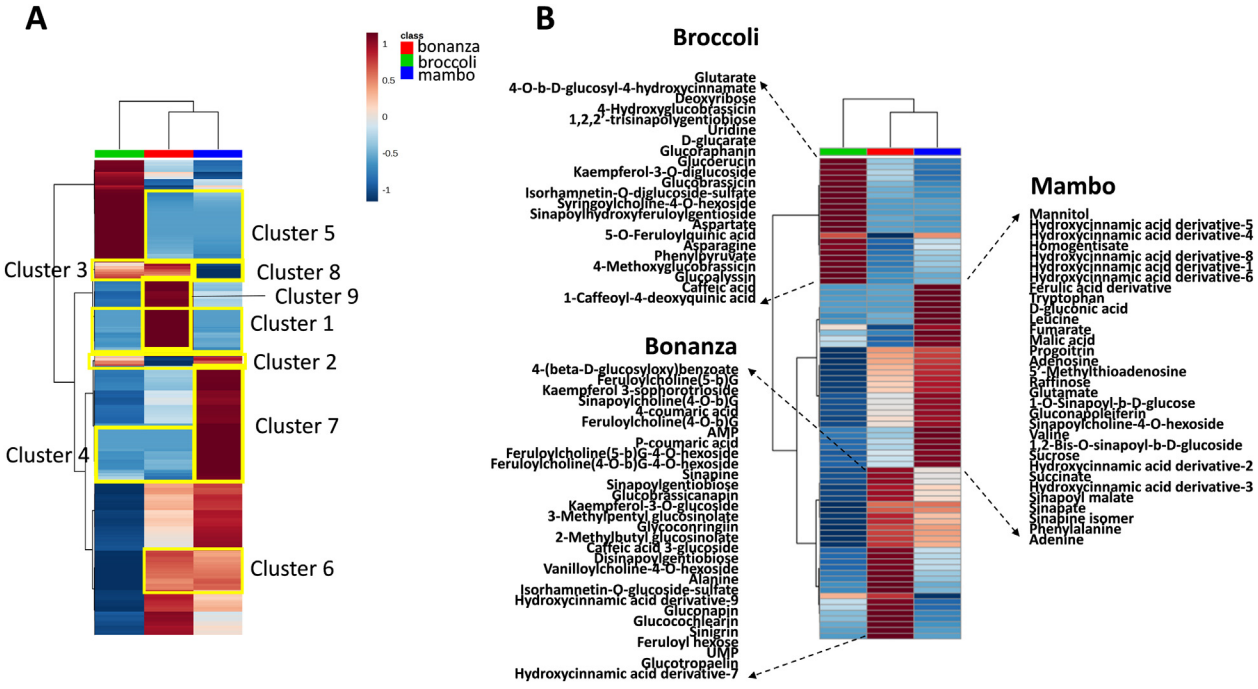

Fig. 1. Hierarchical clustering analysis and heatmap of A) the 4742 ions detected in the *Brassica* extracts, and **B**) of the 84 annotated metabolites (complete list in supplemental Table S8).

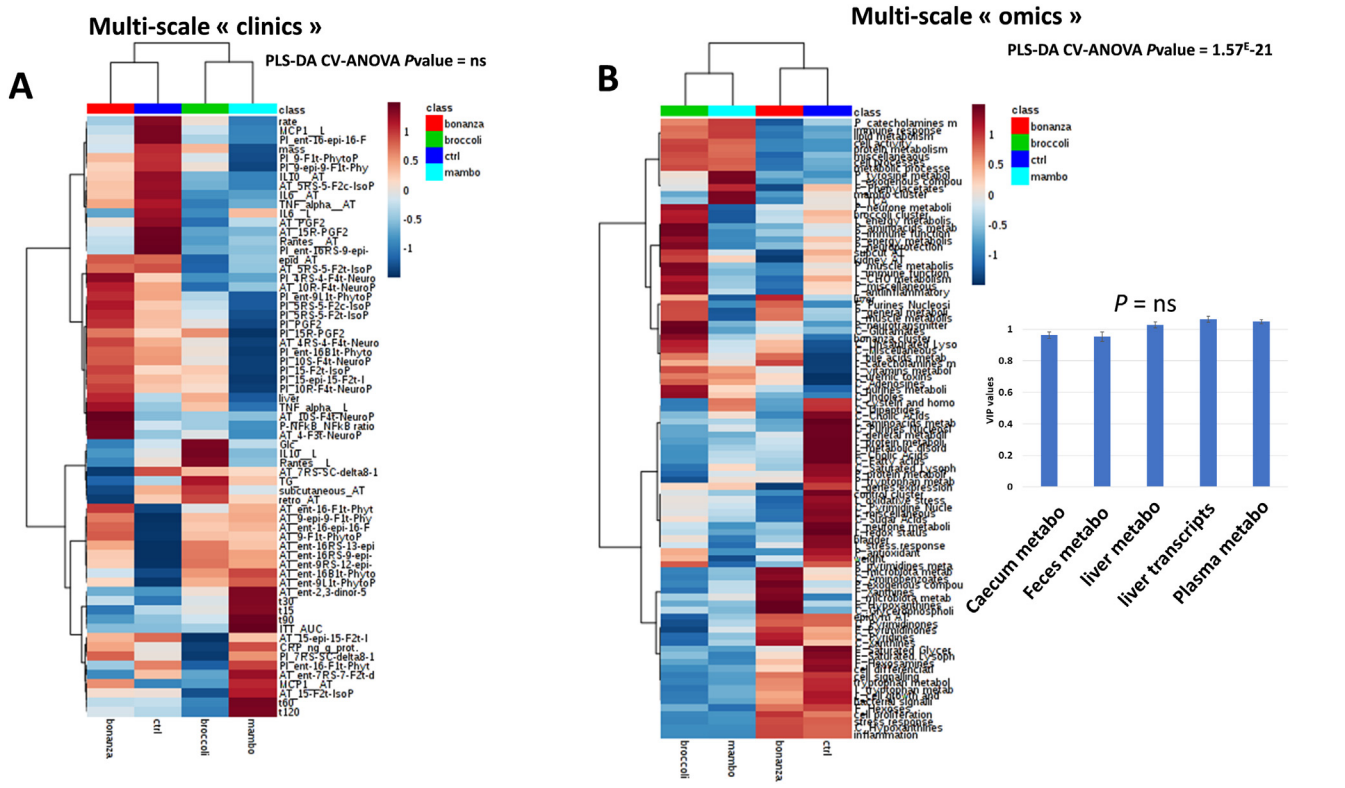

Fig. 2. Expression profiles of the clinical data (A) and of the integrated multiscale 'omics' data (B). measured in the mice fed the control, bonanza, mambo, and broccoli extracts diet (n = 10 per group). The differences in the profiles was determined by cross-validation ANOVA after PLS-DA analysis. The hierarchical analyses used the Pearson distance and the Ward method. The omics data are represented as functional or biochemical set scores calculated by hierarchical PLS-DA (see method and list in the supplemental tables 3 to 7). The mean values of the clinical data or 'omics' functional set scores are depicted as a heatmap, were in each row blue color represents a relative decrease, and red color a relative increase. The insert in B represents the impact of each datasets on the discriminant analysis, e.g., of the various diets on the biological response at each scale analyzed (VIP mean values ± SEM). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Relationship between isoprostanoids and the multi-omics response

Beyond clinical metrics, we also investigated how these plasma isoprostanoid oxidative stress markers were specifically associated to the 'omics' functional set in the mambo diet fed mice compared to the control diet fed mice. For this, the 5 differentially regulated isoprostanoids were combined into a score value using the hierarchical PLS algorithm and correlated to the the 96 'omics' functional sets depicted in Fig. 2B.

Twenty-five omics functions were individually associated to plasma isoprostanoids score (*q*value < 0.05, Fig. 5). These variables were linked to the liver transcriptome and the liver, plasma and gut metabolomes. They represented metabolic regulating functions, cell regulating and signaling functions, but also inflammation and oxidative stress related functions that can be logically associated to the plasma isoprostanoid oxidative stress marker. In that instance, about half of the genes related to the nrf2 antioxidant stress defense genes pathway (Ma, 2013) was found sensitive to our dietary treatments, with the *B. napus* "mambo" and *B. oleacera* "broccoli" sharing the closest profile of expression (Fig. 6). Tryptophan metabolism was also found to be associated to the plasma isoprostanoid oxidative stress marker across all measurements i.e., gut metabolism, transcripts and metabolomics levels (Fig. 5).

4. Discussion

In contrast to other brassicacea such as broccoli (Hannah et al., 2009), the health promoting properties of the polar fraction of *B. napus* has not been studied recently due to the longstanding assumption that it contained high levels of antinutritional compounds. Nevertheless, this assumption is outdated as advances through plant breeding has resulted in the emergence of the double zero seeds, and the discovery of potentially health promoting compounds for humans (Boulghobra et al., 2020; Chen et al., 2014). Therefore, there is a need to reevaluate the nutritional benefit of the polar phytochemical fraction of rapeseed for human health. The content of this polar fraction is highly influenced by agronomical conditions and genetic background (Bennouna et al., 2019), and these factors are important considerations when evaluating the nutritional benefits of these polar phytochemicals, highlighting these factors as an alternative tool for genetic engineering which could allow modulating their content (Björkman et al., 2011). Our present screening of the 8 rapeseed cultivars × 8 cultivation districts highlighted the influence of interactions between the genetic background and the environments on the production of metabolites in *B. napus* seeds (supplemental figure S7). Thus, we selected the 2 most contrasted LCMS *B. napus* metabolite profiles (supplemental figure S4) for nutritional benefit evaluation. Due to the lack of studies evaluating the health benefits of *B. napus* polar extracts, we decided to compare their potential health activity with a commercially available broccoli alcoholic extract belonging to the Brassicaceae family known for its health benefits (Hannah et al., 2009). This comparison was highly relevant as it allowed not only to determine the potential health benefits of *B. napus*, but also the possibility of promoting its polar extracts on a commercial scale as a dietary supplement.

The results obtained during our study, clearly showed that the *B. napus* and broccoli extracts were unlikely to adversely affect feed palatability or protein digestibility since weight gain was similar among all the ob mice including the control group where no plant extract was added.

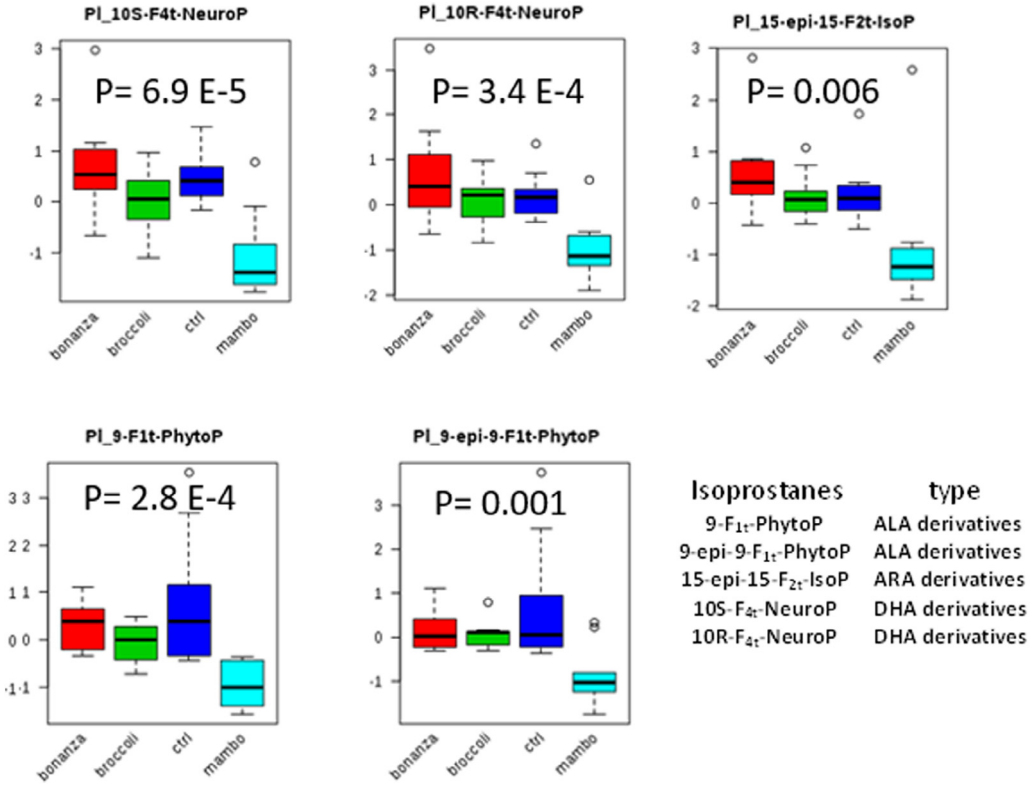

Fig. 3. Plasma (Pl) isoprostanoids significantly changed by the dietary challenges. The P values represent the difference between the mambo treatment with the others (ANOVA, post-hoc tuckey).

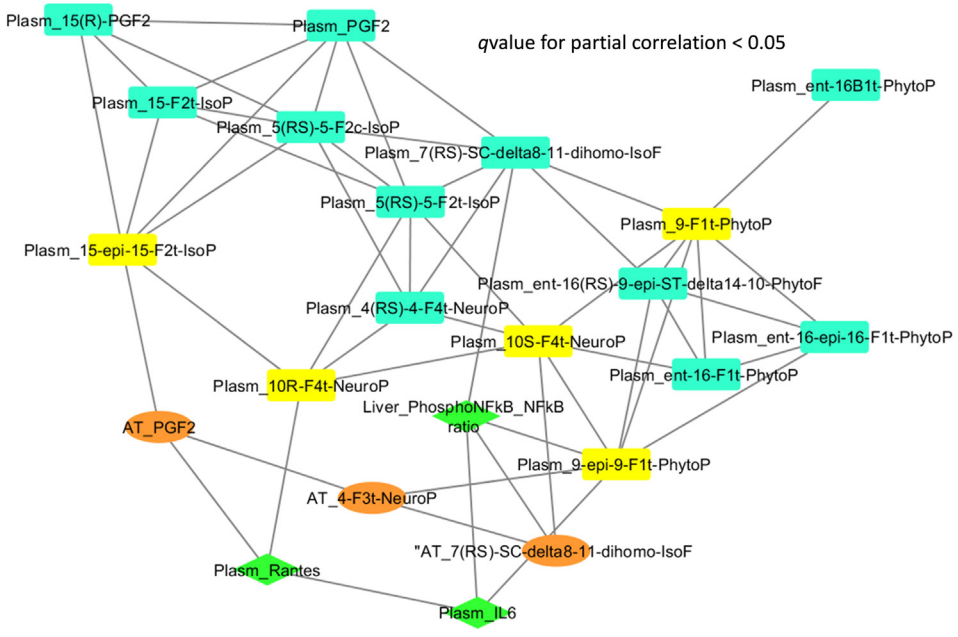

Fig. 4. Subnetwork of the first neighbors of the 5 plasma isoprostanoids decreased by the mambo diet (yellow). This subnetwork is extracted from a wider network including all the clinical parameters and calculated from pairwise partial correlations with a *q*value threshold of 0.05. Each edge represents a correlation (positive in continuous line, negative in dashed line). Plasm, plasma, AT, adipose tissue, PhosphoNFkB_NFkB ratio, ratio of phosphorylated over non-phosphorylated NFkB transcription factor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Due to the lack of studies measuring the health effects of *B. napus* seed extracts, we used an omics multiscale approach to gain a wide overview of the extent of the biological functions that can be specifically impacted by either diet. Results were subsequently combined at each systems biology level to facilitate the interpretation of the complete system. Multiblock PLS or hierarchical PLS enables aggregating

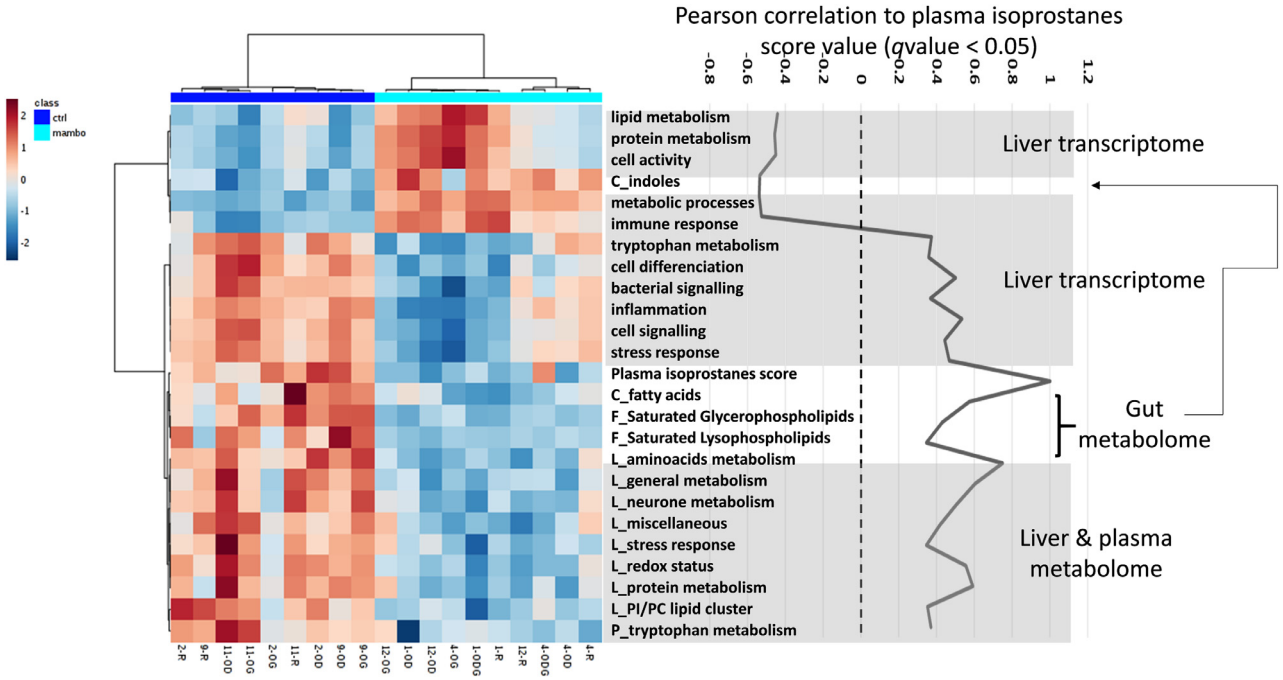

Fig. 5. Individual correlated 'omics' functional sets to the plasma isoprostanoids oxidative stress markers. The expression level of each set is displayed as a heatmap organized according to a hierarchical clustering analysis.

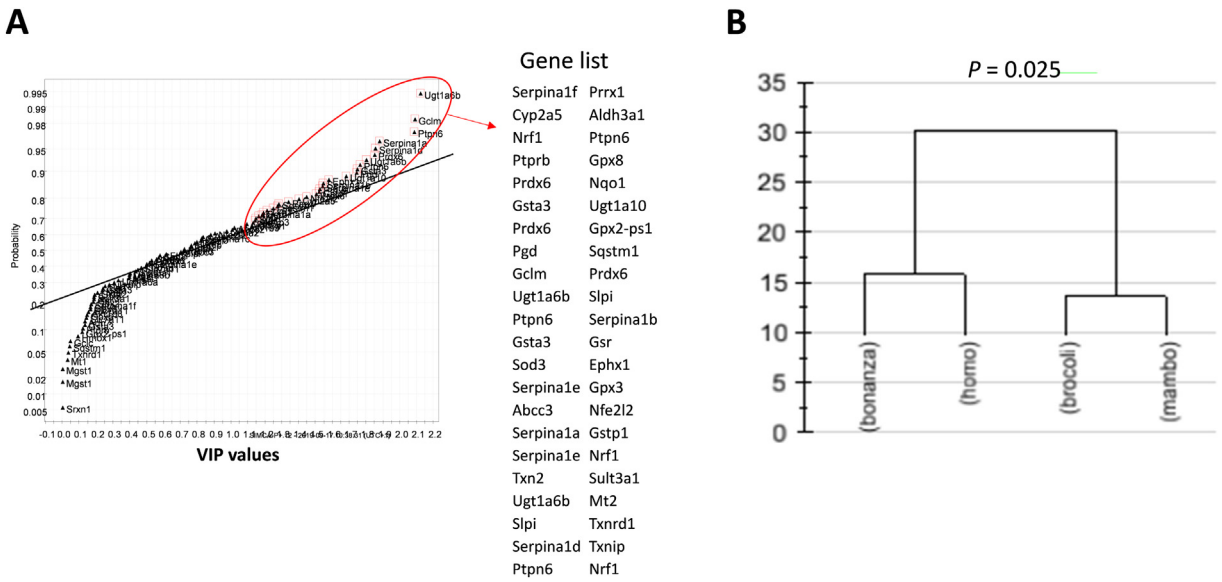

Fig. 6. Regulation of the the nrf2 genes expression across the dietary treatment. **A**, selection of differentially expressed gene probes among the 92 gene probes of the nrf2 pathway. A normal probability plot of each gene variable importance in projection (VIP) value, calculated by PLS-DA using dietary treatment as class, is calculated, and genes VIP deviating from normal distribution (red circle) are selected (VIP > 1.15). **B**, proximity of genes expression profile selected in A determined from hierarchical clustering (Ward method) of c(corr) scores obtained by PLS-DA loadings. The difference across the genes expression profile ($P = 0.025$, obtained by cross-validation ANOVA) is indicated. Dietary treatments are indicated, with "homo" corresponding to control rats.

heterogenous 'omic' data for easier data interpretation (Martin et al., 2015), by creating biologically relevant functional blocks (Martin et al., 2015). The multiblock analysis depicted in a hierarchical clustering output displayed the proximity of the biological response between the mambo and broccoli and/or the control and bonanza. Such proximities were also reflected to a lesser significant level at the clinical scale, indicating that the regulations observed at the molecular level did not translate with the same intensity as it appeared at the clinical phenotype level. Nevertheless, the *B. napus* mambo usually exerted more similar biological effects to that of broccoli extract than to bonanza, which was unexpected since their potential bioactive metabolite profiles suggested a potential similar biological response between the two *B. napus* varieties than that would obtain with the broccoli extract (figure S7). This observation suggests that at least part of these effects is related to common bioactive compounds that are present at similar levels in both *B. napus* mambo and broccoli extracts (Fig. 2). This could be sinapoylhydroxyferuloylgentioside and ferulic acid derivatives, but also other compounds that have not been

identified yet in cluster 2 of Fig. 1. Alternatively, the proximity in the effects between the broccoli and the *B. napus* mambo extracts could occur from the regulation of common mechanisms induced by different bioactives. For instance, in broccoli the most potent bioactive is sulforaphan, a glucosinolate derived from the parent glucoraphanin through microbial or plant myrosinase hydrolytic activity. Sulforaphan is a powerful Nrf2 pathway activator leading to oxidative stress protection (Ruhee & Suzuki, 2020) involved in several chronic diseases that are underlined by oxidative stress and inflammation. Glucoraphanin/-sulforaphan is not represented in *B. napus* seed extracts (Fig. 1). Conversely, the mambo extract contained higher levels of specific phenolic bioactives, such as hydroxycinnamic derivatives, ferulic acid and sinapic derivatives (Fig. 1B), but also other unknown compounds (cluster 7 depicted in Fig. 1). These phenolic compounds are antioxidants also with potent or modulating properties of the Nrf2 signaling for oxidative defense (Ansari, 2017; Lee et al., 2014; Ma, 2013). We found a subset (48%) of genes in the Nrf2 pathway that were modulated by the mambo and broccoli diets, potentially explaining part of the oxidative stress protection observed. Thus, at least part of the clinical and anti-oxidative proximity between the *B napus* mambo and broccoli (Fig. 3 and Fig. 6) could arise from the modulation of Nrf2 signaling, however resulting from different bioactive compounds. Our results also suggest that the specific balance of phenolics found in the mambo extract (plus unidentified) and not in the bonanza extract would exert a more powerful Nrf2-driven antioxidant effect reflected by lower isoprostanoid levels (Fig. 3) than the broccoli bioactives glucoraphanin/-sulforaphan and/or glucobrassicin under our conditions. However, this should be examined in more detail in *in vitro* mechanistic experiments or *in vivo* Nrf2$^{-/-}$ mouse model comparing the specific *B. napus* mambo fraction to the broccoli sulforaphan.

As mentioned earlier, the isoprostanoids quantification results as illustrated in Fig. 3 demonstrate that the mambo bioactive compounds outperformed those of broccoli in antioxidative defense. These isoprostanoids are formed from non-enzymatic reactions induced by reactive oxygen species from α-linolenic (9-F1t) and docosahexaenoic acids (10-F4t) precursors, and from arachidonic acid (15-F2t) (Galano et al., 2017). Due to their recent identification and analytical determination challenges, phytoprostanes (9-F1t) are poorly reported in the literature, contrary to 15-F2t and 10-F4t isoprostanes. Their co-detection with these latter strongly, suggests that they can be also valid marker of oxidative stress. This is also highlighted by the correlation with liver NFKB, a transcription factor sensitive to high oxidative stress, as well as the RANTES and IL6 cytokines.

To further elucidate which other regulations were attached to the antioxidant effect of the mambo extracts, we regressed all the 'omics' data to the oxidative isoprostanoids score value (Fig. 5). This revealed a subset of omics functions related to this marker, notably including liver metabolomic redox and stress responses, and liver transcripts linked to inflammation regulation, that further underlined the impact of the mambo seeds components as antioxidant cell regulators. Other functions were inversely linked to various metabolic regulations in the metabolically dysregulated control mice. Regulations related to cell functional activities were differentially affected along with the improvement of the isoprostanoid oxidative stress status and could be considered beneficial. Noteably the caecal and fecal glycerophospholipids and fatty acids that were also altered by the mambo seeds extract. Generally, such lipids are considered as building blocks for bacterial membranes synthesis (Parsons & Rock, 2013). The difference in membrane lipids between the mambo and control diet would suggest a regulatory role on the gut bacterial turn over and reshaping, which is consistent with the reported action of some polyphenols (Etxeberria et al., 2015). Also, interestingly liver transcripts attached to bacterial signaling were also differentially expressed, suggesting a distinct regulation of the gut-liver axis by the mambo treatment. Tryptophan metabolism was affected in the gut, where the indoles measured mainly occurred from tryptophan and not from glucosinolates (supplemental Table S6 and Table S7), and its metabolism was also highlighted at the liver transcript and plasma metabolome levels. The metabolism of tryptophan plays an important beneficial role in the gut barrier integrity and function (Roager & Licht, 2018), but also in the host, including a role in obesity linked metabolic disorders (Kałużna-Czaplińska, Gątarek, Chirumbolo, Chartrand, & Bjørklund, 2017). Tryptophan metabolites such as those we identified (indoxyl sulfate, tryptamine, kynurenine, xanthurenic acid) can bind and activate the AhR receptor in the intestine and liver, inducing a wide biological response (Hubbard, Murray, & Perdew, 2015), including regulation of inflammation and immune response, cell division and proliferation. This is relevant to our findings associated to tryptophan metabolism in the *ob*-mice, spanning to the regulation of various metabolic processes, cell regulations and cell signaling, as well as microbiota metabolism (dominated by lipid metabolism) and the regulation of inflammatory and stress response processes. Thus, the mambo extract could thus bring other health benefits than the antioxidant stress defense through the modulation of tryptophan metabolism.

While we used brassica myrosinase inactivated extracts in this study, it did not preclude that the hydrolytic bioactive glucosinolate by-products could not be released during digestion. It has previously been observed that glucosinolates can be resistant to digestive enzymes, but susceptible to the myrosinase-like activities of cecal microbiota, and thus explain their appear in plasma (Lai, Miller, & Jeffery, 2010). But one cannot exclude a better biological efficiency especially for the broccoli glucoraphanin-rich extract when initially formed from a release of plant myrosinase and this deserves further consideration in future.

5. Conclusion

We reevaluated the health potential of oilseed rape phytochemicals using a multi-omics approach and compared it to a reference *Brassicacea* (broccoli). This study demonstrated that *B. napus* seeds contained interesting health bioactives, and that the range of plant seeds secondary metabolites level modified by the cultivation conditions or by the genetic background, can greatly mitigate their nutritional health properties. Among the most significant health properties observed to change in this study was the reduction of plasma oxidative stress markers. Omics revealed dietary impacts on subclinical phenotypes comprising more related oxidative stress responses and inflammation, but also extended to metabolic regulations and processes, cell processes and signaling, and gut microbiota lipid and amino-acids metabolic regulations including tryptophan metabolism. We showed that *B. napus* mambo extract (myrosinase free) exerted overall a similar biological response to that of a broccoli (myrosinase free) reference product, with a more pronounced effect involving oxidative stress defense. These findings suggest a potential reassessment of the use of *B. napus* polar extracts for health purposes. Possible applications in the nutraceutical industry but also in animal nutrition could be considered with products occurring from well-controlled upstream agronomical conditions allowing for a constant and desirable quality product.

Finally, the correlation between plant metabolite profile differences and their induced biological effects at different omics-scales provided evidence for a list of bioactive candidates found in these extracts, such as hydroxycinnamic, ferulic and sinapic derivatives, which could be directly associated with the observed biological effects. This should be confirmed using pure compounds.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2021.100011.

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