Food & Function

Bioaccessibility and bioavailability of phenolic compounds in bread: a review

Journal:	*Food & Function*
Manuscript ID	FO-REV-04-2017-000574
Article Type:	Review Article
Date Submitted by the Author:	14-Apr-2017
Complete List of Authors:	Angelino, Donato; University of Parma, Human Nutrition Unit, Department of Food and Drug; University of Parma, The Laboratory of Phytochemicals in Physiology, Department of Food and Drug Cossu, Marta; University of Parma, Human Nutrition Unit, Department of Food and Drug Marti, Alessandra; University of Milan, Department of Food, Environmental and Nutritional Sciences Zanoletti, Miriam; University of Milan, Department of Food, Environmental and Nutritional Sciences Chiavaroli, Laura; University of Parma, Human Nutrition Unit, Department of Food and Drug; University of Toronto, Department of Nutritional Sciences Brighenti, Furio; University of Parma, Human Nutrition Unit, Department of Food and Drug Del Rio, Daniele; University of Parma, Human Nutrition Unit, Department of Food and Drug; University of Parma, The Laboratory of Phytochemicals in Physiology, Department of Food and Drug; The Need for Nutrition Education/Innovation Programme (NNEdPro), Global Centre for Nutrition and Health, St John's Innovation Centre Martini, Daniela; University of Parma, Human Nutrition Unit, Department of Food and Drug; The Laboratory of Phytochemicals in Physiology, Department of Food and Drug, University of Parma

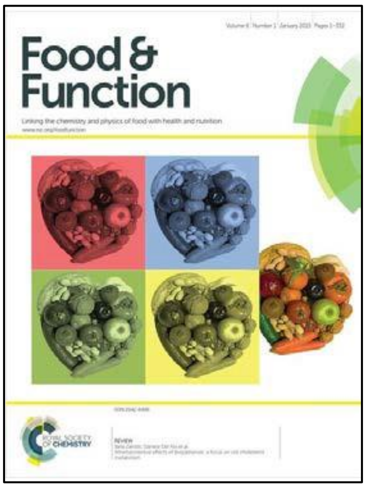
Food & Function

2015 Impact Factor: 2.676

www.rsc.org/foodfunction

REVIEW SUBMISSION

Linking the chemistry & physics of food with health & nutrition



The following article has been submitted to *Food & Function* for consideration as a **Review**.

Food & Function provides a unique venue for physicists, chemists, biochemists, nutritionists and other food scientists to publish work at the interface of the chemistry, physics and biology of food. The journal focuses on food and the functions of food in relation to health; this includes the following:

- Physical properties and structure of food
- Chemistry of food components
- Biochemical and physiological actions
- Nutritional aspects of food

Articles relating purely to food analysis will not be published in *Food & Function* - these can be published in our sister journal, *Analytical Methods*.

***Food & Function* Reviews should bring the reader up to date with research in a particular field, highlighting areas of special excitement and progress. The article should aim to provide an authoritative in-depth discussion of current progress and problems, and should not consist of a laborious account of every paper in the area. Neither should the author concern themselves with providing a comprehensive list of references; those of particular interest and significance are all that are required.**

Thank you for your effort in reviewing this submission. It is only through the continued service of referees that we can maintain both the high quality of the publication and the rapid response times to authors. We would greatly appreciate if you could review this paper in **14 days**. Please let us know if that will not be possible.

Once again, we appreciate your time in serving as a reviewer. To acknowledge this, the Royal Society of Chemistry offers a **25% discount** on our books: http://pubs.rsc.org/bookshop. Please also consider submitting your next manuscript to *Food & Function*.

Best wishes,

Philippa Hughes	Professor Kevin Croft
Executive Editor	Editor-in-Chief

UNIVERSITÀ DI PARMA

DIPARTIMENTO DI SCIENZE DEGLI ALIMENTI E DEL FARMACO

To Prof. Laura Bravo-Clemente,
Institute of Food Science, Technology and Nutrition,
Madrid, Spain

Parma, April 12th 2017

Dear Editor,

following the invitation by the Editorial Office of Food & Function, we are submitting a review manuscript entitled "Bioaccessibility and bioavailability of phenolic compounds in bread: a review" by Angelino and colleagues.

The aim of this review is to provide an overview of the literature related to the bioaccessibility and bioavailability of phenolic compounds in bread. We tried to focus mainly on the potential strategies to improve phenolic bioaccessibility and bioavailability and to the main findings of in vitro and in vivo studies investigating these strategies applied to breads.

We confirm that the paper is not under submission to other journals and that all the authors read and approved the final manuscript.

We hope that you will consider our work for publication in Food & Function.

Regards,

Daniela Martini, PhD

UNIVERSITÀ DI PARMA

DIPARTIMENTO DI SCIENZE DEGLI ALIMENTI E DEL FARMACO

Human Nutrition Unit
Department of Food and Drug, University of Parma
Medical School – Building C, Via Volturno, 39 - 43125 PARMA, Italy
Phone: +39 0521 903913-3841 / Fax: +39 0521 903832
Skype: daniela.martini82

Bioaccessibility and bioavailability of phenolic compounds in bread: a review

Donato Angelino[a,b], Marta Cossu[a], Alessandra Marti[c], Miriam Zanoletti[c], Laura Chiavaroli[a,d], Furio Brighenti[a], Daniele Del Rio[a,b,e], Daniela Martini[a,b,*].

[a] Human Nutrition Unit, Department of Food and Drug, University of Parma, Parma, Italy;

[b] The Laboratory of Phytochemicals in Physiology, Department of Food and Drug, University of Parma, Parma, Italy;

[c] Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy;

[d] Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada;

[e] The Need for Nutrition Education/Innovation Programme (NNEdPro), Global Centre for Nutrition and Health, St John's Innovation Centre, Cambridge, UK.

* Correspondence: daniela.martini@unipr.it; Tel.: +39-0521-903913.

ABSTRACT

Cereal-based products, like breads, are a vehicle for bioactive compounds, including polyphenols. The health effects of polyphenols like phenolic acids (PAs) are dependent on their bioaccessibility and bioavailability. The present review summarizes the current understanding of potential strategies to improve phenolic bioaccessibility and bioavailability and the main findings of *in vitro* and *in vivo* studies investigating these strategies applied to breads, including the use of raw ingredients with greater phenolic content and different pre-processing technologies, such as fermentation and enzymatic treatment of ingredients. There is considerable variability between *in vitro* studies mainly resulting from the use of different methodologies, highlighting the need for standardization. Of the few *in vivo* bioavailability studies identified, acute, single-dose studies demonstrate that modifications to selected raw materials and bioprocessing of bran could increase the bioavailability, but not necessarily net content, of bread phenolics. The two medium term identified dietary interventions also demonstrated greater phenolic content resulting from modification of raw materials used. Overall, findings suggest that several strategies can be used to develop new bread products with greater phenolic bioaccessibility and bioavailability. However, due to the large variability and the few studies available, further investigations are required to better determine the usefulness of these innovative processes.

KEYWORDS: bread, bioaccessibility, bioavailability, phenolic compounds.

INTRODUCTION

Cereal-based products are the most common staple foods globally. Among the wide range of products, bread is one of the most consumed. The estimated bread consumption has been reported to be over 100 g per day (equivalent to approximately 3 slices per day) in many countries[1–3], therefore bread is an important contributor to daily energy intake[4].

Bread products differ widely in shape, size, texture, and sensory characteristics. Part of these differences are ascribable to the type of cereal used for bread-making, which can include rye, barley, oat, and wheat, the latter of which is the most commonly used due to its gluten content, which contributes to good sensory characteristics. Differences can also result from the addition of ingredients, such as seeds, olives and nuts, as well as differences in the bread-making process, such as temperature and the use of yeast versus sourdough. Regardless of these differences, bread is generally characterized by a high carbohydrate and protein content, but it is also a rich source of vitamins (mainly from the B-vitamin group) and minerals (such as iron, calcium, phosphorus, zinc, potassium, and magnesium).

Many bread products are also a good source of bioactive compounds, including fibre and other phytochemicals, specifically those made with wholegrains that consist of the intact, ground, cracked or flaked kernel after the removal of inedible parts such as the hull and husk. Only in wholegrain products are the principal anatomical components, including the starchy endosperm, germ and bran, present in the same relative proportions as in the intact kernel[5]. In the outer layers of the kernels, where the bran is found, there is a high content of bioactive compounds[6].

The consumption of whole grains has been associated with the prevention of chronic diseases, including cardiovascular disease and diabetes[7,8]. Therefore, clinical practice guidelines and dietary guidelines recommend choosing wholegrain products[9–12], which are rich in bioactive compounds, over refined products, in which bioactive compounds are present only in small amounts due to the removal of the seed external layers during milling.

Wholegrain bread products are rich in fibre, particularly insoluble fibre, for which bran represents one of the main sources. Fibre from bread products mainly includes arabinoxylans, a hemicellulose found in plant cell walls and that represent the major component of dietary fibre in cereal grains. The wheat grain also contains aleurone as a monolayer of cells overlying the endosperm, which is rich in fibre and phenolic compounds. Furthermore, breads can be also rich in soluble fibres, like those made with oat and barley as good sources of β-glucans, which are well known to reduce post-prandial blood glucose and blood cholesterol[13–15], risk factors in the development of coronary heart disease (CHD)[16].

Similarly to other cereal-based products, wholemeal bread is generally a good source of phenolic compounds, mainly as esters bound to arabinoxylans[17], with a minor contribution of soluble free or conjugated compounds[18]. Polyphenols exist as secondary metabolites in several different plants, in which they can act as a defence mechanism against parasites and toxic compounds[19,20]. Phenolic compounds are widely diffused in all plant foods including fruits, vegetables and beverages (tea and coffee), the consumption of which may lead to a phenolic intake of ~1000 mg per day, in a typical American diet[21]. Bread products contribute to this daily phenolic intake, especially when they include bran.

Cereal grains constitute a good source of phenolic acids (PAs), in addition to alkylresorcinols and lignans. PAs can be divided in two groups, hydroxycinnamic and hydroxybenzoic acids, deriving from the hydroxylation of the cinnamic or benzoic acid moiety. Hydroxycinnamic acids are the most abundant PAs and chiefly consist of ferulic acid (FA), *p*-coumaric acid (CA), caffeic acid, and sinapic acid (SA). Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic acids.

Polyphenols are not included in the category of micronutrients, as they are not essential for the maintenance of vital functions. However, several studies indicate that phenolic compounds might be responsible for part of the beneficial effects associated with the consumption of plant-based foods, such as the association between fruit and vegetable intake and reduced CVD risk [22]. In particular, *in vitro* studies have demonstrated the involvement of polyphenols and their metabolites in several features linked to prevention of inflammation, oxidative stress and many other recognised pathophysiological processes [23–25]. Furthermore, over the past 20 years, epidemiological studies have demonstrated that the consumption of polyphenol-rich foods, such as fruits, vegetables, cereals, coffee and cocoa, is inversely associated with the risk of many chronic diseases. The first epidemiological study focusing on the protective role of polyphenols on CHD found a 42% reduction in relative risk of CHD mortality when comparing the highest tertile of flavonoid intake to the lowest[26]. Several other epidemiological studies followed, including the Iowa Women's Health

Study (n= 41,836), in which polyphenol intake was inversely associated with inflammation[27], and specifically, whole grain polyphenol intake was inversely associated with the incidence of colorectal cancer[28].

Evidence from human intervention trials on the protective effects of phenol-rich foods against many chronic diseases has been inconsistent, possibly because of differences in food composition, as well as differences in the absorption and metabolism of various phenolic compounds. One of the main issues contributing to the inconsistency in results concerns studies attributing the effects to a single compound or a class of foods, because a single compound can be present in several different foods, and a class of foods can contain mixtures of polyphenols. In addition, the *in vivo* effects of polyphenols are strongly influenced by their bioavailability.

Generally, bioavailability is the fraction of an ingested nutrient or compound that reaches the systemic circulation and may be utilized. Thus, it is multifactorial in that it includes gastrointestinal digestion, absorption, metabolism, tissue distribution, and bioactivity of the nutrient/compound. However, due to the difficulty in investigating the bioactivity, bioavailability is commonly considered both the fraction of a compound as well as the metabolite(s) of that compound that reach the systemic circulation[29].

Bioavailability can be affected by a wide range of factors, not only related to the food (e.g. chemical form of the compound, characteristics of the food matrix), but also to the individual (e.g. gastric emptying, intestinal transit time), resulting in high inter-individual variability[24,30].

Beginning with ingestion and digestion of a food, the food matrix can influence the bioaccessibility of the phenols because the amount that is released from within the matrix will influence the fraction that is made available for intestinal absorption. Effects on bioaccessibility can be evaluated *in vitro* by simulation of gastric and small intestinal digestion[31]. *In vitro* methods are quick and inexpensive ways to estimate the bioaccessibility of a bioactive compound, including changes resulting from variations in the food matrix and food processing. However, these methods cannot completely measure of the bioavailability of bioactives, as this requires *in vivo* methodologies.

The present review summarizes potential strategies, including innovative technologies, that can be applied during the bread-making process in an effort to increase the fraction of phenolic compounds reaching the systemic circulation, and what is currently known about the usefulness of these strategies as assessed in *in vitro* bioaccessibility and *in vivo* bioavailability studies.

POTENTIAL STRATEGIES TO INCREASE PHENOLIC CONTENT IN BREAD PRODUCTS: EFFECTS OF THE BREAD-MAKING PROCESS

Various processing techniques are applied to grains in order to transform the raw materials into finished products with good sensory characteristics and nutritional quality. Since technological processes affect the chemical constituents and physical properties of foods, it is expected they also influence the phenolics within grain products, thus impacting the potential beneficial health effects. The effect of various food-processing methods on phenolic compounds has therefore become an important area of research.

A review of the literature has highlighted three main strategies that can be applied to design phenolic-enriched breads: the first approach focuses on the use of raw materials naturally rich in phenolic compounds; the second focuses on the application of bio-processing techniques on raw materials; and the third focuses on the processing conditions that can be applied during bread-making (Table 1).

Raw materials

Whole grains are a good source of phenolic compounds, mostly concentrated in the bran, but levels of phenolics in the final products can vary widely depending on the raw materials and on the pre-processing techniques. In addition to whole wheat, barley, and rye, minor cereals (e.g. sorghum, millets;[32]), pigmented grains[33–35], and ancient grains (e.g. eikorn, emmer[36] and pseudocereals like buckwheat, quinoa and amaranth)[37] represent a good source of phenolics, thus their use in bread products has increased in the marketplace. Since phenolic compounds are present in the external layers of the kernel, adding bran fractions to refined flour is one of the most common trends to enhance phenolic content in bread products.

Pre-processing techniques

Besides using wheat bran and whole-grain flour, several modifications to pre-processing techniques can be used to influence phenolic content in bread products. A variety of fractionation methods, including both wet extraction and dry fractionation, have been developed for producing milling fractions that are concentrated in phenolic compounds. Among fractionation methods, debranning (also named pearling) is the most widely used. It has been traditionally used as a tool to enhance both hygienic and technological performances of milled flours[38,39]. More recently, debranning has been demonstrated as an effective strategy to produce bran fractions rich in aleurone particles[40], which are particularly rich in phenolics, thus recovering the bioactive compounds that are concentrated in the external layers of grain kernels[41–43].

Regarding to physical treatments, air classification technology is an effective way to separate grain flours into fractions with different sizes, properties, and chemical composition, such as protein, starch, and dietary fibre. When applied to phenolic-rich material, it is a good technique to select fractions with a high content of phenolic compounds[44].

Micronization, also known as ultrafine grinding, is a mechanical treatment, used to change or damage the fibre matrix, causing some phenolics which were linked or embedded into the matrix to be exposed so that the total phenolic content in bran increases[45] , likely due to an increase in extractability.

Lastly, biotechnological processes (*i.e.* germination, fermentation, and enzymatic treatments) have been used to improve the PA content in bran. Germination is the process by which a plant grows from a seed. During germination, high levels of hydrolytic enzymes, such as amylases and proteases, accumulate in the cereal seed, so that the insoluble endosperm starch and protein reserves are hydrolyzed into soluble forms that can be transported to the embryo to meet the needs of the growing plant. A recent review on the effects of grain germination concluded that during this process a net increase in total phenolic content and total antioxidant capacity[46] is observed. It is also thought that germination may increase the extractability of polyphenolic compounds, by releasing bound polyphenols, therefore making them more soluble in extraction solvents.

Fermentation is another beneficial pre-processing technique which effectively releases phenolics from the bran of various grains[47,48]. The enzymes produced by the added microorganisms have the potential to release insoluble bound PAs from bran and thereby improve their bioaccessibility and potential bioavailability[49]. In the case of sourdough fermentation, the effect of the reduction in pH is also important[50,51]. The lower pH during sourdough fermentation favors the activity of hydrolases and can contribute to chemical disintegration of arabinoxylans, and to extensive hydrolysis of both esters and glycosides of PAs[50,51].

Combining fermentation with germination results in an additive effect, since germination results in a higher amount of fermentable sources (sugars and nitrogen) and both increase the concentration of cell wall degrading enzymes, all contributing to increased bioaccessibility of PAs[47].

A third biotechnological process which can be applied during pre-processing is enzymatic treatment, whereby grains or bran are pre-treated with enzymes in a liquid environment. Enzymatic treatment has been reported to free PAs from fibre esters[52], improving the bioavailability of these compounds[53]. Enzymes (e.g. xylanases) are also commonly used in the baking industry, as part of dough conditioners, to improve dough property, baking quality, and shelf-life[54].

Bread-making process

In addition to the formulation and pre-processing of bread products, the bread-making process also influences the content and bioavailability of phenolic compounds in the final bread product.

Bread-making includes several fundamental operations, namely mixing and kneading, fermentation or leavening, and baking, which are indispensable for producing an attractive end product. During mixing, ingredients are evenly distributed and blended. In wheat breads, interaction with water leads to significant structural changes in proteins, resulting in gluten formation; a three-dimensional network structure resulting in a cohesive, completely homogenous, non-sticky mass with well-defined rheological characteristics. These attractive properties depend on the procedure applied and equipment used, as well as on the presence of components, such as phenolics, that may negatively affect gluten viscoelasticity. For example, phenolic compounds can form complexes with proteins, via hydrogen bonding between the hydroxyl groups of the phenols and the carbonyl group of the peptide residue[55–57].

Studies have demonstrated that dough mixing causes an overall decrease in total PAs, such as bound FA, SA and CA, in various grains[50,58,59] reaching up to 50%. However, free FA has been demonstrated to increase significantly in one study showing up to five times the initial level, suggesting that mixing may also facilitate the release of bound phenolic compounds into free and more bioaccessible forms[35,58].

Various mechanisms have been proposed to explain the overall decrease in PAs resulting from dough mixing. High-speed mixing breaks protein disulfide bonds and creates thiol free radicals in gluten, which then react with reducing compounds, like PAs, in flour[60]. Considering the proposed effect of mixing on the formation of bonds between phenolics and proteins, a decrease in phenolic content in various reports may be more accurately described as a reduction in their bioaccessibility and thus extractability[61].

Another proposed effect of mixing on phenolics is the hydrolysis of oxidative enzymes such as oxygenase and peroxidase, that are present in flours, which become active when water is added and thus decrease the amount of phenolics like FA[62].

The leavening and fermentation process increases the original volume of the bread and creates a porous structure, through the action of a leavening agent, usually baker's yeast (i.e. *Saccharomyces cerevisiae*), which converts the fermentable sugars present in the dough into ethanol and CO_2.

The fermentation and leavening process may contribute to an increase in PA bioavailability. Two mechanisms for the fermentation-induced increase in bioaccessibility and bioavailability of phenolic compounds during bread-making have been proposed: i) via the structural breakdown of the cell wall matrix by degrading enzymes present in both grains and microbes activated by the leavening agent[63]; and ii) via the synthesis or enzymatic transformation of various bioactive compounds[47]. However, studies investigating PA content in fermented dough are not consistent. This inconsistency is likely due to differences in the enzymes produced from yeast or other microorganisms and native enzymes present in various types of grains. As an example, rye has been described to have much more native enzymatic activity compared to wheat[35,50,51,59]. In addition, fermentation conditions, particularly temperature, pH, and duration, are contributing factors to PA content. With regards to the fermentation time, prolonged fermentation increases the number of bonds broken between PAs and dietary fibre, thus increasing the bioaccessibility of PAs[35].

The type of fermentation also influences PA content. An alternative to the use of dry yeast is the use of sourdough. Leavening with sourdough consists in the use of a starter, represented by a piece of dough from a previous batch, which is fermented and stored under controlled conditions of temperature and humidity. The intense acidification markedly influences the sensory and shelf-life features of the baked goods. With sourdough, dough acidification and leavening capability is determined by the interactions between lactic acid bacteria and yeasts. This kind of fermentation has a well-established role in improving flavor, structure, and shelf-life of rye and wheat breads.

Sourdough fermentation has been demonstrated to increase the bioaccessibility of PAs as, for example, Liukkonen et al. (2003)[64] found that this type of fermentation increased the content of methanol-extracted phenolic compounds, in addition to demonstrating an increase in antioxidant capacity[43,60]. As mentioned above, low pH favors the hydrolysis of both esters and glycosides of PAs[50,51]. However, different lactic acid bacteria strains exhibit varying abilities in enhancing the extraction of free phenolics, with, for example, the maximum increase in FA - in whole grain barley and oat groat when *Lactobacillus johnsonii* LA1, *Lactobacillus reuteri* SD2112, and *Lactobacillus acidophilus* LA-5 were used[65].

Lastly, baking is considered the most important stage of the bread-making process. During baking, the exchange of heat (as the dough heats up) and material (as the dough loses water/humidity) causes physical, chemical and biochemical changes resulting in the transition from foam to sponge state and the diversification between crust and crumb.

It is assumed that antioxidants, including PAs, contained in grains are lost during thermal treatments, due to degradation, oxidative condensation, or decomposition of thermolabile phenolics caused by high temperature[66,67]. However, the most recent research has reported that baking increases the total PA and FA levels[35,59,68], likely due to the intense heat that makes PAs more bioaccessible. Yu and Beta (2015)[35] found higher contents of soluble FA and *p*-hydroxybenzoic acids in bread crumb compared to crust, suggesting that some free PAs are thermally labile (since there is a higher, more intense heat in bread crust). However, higher levels of insoluble PAs can be found in the crust[35,59,68]. Heat stress could cause degradation of conjugated polyphenolic compounds resulting in an increase in free PAs, which has been demonstrated in wheat[53]. This would improve bioavailability of phenolic compounds since it is believed that free PAs are more readily available than bound PAs[69]. The effect of baking temperature on free or bound PAs can vary due to the nature and source of phenolic compounds as well as the baking method (e.g. yeast vs. sourdough)[51,70].

Unlike temperature, baking time does not seem to affect total PA content of wholegrain bread, as demonstrated in one study comparing breads baked at 10, 20 or 35 minutes[68]. Additionally, Maillard reaction that occurs during baking may contribute to the formation of new phenolic structures[68,71]. Angioloni and Collar (2011)[72] demonstrated that some PAs, such as protocatechuic, syringic, SA and FA, were detected in bread but not in the raw flour. This has also been shown in studies conducted with bread made from pigmented wheat[35] and rye whole meal[50].

Furthermore, one study demonstrated that although there is a measurable decrease in total PA and FA content that occurs during dough preparation, their concentrations significantly increased after baking to levels that surpassed those measured prior to dough preparation[59]. The baking process, however can have different effects depending on the type of grain used. For example, in breads made with pseudocereals (e.g.

amaranth, quinoa, and buckwheat), polyphenol content has generally been found to be reduced in the final bread product when compared to the original grains[66,67].

BIOACCESSIBILITY OF PHENOLIC COMPOUNDS IN BREAD: *In vitro* studies

Methods of assessment of bioaccessibility: Static vs. Dynamic Methods

Bioaccessibility is the determination of the amount of bioactive compounds potentially absorbable from the gut lumen, and can be measured using different methods which simulate *in vivo* digestion. Several i*n vitro* methods have been developed to investigate the effect of the food matrix and of different processing techniques on the ability of nutrients or bioactive compounds, like polyphenols, to become available to absorbtion[73]. These methods try to mimic *in vivo* digestion by simulating the oral, gastric and small intestinal phases and, occasionally, large intestinal fermentation[74].

There are two general categories of methods: static and dynamic (non-static). In static models, products remain largely immobile in a single bioreactor, and the ratios between meal, enzymes, salt, bile acids and all other substrates of the biological digestive reactions are kept constant at each phase of digestion. Static methods can differ in incubation time and characteristics of the digestive juices, namely the concentrations of the enzymes resulting from the preparation, for example by the addition of specific enzymes to inorganic and organic solutions. They can be also adjusted for pH on the basis of the specific gut compartment, as static methods consist of multiple phases, including oral, gastric and intestinal, each of which can vary slightly in different studies.

In the oral phase, the incubation time of the test sample can vary between 2 and 30 minutes[74,75] with either: i) human buffered saliva with phosphate or saline solution[74]; ii) α-amylase solution[75,76]; or iii) saliva solution prepared with different salts and with the addition of α-amylase, uric acid and mucin[77]. Some studies bypass the oral phase[72,78] possibly because a significant contribution to the digestive process is not expected in this stage due to the short time during which food is in contact with saliva in *in vivo* conditions[79].

In the gastric phase, a pepsin solution is normally used and incubation time can vary between 1 and 2 hours[72,74–78]. The addition of mucin has also been reported[77]. Furthermore, hydrochloric acid is commonly used to more accurately simulate *in vivo* gastric conditions[79].

In the intestinal phase, neutralization as well as incubation with pancreatic enzymes is set up. The enzymes used in most studies include pancreatin[74], a bile/pancreatin solution[72,76,78], or a duodenal juice including pancreatin, lipase and bile[77]. Incubation time can vary from 2 to over 24 hours[74,76,77].

After gastrointestinal digestion simulation, the point at which bioaccessibility determination of compounds of interest occurs can also vary. One method is to centrifuge or filtrate the sample mixture to measure the bioaccessibility of compounds based on the levels present in the supernatant. An alternative method includes the use of a dialysis membranes, which allows for the discrimination between high and low molecular weight components[31]. When a dialysis tube is used, the undigested material (the fraction remaining inside the tube) can be analysed for the content of the nutrient/bioactive compound under study (e.g. PAs) and then the bioaccessibility can be obtained as a difference from that measured in the sample before digestion[74,76]. The time at which the dialysis tube is used may vary, as in some works it is used immediately after the gastric phase[74] while in others after the intestinal digestion phase[76].

In general, static methods are quick, cost-effective and can be used to assess effects on several nutrients and bioactive compounds resulting from changes made to the food matrix, by changing the raw materials or processing techniques used, compared to the reference material or to the original food matrix.

The main limitations of static methods are that they do not provide the most accurate simulation of the complex dynamic physiological processes occurring during *in vivo* conditions. This has led to the development of dynamic (non-static) digestion models. A common and very sophisticated gut model to simulate the human digestive system was developed by The Netherland Organization for Applied Scientific Research[80]. Their commercial gastrointestinal model, also known as the TIM system, is a multi-compartmental dynamic computer-controlled model that has been successfully used to study the bioaccessibility of many compounds including vitamins and minerals, as well as phenolics[81,82]. The TIM system simulates the dynamic conditions occurring in the four main gastrointestinal compartments: stomach, duodenum, jejunum and ileum. All parameters, including gastric and small intestinal transit, flow rate, composition of digestive fluids, temperature, pH, and removal of water and metabolites, are all remote-computer controlled. In the jejunal and ileal compartments, a dialysis system allows for the removal of digestion products, isolating the dialysate fraction, which contains the bioaccessible products from the "unabsorbed" sample.

Overall, the use of realistic concentrations of digestive enzymes, pH levels, transit times appropriate to each digestion step, and salt concentrations, among other factors, contribute to a more accurate simulation of the gastrointestinal tract. Furthermore, the removal of the products of digestion and the appropriate mixing at each stage of digestion in the use of dynamic methods may represent crucial points in mimicking physiological conditions *in vivo*.

In vitro studies investigating effects of altering raw materials on phenolic bioaccessibility

Table 2 shows the main findings of all studies identified in the literature and evaluating the bioaccessibility of phenolic compounds in bread. Among the different potential strategies to apply in the bread-making process to increase the bioaccessibility of phenolic compounds in breads, as summarized in Table 1, the efficacy of using different raw materials has been the most investigated. In particular, the majority of the studies has explored the bioaccessibility in breads made by using different types of cereals or pseudocereals. As expected, wheat-based breads (both white and wholegrain) were the most investigated (in all 9 studies), with few investigating rye (2/9), oat (1/9) and barley (1/9), either alone or mixed. Among pseudocereals, buckwheat breads were analysed in two out of the nine studies.

Almost all studies included wheat bread as an internal control to be compared with breads made with different cereals. Generally, white bread is characterized by a low bioaccessibility of PAs, partially ascribable to the very low FA content in the samples[83], especially in its free form. Three studies, investigated the bioaccessibility of PAs in white breads following digestion by the dynamic TIM system, expressing results as the percentage of PAs in the dialysate in relation to the original sample[69,83,84]. In the first study by Mateo Anson et al. (2009 a)[69], FA was undetected in the dialysate-samples, whereas in the second study, conducted by the same authors[84], 4.9% FA bioaccessibility was reported. CA and SA were measured in the second study[84], but they were not detected in the dialysate, post-digestion. The third study by Hemery and colleagues (2010)[83] found a 10.2% FA bioaccessibility.

In another study conducted by Angioloni and Collar (2011)[72], the authors found a 58% bioaccessibility of the total phenolics (measured as Total Phenolic Content, TPC) in the supernatant from static *in vitro* digestion of wheat bread. This is similar to a second study conducted by the same authors, in which they found ~84% TPC bioaccessibility in wheat bread, although in this latter study the percentage bioaccessibility was calculated from the initial TPC in flour as opposed to the bread, as is typically done[78].

The differences in bioaccessibility in white wheat bread found in the latter two studies (58% and 84% for TPC) compared to the former three studies (0% FA, 4.9% FA and 10.2% FA) may be linked to the former three measuring FA only, using chromatographic methods, while the latter two measured total phenolics using the Folin-Ciocalteau method. In addition to potential differences due to type of *in vitro* method (*i.e.* static vs. dynamic), further sources of variability might include the phenolic content in the raw materials, as well as the state of the test samples used for post- digestion measurements (*i.e.* dialysate samples in former 3 versus supernatant and precipitate used in the latter 2 studies).

Three studies compared the bioaccessibility of phenolic compounds in white bread with respect to whole wheat bread[69,75,83]. As expected, the whole wheat breads had higher initial PA content, due to the preservation of the outer layers of the kernels (e.g. 9-12-fold higher FA content in whole wheat versus white bread). This contributed to a greater net content of bioaccessible PAs, demonstrating how the use of different raw materials is a valid strategy for this purpose. However, although the net content of bioaccessible PAs in whole wheat bread is higher than that of white bread, the bioaccessibility was higher in white breads compared to whole wheat (e.g. 4.9% versus 1.1% in Mateo Anson et al. (2009b)[84] and 10.2% FA vs. 2.9% FA in Hemery et al. (2010)[83]). Nevertheless, other studies have observed much higher bioaccessibility for specific PA in whole wheat breads. For example, Dall'Asta, *et al.* (2016)[74] found a 13.1% FA bioaccessibility in whole grain bread, but this may be due to differences in the methods used, with this latter study using a static digestion model. Variations in bioaccessibility in whole wheat breads may also differ due to the type of whole wheat or whole grain bread used, as the former two studies produced breads from flour at lab level, while the latter used a commercial whole grain bread which may have been exposed to different, perhaps greater, degrees of processing. Furthermore, there seem to be no differences in bioaccessibility for different types of PA. For example, FA appears to have lower percentage bioaccessibility compared to CA and SA, regardless of the analytical method[74,83]. This may be due to different distributions of phenolic compounds in the free, conjugated, and bound forms.

Szawara-Nowak and colleagues (2016)[75], following *in vitro* digestion of white wheat bread, found a soluble fraction of these compounds quite comparable to the content in dark wheat bread (~9 mg rutin equivalent/g dry weight). They reported, for both white and dark bread, an exceptionally large increase in rutin post versus pre-digestion (~20 and ~9 fold, respectively), which is much greater compared to any other study.

Similar unexpected increases following digestion were also found with increasing substitution of buckwheat flour (both white and roasted) in white and dark breads[75]. Authors hypothesized this may be due to an increase in the extractability of phenolic compounds resulting from the parameters set in their *in vitro* digestion, including pH, temperature, incubation times, and extraction solvent.

As reported in Table 1, a strategy to increase PA content in bread includes modifications to the raw materials. The use of different types of cereals or pseudocereals, or a mixture of them, is a strategy to increase PA content which has become increasingly common[85]. In the above-mentioned study, Angioloni and Collar (2011)[72] assessed the differences in TPC in breads made with oat, rye, buckwheat and wheat flours. Among the four breads made with 100% of one single type of flour, the TPC (measured by Folin-Ciocalteau method) in the initial bread was highest in buckwheat (808 mg GAE/kg), followed by wheat (685 mg GAE/kg), oat (643 mg GAE/kg) and rye (536 mg GAE/kg). Following *in vitro* digestion, although the bioaccessibility of TPC was greatest in the rye bread (62%), the net PA content was greatest in the 100% wheat bread (401 mg GAE/kg) with 58% bioaccessibility, followed by buckwheat (366 mg GAE/kg; 45%), rye (334 mg GAE/kg; 62%) and then oat (264 mg GAE/kg; 41%). The lower bioaccessibility in the buckwheat and oat breads may be due to their substantially greater fibre and protein contents in the respective flours (13.8% and 17.4%, and 18.9% and 21.5%, respectively) compared to the white wheat and rye breads (2.2% and 12.6%, and 14.6% and 9.6%, respectively). The higher fibre and protein content may partially prevent the digestive enzymes to free bound PAs, thus limiting their bioaccessibility. The same study also assessed blends of flours, specifically the multigrain bread "blend 15%" (oat:rye:buckwheat:wheat 15:15:15:55, "blend 20%" (20:20:20:40) and "blend 25%" (25:25:25:25), where the TPC in the initial bread increased with the increase in wheat flour replacement (from 592 to 745 to 916 mg GAE/kg). Interestingly, the higher the substitution level of wheat flour by minor cereal and pseudocereal, the lower the percentage of TPC bioaccessibility, with the highest value (80%) reached with the 15% blend. However, the net TPC was comparable between the 3 blends (472, 549, 504mg GAE/kg corresponding to the 15, 20, 25% blends), and was actually greater than any of the 100% breads (401, 366, 334 and 264 mg GAE/kg, for wheat, buckwheat, rye and oat breads, respectively). Therefore, there may be some influential effect on PA bioaccessibility resulting from mixed grains, regardless of the actual quantities of each individual type. Comparing the 100% wheat flour bread with the 15% blend, which was 55% wheat flour, it is interesting to notice that that the initial TPC content of the 100% wheat flour bread was higher (685 vs. 592mg GAE/kg TPC), yet the final bread TPC is higher in the 15% blend (472 vs. 401mg GAE/kg).

In another study by the same authors, a 40% barley bread (made by replacing 40% wheat flour with barley flour) showed no difference in net TPC (597 vs. 598 mg/100g, respectively) and a much lower % bioaccessibility (60% vs. 84%, respectively, although the difference in TPC in the flour was higher (1003 mg/100g vs. 713 mg/100g)[78]. Perhaps the specific barley flour used, commercial barley flour, had low bioaccessibility due to greater fibre content (4.01 vs. 1.15 g/100g, in the respective breads). When the type of barley flour was changed to a high β-glucan barley flour, the percentage bioaccessibility was still lower compared to the bread made from refined common wheat flour (42% vs. ~84%, respectively), again likely due to the higher fibre content (11.91 vs. 1.15 g/100g in the respective breads). However, the net TPC was much greater in the high β-glucan barley bread compared to the 100% white wheat bread (857 vs. 598 mg/100g, respectively) because the TPC in the raw flour was ~3-fold higher (2197 vs. 713 mg/100g, respectively). Beta-glucan is a soluble, viscous-type fibre, which may therefore contribute to the low PA bioaccessibility since β-glucans can produce viscous gels able to entrap nutrients and phytochemicals, including phenolics, as previously hypothesized[72]. This may also explain part of the particularly lower bioaccessibility in the oat bread found in the study discussed above[72], since oats are also a rich source of β-glucan soluble fibre. Overall, these studies demonstrate that the types of grain flour used in blends may be influential on PA bioaccessibility.

Another way to increase PA content in breads by modifications of raw materials includes the addition of selected fractions from the original grain. One of the most commonly used fractions is the cereal bran, as it is a recognized source of phenolics, including PAs. Mateo Anson *et al.* (2009b)[84] compared a wholemeal bread to a wholemeal bread added with native wheat bran. Although they found the same FA bioaccessibility in both breads (1.1%), the net FA content in the wholemeal bread plus bran was greater, since the bread plus bran had a greater initial content of FA (1300 μg/g vs. 800 μg/g). The potential reason why the FA bioaccessibility was the same between the breads is because the bioaccessibility of FA is mainly associated with the amount of free FA present in breads, and the FA in the bran is mostly bound. Mateo Anson *et al.* (2009b)[84] demonstrated a strong correlation between the amount of free FA and bioaccessibility among five breads. This hypothesis is further supported by the study of Koistinen *et al.* (2017)[76], where the authors compared wheat bread made with bioprocessed rye bran to the same bread made with native rye bran and found that FA bioaccessibility was significantly greater in the bread with the bioprocessed rye bran (88% vs. 51%, respectively). This was also reflected in the bioaccessibility of total PAs (89% vs. 53%, respectively). The bioaccessibility was not directly calculated in this study. However, by calculating it as the difference between polyphenol content in the original sample and the residue of the enzymatic digestion[86], percentage bioaccessibility of PAs was inferred.

In addition to the use of bran, bread can be enriched with the polyphenol-rich aleurone fraction, as was investigated in two studies[69,74]. In the study by Mateo Anson *et al.* (2009a)[69], the addition of aleurone resulted in a substantial increase in initial FA in the bread compared to the white bread (2290 µg/g and 33.5 µg/g, respectively). After *in vitro* dynamic digestion, an increase in FA bioaccessibility was detected in the aleurone-enriched bread (0.57%) compared to white bread (not detected). Furthermore, Mateo Anson *et al.* (2009a)[69] demonstrated that the aleurone-enriched bread had a level of FA bioaccessibility that was ~60% lower than that found in a raw flour which had free FAs added (which was used as a "positive" control). In the aleurone-enriched bread, the majority of FA was present in bound form and only 20 µg/g as free FAs. Considering that only free and conjugated phenolic compounds are readily available for absorption, these results further support the consideration that free phenolic compounds are the major contributors to the bioaccessibility of PAs. Conversely, bound phenolics, being largely attached to undigested cell wall polysaccharides, are mainly retained into the material reaching the colon.

The static model study by Dall'Asta *et al.* (2016)[74] showed instead that aleurone-enriched bread resulted in bioaccessibility values 2.5-fold to 4.4-fold greater compared to whole grain bread for various PAs, including a 3-fold greater bioaccessibility for FA. These results are particularly interesting, since the aleurone bread had approximately half the amount of PAs compared to the wholegrain bread (total FA 70.67 vs. 144.78 mg/100g, respectively). Although the results from this latter study contrast the ones of Mateo Anson *et al.* (2009a)[69], they are supported by a previous study where it was reported that, in addition to the free form, a relevant percentage of the bound fraction may become available for absorption following digestion[87]. The mechanisms through which aleurone additions may influence PA bioaccessibility in the two studies may be ascribable to several factors. In addition to the differences between the *in vitro* method used (TIM versus static), the studies differed in the applied digestion length (6 versus 24 hours), in the aleurone content (22%

vs. 9.3% aleurone flour in the final dough), and in the kind of phenolic compounds considered (*i.e.* the consideration of di- and tri-FA in the work of Dall'Asta *et al.* (2016)[74]). Regardless, both studies demonstrate that the use of the polyphenol-rich aleurone fraction may represent a valuable source of phenolics and as an attractive strategy for producing breads with bioaccessible PAs, along with the advantage of more acceptable sensory characteristics.

In vitro bioaccessibility studies investigating effects of pre-processing techniques in bread-making on polyphenolic content

Beyond using different raw materials to influence PA content, innovative technologies have been developed, including pre-processing techniques, with the aim to improve the release of bound phenolic compounds and thus their bioaccessibility. Biotechnological processing and dry-fractionation of wheat bran are two types of technologies that have thus far been investigated in *in vitro* digestion studies assessing bioaccessibility of phenolic compounds in bread[76,83,84].

Fermentation and enzymatic treatment are two biotechnological processing techniques applied during bread-making, which have been investigated on their effect on the bioaccessibility of FA, CA and SA. One study compared a wholemeal bread with native wheat bran to one where the wheat bran had been fermented and to another where the wheat bran had been both fermented and enzymatically treated with xylanase, β-glucanase, α-amylase, cellulase and ferulic acid esterase[84]. All three breads had the same initial content of FA, CA and SA. However, after a dynamic digestion method was applied, the bioaccessibility of FA was twice as high in the bread with fermented wheat bran and 5-fold higher in the bread with fermented and enzymatically treated wheat bran, compared to the bread with native wheat bran. A slightly smaller but similar trend was observed for CA and SA. The great increase in bioaccessibility in the bread with bioprocessed bran may be due to the hydrolysis of different wheat fibre polymers resulting from to the hydrolytic enzymes, which may lead to a structural breakdown of bran cell walls.

Mandak and Nystrom (2013)[77] also evaluated the effect of enzymatic treatment, and assessed the bioaccessibility of steryl ferulates, which are phytosterols that can be esterified to FA, in breads made with two types of wheat flour, either with or without the use of the enzymes cellulose or xylanase, alone or in combination. The bioaccessibility of steryl ferulate (calculated as the percentage in the supernatant compared to the total extractable amount) was generally very low (0.01-0.25%), although when both enzymes were used, bioaccessibility increased from 0.01 to 0.25% in wholegrain breads, but only from 0.09 to 0.10% in baking flour breads. The differences in effect of enzymatic treatment seen in this study versus the study by Mateo Anson *et al.* (2009b)[84] may be: i) the specificity in the phenolic compounds assessed (steryl ferulates vs. PAs); ii) the specific enzymes used and the number and combination of them (xylanase and cellulase vs. β-glucanase, xylanase, α-amylase and ferulic acid esterase); iii) the method of bread preparation (direct incorporation of the enzymes to the flour vs. preliminary bioprocessing of bran); and iv) the digestion method employed (static vs. dynamic).

As previously mentioned, Koistinen *et al.* (2017)[76] recently investigated the bioaccessibility of phenolic compounds in a bioprocessed (by enzymatic treatment and fermentation) rye bran added to wheat bread, and found a stunning 88% bioaccessibility of FAs. Bioaccessibility was therefore much higher than that of the two previous studies, possibly because a considerable amount of phenolic bound compounds became available due to the addition of enzymes and the activation of endogenous enzymes resulting from fermentation.

The bioaccessibility of PAs in bread was also increased when wheat bran was dry-fractionated. Hemery *et al.* (2010)[83] analysed free, conjugated, bound and total FA, SA and CA in bread made following bran ultra-fine grinding and bran electrostatic separation. They found that the finer the bran particles in bran-rich breads, the more bioaccessible the PAs (following Tiny-TIM digestion), with a very strong correlation between FA bioaccessibility and the proportion of small particles (10-20 μm diameter). The bioaccessibility of SA was generally much higher than that of CA or FA (26-33% versus 6-13% and 2.5-3.4%), likely because SA is mainly present in the conjugated form and within the aleurone grains[88]. Furthermore, although the breaking of covalent bonds during extensive milling contributes to increased bioaccessibility[89], the particle size of the samples seems to play a role in determining the bioaccessibility of phenolic compounds, possibly through an improvement of the extractability resulting from micronization[90]. The described study also found SA bioaccessibility was highly correlated to the proportion of small particles (<10um diameter), and the authors furthermore evaluated also bread made with positive and negative fractions obtained by electrostatic separation of bran, after the highest level of grinding (cryo-ultrafine), and demonstrated these to have the highest amount of bioaccessible PAs. The charge of these particles was influenced by the type of cell walls (branched and cross-linked vs. linear oligosaccharides), with separation between fibre-rich particles of pericarp (outer cell wall), rich in highly branched and cross-linked arabinoxylans (negatively charged) and particles rich in β-glucan, FA and CA from aleurone cell walls (positively charged)[91]. These results provide insights for the improvement of electrostatic separation processes able to select specific fractions rich in free and conjugated PAs[40].

Overall, the studies investigating the bioaccessibility of phenolic compounds in bread suggest alterations, such as the incorporation of polyphenol-rich raw materials and, especially, the application of different bio-processing techniques represent promising strategies to increase the amount of bioaccessible phenolic compounds in bread. The significant variations among the *in vitro* methods used impede a proper comparison of the results across studies and make the possibility to deduce general findings very difficult. To circumvent this, Minekus *et al*. 2014[92] recently published an international consensus paper aimed at introducing a standardised *in vitro* digestion method to analyse food, providing recommendations for every step of digestion. Adoption of this standardized method will assist in comparison of multiple study results in the future, allowing for clearer conclusions to be drawn.

BIOAVAILABILITY OF PHENOLIC COMPOUNDS IN BREAD: *in vivo* studies

Determining the content of bioactive compounds in food products or their sole bioaccessibility *in vitro* is not sufficient per se to predict their potential health effects *in vivo*. Therefore, *in vivo* studies are important to determine the bioavailability of PAs in order to understand the amount of PA actually absorbed post-ingestion, becoming therefore available to elicit health effects.

A review of the literature identified 5 studies investigating the bioavailability of PAs from standard versus bioprocessed bread (Table 3). The most common methodology used *in vivo* to assess phenolic bioavailability is represented by acute studies, where subjects are provided a single-dose of the test food and biological samples (e.g. blood, urine) are collected pre- and post-consumption. The changes, therefore, reflect the ability to absorb polyphenols from a complex food matrix[93]. Three out of the five identified studies were single-dose acute studies, with 2 evaluating the bioavailability of phenolics in bread in urine and plasma[49,94] and 1 in urine alone[95].

Bioavailability was calculated in all studies as the ratio between the amount of the excreted phenolic compounds and the amount provided with in the fed bread sample. Bresciani *et al.* (2016)[94] specifically detected and quantified secondary metabolites of phenolic compounds and described the bioavailability as the sum of these conjugated metabolites, while Lappi et al. (2013)[95] and Mateo Anson et al. (2011)[49] performed an enzymatic hydrolysis of the urinary sample by using a mixture of β-glucuronidase and sulfatase from *Helix pomatia*. This reaction allows to cleave the glucuronic and sulfonic moieties of the phase II metabolites and to detect the only aglycones, to which the bioavailability is accounted for.

As discussed, raw materials as well as bioprocessing techniques in bread-making play important roles in the bioavailability of phenolic compounds in breads. Product innovation in these acute studies was based on three main strategies: i) the addition of aleurone fraction to commercial wheat breads[94]; ii) bioprocessing of wheat bran added to a whole grain bread[49]; and iii) the use of rye bread and rye bran[95].

All 3 acute studies evaluated the urinary bioavailability of FA. Bresciani et al. (2016)[94] fed healthy volunteers, on three separate days, a wholegrain bread and a 6% w/w aleurone-enriched bread at two different servings of 94 g and 190 g, containing 43 mg and 87 mg total FA, respectively. Results showed a significant 2-fold higher FA bioavailability (as the sum of FA metabolites ferulic acid-4'-O-sulfate, dihydroferulic acid-4'-O-sulfate, and dihydroferulic acid-O-glucuronide) in urine of volunteers fed with the single portion of the aleurone-enriched bread compared to wholegrain bread and to the double portion of aleurone-enriched bread. Intriguingly, no significant difference was found in urinary FA bioavailability between the double portion of aleurone-enriched and wholegrain breads (~5% and ~4%, respectively). The authors commented that the higher bioavailability derived from the lower ferulic consumption in the single compared to double portion of aleurone-enriched bread may be due by a reduction in the capacity to metabolize and absorb PAs as intake increases.

Mateo Anson et al. (2011)[49] demonstrated similar results when breads were standardized to contain the same initial total PA amount. Specifically, they found 10% FA bioavailability in the bread with bioprocessed bran compared to 4% in the whole wheat control bread with native bran (21.34 mg/24h vs. 9.89 mg/24h FA in urine, $p < 0.05$). Furthermore, Lappi et al. (2013)[95] found a 2.5-fold greater urinary FA excretion after consumption of whole wheat bread with bioprocessed rye bran compared to the same whole wheat bread with native rye bran and with control wheat. For a thorough comprehension of the results of this study, it is important to consider the initial amount of FA in the fed bread. Indeed, the control wheat bread in this study showed a 3.2% FA bioavailability, as per excretion in urine, even if the initial FA intake was much lower compared to both the rye or the bioprocessed rye brans. Thus, the total FA urinary excretion was lower (0.27 mg/d in control whole wheat bread vs. 1.66 mg/d from bioprocessed rye bran bread vs. 0.45 mg/d in native rye bran bread, corresponding to 3.2%, 1% and 0.4% FA bioavailability, respectively). Therefore, although the percentage bioavailability may be higher, if the initial intake is lower, the total amount absorbed may nevertheless be lower.

The application of bioprocessing techniques to breads, similarly, elicited increased bioavailability for SA and other PAs. The study by Mateo Anson and colleagues (2011)[49] found that the amount of SA in 24-hour urine corresponded to a 15% and 7% bioavailability in bioprocessed bran and control breads, respectively. However, the bioavailability for CA equalled 2% for both the bioprocessed and control breads. Lappi *et al.* (2013)[95] showed a 0.6% SA bioavailability for the bioprocessed bread compared to a 2.8% for white wheat bread, and generally all three breads were characterised by a ~4-fold lower SA bioavailability compared to the white wheat bread. In spite of this, the bioprocessed bread showed the highest excreted SA net amount (0.23 mg vs. 0.06-0.12 mg in the other three breads). Similar results were found for CA bioavailability. Intriguingly, Mateo Anson *et al.* (2011)[49] evaluated the percentage vanillic acid bioavailability based on 24-hour urine excretion and demonstrated 160% and 104% bioavailability in the bioprocessed and control breads, respectively, and both had similar initial concentrations in breads (0.018 mg/g and 0.017 mg/g, respectively), thus the bioprocessed bran bread resulted in greater vanillic acid absorption. Authors did not provide a possible explanation for such high recoveries, which could be at least partially attributable to an insufficient initial extraction of phenolics from the bread.

Two studies also evaluated blood concentrations of phenolic compounds after bread consumption[49,94] and both demonstrated increased hippuric and hydroxyhippuric acid plasma levels after bread consumption. However, being degradation products from several different metabolic pathways, these two catabolites cannot be considered uniquely associated to polyphenol metabolism[22]. The second most concentrated polyphenol compound in plasma was FA, together with its main phase II conjugates. Bresciani et al. (2016)[94] found concentrations of the main FA metabolites (ferulic acid- 4'-O-sulfate and dihydroferulic acid-4'-O-sulfate) ranging from 66 to 100 nmol/L at 90 minutes after bread intake, with no significant differences among the various breads. Mateo Anson *et al.* (2011)[49], however, found a significantly higher plasma FA concentration from bioprocessed bread (2.7 µmol/L) compared to control bread (0.9 µmol/L). The contrast of these results may be explained by the different initial intakes of FA, as, although the concentration of FA in breads were similar, in the latter study[49] the subjects consumed 3 times as much bread (300 g vs. 94 g) and thus had a 3-fold higher FA intake. Two studies investigated the consumption of rye bran breads in the context of a dietary intervention. The study by Harder *et al.* (2004)[96] compared 250 g/d of rye bran-enriched products with 250 g/d of control wheat products (Vitacell®) consumed for 6 weeks in a randomized, crossover designed intervention with a 4-week washout in 18 healthy postmenopausal women. Juntunen *et al.* (2000)[97] similarly compared the consumption of 4-5 slices/d of rye bread with wheat bread for 4 weeks in a randomized, crossover design with a 4-week washout in 43 healthy volunteers (Table 3). Although it was not possible to calculate the bioavailability of phenolic compounds in the breads because measurements would have had to include phenolics found in foods consumed during the rest of the daily diet, measurements of phenolic metabolites in blood (plasma) and urine samples were compared after consumption of the different bread interventions. Moreover, in the study by Harder *et al.* (2004)[96], in addition to rye bread, the authors also included rye-enriched muffins and crisp bread products, thus making the FA amount found in biological samples not originating solely from bread. These authors measured FA concentration in 48-hour urine collections and found urinary FA excretion was ~2 mg/24hour for the habitual diet (*i.e.* at baseline) and at the end of 6-weeks after the incorporation of white wheat bread (Vitacell®). However, at the end of 6-weeks of the intervention with rye bran-enriched bread products, FA excretion was 2.5-fold higher ($p < 0.05$) compared with both the control wheat bread intervention (40.2% higher, $p = 0.001$) and the baseline diet (39.8% higher, $p = 0.002$). Considering the 10.2 mg FA/day intake during the rye bran intervention, the study demonstrated a recovery of 28% of FA metabolites.

Juntunen *et al.* (2000)[97] considered the plant lignans, secoisolariciresinol (SECO) and matairesinol (MAT), which are found in large quantities in rye cereal-based products and bio-transformed by gut microbiota into enterodiol and enterolactone (ENL), respectively, and the latter finally oxidized to ENL. After a 4-week dietary intervention on either wheat or rye bread consumption, total ENL excretion in 24-hour urine samples almost doubled after rye bread consumption (6.8 µmol/day for men and 7.8 µmol/day for women) compared to the period with wheat control bread (4.0 µmol/day for men and 3.7 µmol/day for women). However, 24-hour urine ENL concentration at the end of the rye intervention was not significantly different from the baseline. Furthermore, there was no correlation between the intake of rye bread or plant lignans and ENL urinary excretion, which is interesting considering the intake of rye bread was more than double during the rye bread intervention compared to the habitual diet. Additionally, no difference in serum ENL concentrations between pre- and post-rye intervention was observed and again, there was no correlation between rye intake and serum ENL concentration. It is possible that a plateau of ENL is physiologically reached independently from the intake of rye bread.

CONCLUSIONS AND REMARKS

Phenolic compounds are recognized for several beneficial effects on human health. These effects depend not only on their content in food products but also on their ability to be absorbed and become available within the human body. For this reason, *in vitro* and *in vivo* studies have been performed with the aim of investigating the bioaccessibility and bioavailability of phenolic compounds, respectively, suggesting that the use of specific raw materials (e.g. cereals/pseudocereals as alternatives to wheat, or specific cereal fractions) or of pre-processing techniques might represent valuable strategies for enhancing the phenolic content in the raw materials and for increasing the amount of bioaccessible and bioavailable compounds.

Unequivocal conclusions could not be drawn at present, as the available studies widely differ for fed amounts of phenolic compounds and, more importantly, for the methodologies applied. This highlights a great need for standardization of methodologies used in *in vitro* studies in order to be able to compare results and draw conclusions on the potential usefulness of the application of innovative techniques to improve phenolic bioaccessibility. The few *in vivo* studies identified also highlight the need for further research to be carried out in this area to assess the effectiveness of the application of new strategies in the bread-making process on phenolic bioavailability. With the ultimate goal of eliciting health benefits, intervention trials will be required to assess if strategies that demonstrate effectiveness at increasing phenolics bioavailability translate then to improvements in health outcomes in humans.

The authors declare no conflict of interest.

REFERENCE LIST

1 C. Leclercq, D. Arcella, R. Piccinelli, S. Sette, C. Le Donne and A. Turrini, The Italian National Food Consumption Survey INRAN-SCAI 2005-06: main results in terms of food consumption, *Public Health Nutr.*, 2009, **12**, 2504–2532.

2 C. T. M. van Rossum, E. J. M. Buurma-Rethans, F. B. C. Vennemann, M. Beukers, H. A. M. Brants, E. J. de Boer and M. C. Ockém, *The diet of the Dutch. Results of the first two years of the Dutch National Food Consumption Survey 2012-2016*, National Institute for Public Health and the Environment, Bilthoven, The Netherlands, 2016.

3 T. Heuer, C. Krems, K. Moon, C. Brombach and I. Hoffmann, Food consumption of adults in Germany: results of the German National Nutrition Survey II based on diet history interviews, *Br. J. Nutr.*, 2015, **113**, 1603–1614.

4 E. Ruiz, J. M. Avila, T. Valero, S. Del Pozo, P. Rodriguez, J. Aranceta-Bartrina, A. Gil, M. Gonzàlez-Gross, R. M. Ortega, L. Serra-Majem and G. Varela-Moreiras, Energy intake, profile, and dietary sources in the spanish population: Findings of the ANIBES study, *Nutrients*, 2015, **7**, 4739–4762.

5 J. W. Van Der Kamp, K. Poutanen, C. J. Seal and D. P. Richardson, The HEALTHGRAIN definition of 'whole grain', *Food Nutr. Res.*, 2014, **58**, 22100–22108.

6 K. A. Harris and P. M. Kris-Etherton, Effects of whole grains on coronary heart disease risk, *Curr. Atheroscler. Rep.*, 2010, **12**, 368–376.

7 G. Tang, D. Wang, J. Long, F. Yang and L. Si, Meta-Analysis of the Association Between Whole Grain Intake and Coronary Heart Disease Risk, *Am. J. Cardiol.*, 2015, **115**, 625–629.

8 D. Aune, T. Norat, P. Romundstad and L. J. Vatten, Whole grain and refined grain consumption and the risk of type 2 diabetes: A systematic review and dose-response meta-analysis of cohort studies, *Eur. J. Epidemiol.*, 2013, **28**, 845–858.

9 INRAN, Linee guida per una sana alimentazione italiana. Revisione 2003, http://www.salute.gov.it/imgs/C_17_pubblicazioni_652_allegato.pdf, (02/2017).

10 O. K. Chun, S. J. Chung and W. O. Song, Estimated dietary flavonoid intake and major food sources of U.S. adults, *J. Nutr.*, 2007, **137**, 1244–1252.

11 US Department of Health and Human Services and US Department of Agriculture, 2015-2020 Dietary Guidelines for Americans. 8th Edition. January 2016, https://health.gov/dietaryguidelines/2015/resources/2015-2020_Dietary_Guidelines.pdf, (02/2017).

12 T. J. Anderson, J. Grégoire, G. J. Pearson, A. R. Barry, P. Couture, M. Dawes, G. A. Francis, J. Genest, S. Grover, M. Gupta, R. A. Hegele, D. C. Lau, L. A. Leiter, E. Lonn, G. B. J. Mancini, R. McPherson, D. Ngui, P. Poirier, J. L. Sievenpiper, J. A. Stone, G. Thanassoulis and R. Ward, 2016 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the Adult, *Can. J. Cardiol.*, 2016, **32**, 1263–1282.

13 K. M. Behall, D. J. Scholfield and J. Hallfrisch, Diets Containing Barley Significantly Reduce Lipids in Mildly Hypercho-lesterolemic Men and Women, *Am. J. Clin. Nutr.*, 2004, **80**, 1185–1193.

14 B. M. Davy, K. P. Davy, R. C. Ho, S. D. Beske, L. R. Davrath and C. L. Melby, High-fiber oat cereal compared with wheat cereal consumption favorably alters LDL-cholesterol subclass and particle numbers in middle-aged and older men, *Am. J. Clin. Nutr.*, 2002, **76**, 351–358.

15 M. Kristensen and S. Bügel, A diet rich in oat bran improves blood lipids and hemostatic factors, and reduces apparent energy digestibility in young healthy volunteers, *Eur. J. Clin. Nutr.*, 2011, **65**, 1053–1058.

16 European Food Safety Authority (EFSA), Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations, *EFSA J.*, 2011, **9**, 2207.

17 L. Stevenson, F. Phillips, K. O'Sullivan and J. Walton, Wheat bran: its composition and benefits to health, a European perspective, *Int. J. Food Sci. Nutr.*, 2012, **63**, 1001–1013.

18 L. Li, P. R. Shewry and J. L. Ward, Phenolic acids in wheat varieties in the healthgrain diversity screen, *J. Agric. Food Chem.*, 2008, **56**, 9732–9739.

19 S. Hättenschwiler and P. M. Vitousek, The role of polyphenols in terrestrial ecosystem nutrient cycling, *Trends Ecol. Evol.*, 2000, **15**, 238–242.

20 J. E. Beart, T. H. Lilley and E. Haslam, Plant polyphenols-secondary metabolism and chemical defence: Some observations, *Phytochemistry*, 1985, **24**, 33–38.

21 A. Scalbert and G. Williamson, Chocolate: Modern Science Investigates an Ancient Medicine, *J.*

Med. Food, 2000, **3**, 121–125.

22 D. Del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges and A. Crozier, Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases, *Antioxid. Redox Signal.*, 2013, **18**, 1818–1892.

23 L. Mele, S. Carobbio, N. Brindani, C. Curti, S. Rodriguez-Cuenca, G. Bidault, P. Mena, I. Zanotti, M. Vacca, A. Vidal-Puig and D. Del Rio, Phenyl-γ-valerolactones, flavan-3-ol colonic metabolites, protect brown adipocytes from oxidative stress without affecting their differentiation or function, *Mol. Nutr. Food Res.*, 2017, DOI:10.1002/mnfr.201700074.

24 A. Rodriguez-Mateos, D. Vauzour, C. G. Krueger, D. Shanmuganayagam, J. Reed, L. Calani, P. Mena, D. Del Rio and A. Crozier, Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update, *Arch. Toxicol.*, 2014, **88**, 1803–1853.

25 S. V Joseph, I. Edirisinghe and B. M. Burton-Freeman, Fruit Polyphenols: A Review of Anti-inflammatory Effects in Humans, *Crit. Rev. Food Sci. Nutr.*, 2016, **56**, 419–444.

26 M. G. Hertog, E. J. Feskens, P. C. Hollman, M. B. Katan and D. Kromhout, Dietary flavonoids and cancer risk in the Zutphen Elderly Study, *Nutr. Cancer*, 1994, **22**, 175–184.

27 D. R. Jacobs Jr., K. A. Meyer, L. H. Kushi and A. R. Folsom, Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: The Iowa women's health study, *Am. J. Clin. Nutr.*, 1998, **68**, 248–257.

28 N. Shivappa, A. E. Prizment, C. K. Blair, D. R. Jacobs, S. E. Steck and J. R. Hébert, Dietary Inflammatory Index and Risk of Colorectal Cancer in the Iowa Women's Health Study, *Cancer Epidemiol. Biomarkers Prev.*, 2014, **23**, 2383–2392.

29 B. Holst and G. Williamson, Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants, *Curr. Opin. Biotechnol.*, 2008, **19**, 73–82.

30 J. A. Domínguez-Avila, A. Wall-Medrano, G. R. Velderrain-Rodríguez, C.-Y. O. Chen, N. J. Salazar-López, M. Robles-Sánchez and G. A. González-Aguilar, Gastrointestinal interactions, absorption, splanchnic metabolism and pharmacokinetics of orally ingested phenolic compounds, *Food Funct.*, 2017, **8**, 15–38.

31 P. Etcheverry, M. A. Grusak and L. E. Fleige, Application of in vitro bioaccessibility and

bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B 6, B 12, D, and E, *Front. Physiol.*, 2012, **3**, 1–22.

32 J. R. Taylor and K. G. Duodu, Effects of processing sorghum and millets on their phenolic phytochemicals and the implications of this to the health-enhancing properties of sorghum and millet food and beverage products, *J. Sci. Food Agric.*, 2015, **95**, 225–237.

33 E. S. M. Abdel-Aal, T. M. Choo, S. Dhillon and I. Rabalski, Free and bound phenolic acids and total phenolics in black, blue, and yellow barley and their contribution to free radical scavenging capacity, *Cereal Chem.*, 2012, **89**, 198–204.

34 E. S. M. Abdel-Aal, P. Hucl, J. Shipp and I. Rabalski, Compositional differences in anthocyanins from blue- and purple-grained spring wheat grown in four environments in central Saskatchewan, *Cereal Chem.*, 2016, **93**, 32–38.

35 L. Yu and T. Beta, Identification and antioxidant properties of phenolic compounds during production of bread from purple wheat grains, *Molecules*, 2015, **20**, 15525–15549.

36 E.-S. M. Abdel-Aal and I. Rabalski, Bioactive Compounds and their Antioxidant Capacity in Selected Primitive and Modern Wheat Species, *Open Agric. J.*, 2008, **2**, 7–14.

37 L. Alvarez-Jubete, E. K. Arendt and E. Gallagher, Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients, *Trends Food Sci. Technol.*, 2010, **21**, 106–113.

38 G. Bottega, R. Caramanico, M. Lucisano, M. Mariotti, L. Franzetti and M. Ambrogina Pagani, The debranning of common wheat (Triticum aestivum L.) with innovative abrasive rolls, *J. Food Eng.*, 2009, **94**, 75–82.

39 J. E. Dexter and P. J. Wood, Recent applications of debranning of wheat before milling, *Trends Food Sci. Technol.*, 1996, **7**, 35–41.

40 Y. Hemery, X. Rouau, V. Lullien-Pellerin, C. Barron and J. Abecassis, Dry processes to develop wheat fractions and products with enhanced nutritional quality, *J. Cereal Sci.*, 2007, **46**, 327–347.

41 M. Blandino, V. Sovrani, F. Marinaccio, A. Reyneri, L. Rolle, S. Giacosa, M. Locatelli, M. Bordiga, F. Travaglia, J. D. Coïsson and M. Arlorio, Nutritional and technological quality of bread enriched with an intermediated pearled wheat fraction, *Food Chem.*, 2013, **141**, 2549–2557.

42 M. Zanoletti, P. Abbasi Parizad, V. Lavelli, C. Cecchini, P. Menesatti, A. Marti and M. A. Pagani,

Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production, *LWT - Food Sci. Technol.*, 2017, **75**, 663–669.

43 D. Martini, M. G. D'Egidio, I. Nicoletti, D. Corradini and F. Taddei, Effects of durum wheat debranning on total antioxidant capacity and on content and profile of phenolic acids, *J. Funct. Foods*, 2015, **17**, 83–92.

44 V. Verardo, A. M. Gómez-Caravaca, E. Marconi and M. F. Caboni, Air classification of barley flours to produce phenolic enriched ingredients: Comparative study among MEKC-UV, RP-HPLC-DAD-MS and spectrophotometric determinations, *LWT - Food Sci. Technol.*, 2011, **44**, 1555–1561.

45 K. X. Zhu, S. Huang, W. Peng, H. F. Qian and H. M. Zhou, Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber, *Food Res. Int.*, 2010, **43**, 943–948.

46 F. Hübner and E. K. Arendt, Germination of cereal grains as a way to improve the nutritional value: a review, *Crit. Rev. Food Sci. Nutr.*, 2013, **53**, 853–861.

47 K. Katina, K. H. Liukkonen, A. Kaukovirta-Norja, H. Adlercreutz, S. M. Heinonen, A. M. Lampi, J. M. Pihlava and K. Poutanen, Fermentation-induced changes in the nutritional value of native or germinated rye, *J. Cereal Sci.*, 2007, **46**, 348–355.

48 L. Zhang, W. Gao, X. Chen and H. Wang, The effect of bioprocessing on the phenolic acid composition and antioxidant activity of wheat bran, *Cereal Chem.*, 2014, **91**, 255–261.

49 N. Mateo Anson, A. M. Aura, E. Selinheimo, I. Mattila, K. Poutanen, R. van den Berg, R. Havenaar, A. Bast and G. R. M. M. Haenen, Bioprocessing of wheat bran in whole wheat bread increases the bioavailability of phenolic acids in men and exerts antiinflammatory effects ex vivo, *J. Nutr.*, 2011, **141**, 137–143.

50 H. Boskov Hansen, M. F. Andreasen, M. M. Nielsen, L. M. Larsen, K. E. Bach Knudsen, A. S. Meyer, L. P. Christensen and A. Hansen, Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making, *Eur. Food Res. Technol.*, 2002, **214**, 33–42.

51 I. Konopka, M. Tańska, A. Faron and S. Czaplicki, Release of free ferulic acid and changes in antioxidant properties during the wheat and rye bread making process, *Food Sci. Biotechnol.*, 2014, **23**, 831–840.

52 H. R. Sørensen, A. S. Meyer and S. Pedersen, Enzymatic hydrolysis of water-soluble wheat

arabinoxylan. 1. Synergy between α-L-arabinofuranosidases, endo-1,4-β-xylanases, and β-xylosidase activities, *Biotechnol. Bioeng.*, 2003, **81**, 726–731.

53 J. Moore, Z. Cheng, L. Su and L. Yu, Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran, *J. Agric. Food Chem.*, 2006, **54**, 9032–9045.

54 M. S. Butt, M. Tahir-Nadeem, Z. Ahmad and M. T. Sultan, Xylanases and their applications in baking industry, *Food Technol. Biotechnol.*, 2008, **46**, 22–31.

55 F. Shahidi and M. Naczk, in *Food phenolics: Sources, chemistry, effects and applications*, Technomic Publisher, Lancester, PA, 1995, pp. 171–191.

56 C. M. G. C. Renard, A. Baron, S. Guyot and J. F. Drilleau, Interactions between apple cell walls and native apple polyphenols: Quantification and some consequences, *Int. J. Biol. Macromol.*, 2001, **29**, 115–125.

57 M. P. Almajano, M. E. Delgado and M. H. Gordon, Changes in the antioxidant properties of protein solutions in the presence of epigallocatechin gallate, *Food Chem.*, 2007, **101**, 126–130.

58 E. S. M. Abdel-Aal and I. Rabalski, Effect of baking on free and bound phenolic acids in wholegrain bakery products, *J. Cereal Sci.*, 2013, **57**, 312–318.

59 Y. Lu, D. Luthria, E. P. Fuerst, A. M. Kiszonas, L. Yu and C. F. Morris, Effect of processing on phenolic composition of dough and bread fractions made from refined and whole wheat flour of three wheat varieties, *J. Agric. Food Chem.*, 2014, **62**, 10431–10436.

60 G. M. Jackson and R. C. Hoseney, Effect of endogenous phenolic acids on the mixing properties of wheat flour doughs, *J. Cereal Sci.*, 1986, **4**, 79–85.

61 K. G. Duodu, Effects of Processing on Phenolic Phytochemicals in Cereals and Legumes, *Cereal Foods World*, 2014, **59**, 64–70.

62 R. Hilhorst, B. Dunnewind, R. Orsel, P. Stegeman, T. Vliet, H. Gruppen and H. A. Schols, Baking Performance, Rheology, and Chemical Composition of Wheat Dough and Gluten Affected by Xylanase and Oxidative Enzymes, *J. Food Sci.*, 1999, **64**, 808–813.

63 T. M. Dordević, S. S. Šiler-Marinković and S. I. Dimitrijević-Branković, Effect of fermentation on antioxidant properties of some cereals and pseudo cereals, *Food Chem.*, 2010, **119**, 957–963.

64 K.-H. Liukkonen, K. Katina, A. Wilhelmsson, O. Myllymaki, A.-M. Lampi, S. Kariluoto, V.

Piironen, S.-M. Heinonen, T. Nurmi, H. Adlercreutz, A. Peltoketo, J.-M. Pihlava, V. Hietaniemi and K. Poutanen, Process-induced changes on bioactive compounds in whole grain rye, *Proc. Nutr. Soc.*, 2003, **62**, 117–122.

65 A. S. Hole, I. Rud, S. Grimmer, S. Sigl, J. Narvhus and S. Sahlstrøm, Improved bioavailability of dietary phenolic acids in whole grain barley and oat groat following fermentation with probiotic Lactobacillus acidophilus, Lactobacillus johnsonii, and Lactobacillus reuteri, *J. Agric. Food Chem.*, 2012, **60**, 6369–6375.

66 M. Vogrinčič, M. Timoracka, S. Melichacova, A. Vollmannova and I. Kreft, Degradation of rutin and polyphenols during the preparation of tartary buckwheat bread, *J. Agric. Food Chem.*, 2010, **58**, 4883–4887.

67 L. Alvarez-Jubete, H. Wijngaard, E. K. Arendt and E. Gallagher, Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking, *Food Chem.*, 2010, **119**, 770–778.

68 P. Gélinas and C. M. McKinnon, Effect of wheat variety, farming site, and bread-baking on total phenolics, *Int. J. Food Sci. Technol.*, 2006, **41**, 329–332.

69 N. Mateo Anson, R. van den Berg, R. Havenaar, A. Bast and G. R. M. M. Haenen, Bioavailability of ferulic acid is determined by its bioaccessibility, *J. Cereal Sci.*, 2009, **49**, 296–300.

70 V. Menga, C. Fares, A. Troccoli, L. Cattivelli and A. Baiano, Effects of genotype, location and baking on the phenolic content and some antioxidant properties of cereal species, *Int. J. Food Sci. Technol.*, 2010, **45**, 7–16.

71 A. Michalska, M. Amigo-Benavent, H. Zielinski and M. D. del Castillo, Effect of bread making on formation of Maillard reaction products contributing to the overall antioxidant activity of rye bread, *J. Cereal Sci.*, 2008, **48**, 123–132.

72 A. Angioloni and C. Collar, Polyphenol composition and 'in vitro' antiradical activity of single and multigrain breads, *J. Cereal Sci.*, 2011, **53**, 90–96.

73 M. Alminger, A.-M. Aura, T. Bohn, C. Dufour, S. N. El, A. Gomes, S. Karakaya, M. C. Martínez-Cuesta, G. J. McDougall, T. Requena and C. N. Santos, In Vitro Models for Studying Secondary Plant Metabolite Digestion and Bioaccessibility, *Compr. Rev. Food Sci. Food Saf.*, 2014, **13**, 413–

436.

74 M. Dall'Asta, L. Bresciani, L. Calani, M. Cossu, D. Martini, C. Melegari, D. Del Rio, N. Pellegrini, F. Brighenti and F. Scazzina, In vitro bioaccessibility of phenolic acids from a commercial aleurone-enriched bread compared to a whole grain bread, *Nutrients*, 2016, **8**, 42.

75 D. Szawara-Nowak, N. Bączek and H. Zieliński, Antioxidant capacity and bioaccessibility of buckwheat-enhanced wheat bread phenolics, *J. Food Sci. Technol.*, 2016, **53**, 621–630.

76 V. M. Koistinen, E. Nordlund, K. Katina, I. Mattila, K. Poutanen, K. Hanhineva and A.-M. Aura, Effect of bioprocessing on the in vitro colonic microbial metabolism of phenolic acids from rye bran fortified breads, *J. Agric. Food Chem.*, 2017, **65**, 1854–1864.

77 E. Mandak and L. Nyström, Influence of baking and in vitro digestion on steryl ferulates from wheat, *J. Cereal Sci.*, 2013, **57**, 356–361.

78 C. Collar and A. Angioloni, Nutritional and functional performance of high β-glucan barley flours in breadmaking: Mixed breads versus wheat breads, *Eur. Food Res. Technol.*, 2014, **238**, 459–469.

79 H. Verhoeckx, K., Cotter, P., López-Expósito, I., Kleiveland, C., Lea, T., Mackie, A., Requena, T., Swiatecka, D., Wichers, *The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models*, Springer, London, 2015.

80 M. Minekus, P. Marteaul, R. Havenaarl and J. H. J. Huis in't Veld, A Multicompartmental Dynamic Computer- controlled Model Simulating the Stomach and Small Intestine, *Atla*, 1995, **23**, 197–209.

81 M. Larsson, M. Minekus and R. Havenaar, Estimation of the bioavailability of iron and phosphorus in cereals using a dynamic in vitro gastrointestinal model, *J. Sci. Food Agric.*, 1997, **74**, 99–106.

82 M. Verwei, A. P. Freidig, R. Havenaar and J. P. Groten, Predicted serum folate concentrations based on in vitro studies and kinetic modeling are consistent with measured folate concentrations in humans, *J. Nutr.*, 2006, **136**, 3074–3078.

83 Y. M. Hemery, N. M. Anson, R. Havenaar, G. R. M. M. Haenen, M. W. J. Noort and X. Rouau, Dry-fractionation of wheat bran increases the bioaccessibility of phenolic acids in breads made from processed bran fractions, *Food Res. Int.*, 2010, **43**, 1429–1438.

84 N. Mateo Anson, E. Selinheimo, R. Havenaar, A. M. Aura, I. Mattila, P. Lehtinen, A. Bast, K. Poutanen and G. R. M. M. Haenen, Bioprocessing of wheat bran improves in vitro bioaccessibility

and colonic metabolism of phenolic compounds, *J. Agric. Food Chem.*, 2009, **57**, 6148–6155.

85 H. Zieliński and H. Kozłowska, Antioxidant Activity and Total Phenolics in Selected Cereal Grains and Their Different Morphological Fractions, *J. Agric. Food Chem.*, 2000, **48**, 2008–2016.

86 F. Saura-Calixto, J. Serrano and I. Goñi, Intake and bioaccessibility of total polyphenols in a whole diet, *Food Chem.*, 2007, **101**, 492–501.

87 M. Zaupa, F. Scazzina, M. Dall'Asta, L. Calani, D. Del Rio, M. A. Bianchi, C. Melegari, P. De Albertis, G. Tribuzio, N. Pellegrini and F. Brighenti, In vitro bioaccessibility of phenolics and vitamins from durum wheat aleurone fractions, *J. Agric. Food Chem.*, 2014, **62**, 1543–1549.

88 C. Antoine, S. Peyron, V. Lullien-Pellerin, J. Abecassis and X. Rouau, Wheat bran tissue fractionation using biochemical markers, *J. Cereal Sci.*, 2004, **39**, 387–393.

89 V. Van Craeyveld, U. Holopainen, E. Selinheimo, K. Poutanen, J. A. Delcour and C. M. Courtin, Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation, *J. Agric. Food Chem.*, 2009, **57**, 8467–8473.

90 L. R. Brewer, J. Kubola, S. Siriamornpun, T. J. Herald and Y. C. Shi, Wheat bran particle size influence on phytochemical extractability and antioxidant properties, *Food Chem.*, 2014, **152**, 483–490.

91 Y. Hemery, U. Holopainen, A. M. Lampi, P. Lehtinen, T. Nurmi, V. Piironen, M. Edelmann and X. Rouau, Potential of dry fractionation of wheat bran for the development of food ingredients, part II: Electrostatic separation of particles, *J. Cereal Sci.*, 2011, **53**, 9–18.

92 M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrì, R. Boutrou, F. M. Corredig, D. Dupont, F. C. Dufour, L. Egger, M. Golding, L. S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. Mcclements, O. Enard, I. Recio, C. N. Santos, R. P. Singh, G. E. Vegarud, M. S. J. Wickham, W. Weitschies and A. Brodkorb, A standardised static in vitro digestion method suitable for food – an international consensus, *Food Funct.*, 2014, **5**, 1113–1124.

93 M. D'Archivio, C. Filesi, R. Varì, B. Scazzocchio and R. Masella, Bioavailability of the polyphenols: Status and controversies, *Int. J. Mol. Sci.*, 2010, **11**, 1321–1342.

94 L. Bresciani, F. Scazzina, R. Leonardi, E. Dall'Aglio, M. Newell, M. Dall'Asta, C. Melegari, S. Ray,

F. Brighenti and D. Del Rio, Bioavailability and metabolism of phenolic compounds from wholegrain wheat and aleurone-rich wheat bread, *Mol. Nutr. Food Res.*, 2016, **60**, 2343–2354.

95 J. Lappi, A.-M. Aura, K. Katina, E. Nordlund, M. Kolehmainen, H. Mykkänen and K. Poutanen, Comparison of postprandial phenolic acid excretions and glucose responses after ingestion of breads with bioprocessed or native rye bran, *Food Funct.*, 2013, **4**, 972–981.

96 H. Harder, I. Tetens, M. B. Let and A. S. Meyer, Rye bran bread intake elevates urinary excretion of ferulic acid in humans, but does not affect the susceptibility of LDL to oxidation ex vivo, *Eur. J. Nutr.*, 2004, **43**, 230–236.

97 K. S. Juntunen, W. M. Mazur, K. H. Liukkonen, M. Uehara, K. S. Poutanen, H. C. Adlercreutz and H. M. Mykkänen, Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women, *Br. J. Nutr.*, 2000, **84**, 839–846.

98 L. Dykes and L. Rooney, Phenolic Compounds in Cereal Grains and Their Health Benefits, *Cereal Foods World*, 2007, **52**, 105–111.

99 N. Rosa-Sibakov, K. Poutanen and V. Micard, How does wheat grain, bran and aleurone structure impact their nutritional and technological properties?, *Trends Food Sci. Technol.*, 2015, **41**, 118–134.

100 K. Poutanen, L. Flander and K. Katina, Sourdough and cereal fermentation in a nutritional perspective, *Food Microbiol.*, 2009, **26**, 693–699.

Table. 1 Potential strategies to increase bioaccessibility and bioavailability of phenolic compounds in bread

STRATEGY			REASON/MECHANISM	References
Raw materials	Type of grain/cereal	Whole grains	Keeping all the anatomic parts of the kernel, where phenolic compounds are located	Hemery *et al*. (2007)[40]
		Rye, Barley	Raw material naturally rich in phenolic compounds	Dykes and Rooney (2007)[98]
		Minor cereals		Taylor and Duodu (2015)[32]
		Pseudocereals		Alvarez-Jubete *et al*. (2010a)[67]
		Ancient grains		Abdel-Aal and Rabalski (2008)[36]
		Pigmented grains		Abdel-Aal *et al*. (2012)[33] Abdel-Aal *et al*. (2016)[34] Yu and Beta (2015)[35]
	Selected fractions	Bran	Anatomic parts of the kernel, rich in phenolic compounds	Rosa-Sibakov *et al*. (2015)[99]
		Aleurone layer		
Pre-processing	Fractionation	De-branning	Selection of phenolic-rich fractions	Blandino *et al*. (2013)[41] Martini *et al*. (2015)[43] Zanoletti *et al*. (2017)[42]
	Physical treatment	Air classification	Selection of phenolic-rich layers	Verardo *et al*. (2011)[44]
	Mechanical treatment	Micronization	Ultrafine grinding which damages the fiber matrix and increases the phenolic compounds available for extraction	Zhu *et al*. (2010)[45]
	Bio-technological processes	Germination	Metabolic changes and/or increase in extractability by the activation of endogenous enzymes which break the bonds of bound phenolic compounds	Hubner and Arendt (2013)[46] Alvarez-Jubete *et al*. (2010b)[67]
		Fermentation/leavening	Release of insoluble bound phenolic compounds by activity of exogenous enzymes	Katina *et al*. (2007)[47] Zhang *et al*. (2014)[48] Poutanen *et al*. (2009)[100]

		Enzymatic treatment	Addition of enzymes which act to increase free phenolic compounds available for extraction	Sørensen *et al*. (2003)[52] Moore *et al*. (2006)[53]
Bread-making process	Mixing and kneading		Release of bound phenolic compounds into free forms by mechanical action and/or activation of oxygenase and peroxidase	Hilhorst *et al*. (1999)[62] Abdel-Aal and Rabalski (2013)[58]
	Fermentation/ Leavening	Length of fermentation	Prolonged fermentation time increase the phenolic compounds available for extraction	Yu and Beta (2015)[35]
		Type of fermentation (sourdough vs dry yeast)	Increase in the release of insoluble bound phenolic compounds during sourdough fermentation favoured by the lowering of pH	Boskov Hansen *et al*. (2002)[50] Konopka *et al*. (2014)[51]
	Baking	Temperature	Possible decrease in phenolic content due to degradation (thermal labile) Possible increase in phenolic bioaccessibility due to the release resulting from intense heat E.g. The upper crust, exposed to the greatest heat, generally has the highest level of phenolic compounds	Vogrincic et al. (2010)[66] Alvarez-Jubete et al. (2010)[67] Lu et al. (2014)[59] Gélinas and McKinnon (2006)[68] You and Beta (2015)[35]
		Maillard Reactions	May result in newly generated phenolic compounds	Gelinas and McKinnon (2006)[68] Michalska et al. (2008)[71]
		Time	No known effect	Gélinas and McKinnon (2006)[68]

Table 2: Summary of in vitro studies investigating the bioaccessibility of PAs resulting from the alterations to the bread-making process

Reference (Method)	Type of grain	Type of bread	Phenols analyzed	Initial phenolic content‡		Main findings	
				Total	Free	Net Content	% bioaccessibility
Mateo Anson *et al.* (2009)[69] (Dynamic- TIM)	Wheat	a) White bread b) Aleurone-enriched bread (50% flour replacement; 22% in final dough)	- FA	µg/g FA a) 33.5 b) 2290	µg/g FA a) 2.4 b) 20	a) not detectable b) 0.69 mg free	a) not detectable b) 0.57% free
Angioloni and Collar (2011)[72] (Static)	Wheat, oat, rye, buckwheat	a) White wheat bread b) Buckwheat bread c) Rye bread d) Oat bread e) Blend 15%, Multigrain bread (oat:rye:buckwheat: wheat 15:15:15:55): f) Blend 20%, Multigrain bread (oat:rye:buckwheat: wheat 20:20:20:40): g) Blend 25%, Multigrain bread (oat:rye:buckwheat: wheat 25:25:25:25)	- TPC	mg GAE/kg a) 685 b) 808 c) 536 d) 643 e) 592 f) 745 g) 916	n/a	GAE mg/kg a) 401 b) 366 c) 334 d) 264 e) 472 f) 549 g) 504	a) 58% b) 45% c) 62% d) 41% e) 80% f) 74% g) 55%
Collar and Angioloni (2014)[78] (Static)	Wheat, barley	a) White wheat bread b) 40% barley bread (40% wheat replaced with commercial barley flour) c) 40% high beta-glucan barley bread (40% wheat replaced with high β-glucan barley flour)	- TPC	mg/100g dw in flour a) 713 b) 1003 c) 2197	n/a	mg/100g bread as is a) 598 b) 597 c) 857	a) ~84%* b) ~60%* c) 42%* **based on initial content in flours*
Szawara-Nowak *et al.* (2016)[75] (Static)	Wheat, buckwheat	a) White wheat bread b) Dark wheat bread c) White wheat bread with white buckwheat flour	- TPC	a) 0.38 mg rutin eq./g dw b) 1.8 mg rutin eq./g dw	n/a	Soluble fraction: a) ~9 mg rutin eq./g dw b) ~9 mg rutin eq./g dw	*(Extrapolated)* a) ~20 folds b) ~5 folds

		(substitution from 10% to 50%) d) White wheat bread with white roasted buckwheat groats (substitution from 10% to 50%) e) Dark wheat bread with white buckwheat flour (substitution from 10% to 50%) f) Dark wheat bread with white roasted buckwheat groats (substitution from 10% to 50%)		c) ~8-fold increase with 50% substitution as compared to a) d) ~11-fold increase with 50% substitution as compared to a) e) f) increase from 1.5 to 3 times as compared to b)		c) up to ~11 mg rutin eq./g dw (50% substitution) d) up to ~12.5 mg rutin eq./g dw (50% substitution) e) up to ~10.5 mg rutin eq./g dw (50% substitution) f) up to ~10.5 mg rutin eq./g dw (50% substitution)	c) ~3.5 to ~6 folds d) ~3 to ~5 folds e) ~3 to ~6 folds f) ~2.5 to ~4.5 folds
Dall'Asta *et al.* (2016)[74] (Static)	Wheat	a) Whole grain bread (commercial) b) Aleurone-enriched bread (commercial)	- FA - CA - SA - CFA	mg/100g dw a) 144.78 FA 1.51 CA 3.08 SA 0.83 CFA b) 70.67 FA 0.87 CA 3.96 SA 0.28 CFA	mg/100g dw a) 0.71 FA 0.02 CA ND SA 0.04 CFA b) 0.41 FA 0.05 CA 0.12 SA 0.02 CFA	mg/100g dw *(calculated)* a) 18.97 FA 0.15 CA 0.99 SA 0.16 CFA b) 28.76 FA 0.26 CA 3.15 SA 0.23 CFA	a) 13.1% FA 10.1% CA 32.2% SA 19.2% CFA b) 40.7% FA 29.5% CA 79.5% SA 83.3% CFA
Mateo Anson *et al.* (2009)[84] (Dynamic, TIM)	Wheat	a) White bread b) Whole-meal bread c) Whole-meal bread with native wheat bran d) Whole-meal bread with	- FA - CA - SA	µg/g dw a) 86 FA 2 CA 9 SA b) 810 FA 20 CA 70 SA c) 1300 FA 40 CA 130 SA d) 1300 FA	µg/g free a) 3.6 FA 0.8 CA 0.9 SA b) 13 FA 0.9 CA 3.5 SA c) 12 FA 1.2 CA 4.6 SA d) 42 FA	µg/g *(calculated)* a) 4.2 FA n/a CA n/a SA b) 8.91 FA n/a CA n/a SA c) 14.3 FA 2.08 CA 2.73 SA	a) 4.9% FA n/a CA n/a SA b) 1.1% FA n/a CA n/a SA c) 1.1% FA 5.2% CA 2.1% SA d) 2.2% FA

		fermented wheat bran e) Whole-meal bread with fermented and enzymatic treated bran		40 CA 130 SA e) 1300 FA 40 CA 130 SA	1.5 CA 9.6 SA e) 100 FA 3.0 CA 9.9 SA	d) 28.6 FA n/a CA n/a SA e) 71.5 FA 3.96 CA 6.5 SA	n/a CA n/a SA e) 5.5% FA 9.9% CA 5.0 % SA
Hemery *et al.* (2010)[83] (Dynamic, Tiny-TIM)	Wheat	a) White bread b) Whole bread (100% wheat grain) c) "Amb, medium" d) "Amb, fine" e) "Amb, ultrafine" f) "Cyro, ultrafine" g) "FES positive" h) "FES middle" i) "FES negative" ** coarse bran increasingly processed from c-f; FES=cryo particles separated by charge; middle is mixed*	- FA - CA - SA	µg/g dw a) 62.6 FA 2.5 CA 3.2 SA b) 793.2 FA 23.5 CA 40.7 SA c) 865.4 FA 24.7 CA 44.7 SA d) 898.5 FA 26.6 CA 41.0 SA e) 899.4 FA 26.8 CA 42.5 SA f) 869.8 FA 25.6 CA 44.2 SA g)1072.7 FA 30.9 CA 41.3 SA h)763.8 FA 22.0 CA 48.1 SA i) 625.8 FA 25.9 CA 38.1 SA	µg/g dw a) 1.2 FA 0.12 CA 0.09 SA b) 8.2 FA 0.28 CA 1.68 SA c) 12.4 FA 0.48 CA 1.17 SA d) 14.6 FA 0.55 CA 1.21 SA e) 15.6 FA 0.53 CA 1.17 SA f) 16.4 FA 0.56 CA 1.21 SA g) 12.4 FA 0.47 CA 1.36 SA h) 17.9 FA 0.65 CA 1.01 SA i) 15.5 FA 0.56 CA 1.28 SA	µg/g dw a) 6.4 FA 0.87 CA 3.3 SA b) 22.7 FA 1.38 CA 18.1 SA c) 21.7 FA 1.49 CA 12.2 SA d) 26.2 FA 1.83 CA 13.8 SA e) 30.7 FA 2.47 CA 15.4 SA f) 26.7 FA 3.32 CA 11.7 SA g) 31.8 FA 3.59 CA 1.67 SA h) 23.0 FA 3.51 CA 9.8 SA i) 32.1 FA 3.93 CA 22.7 SA	a) 10.2% FA 35% CA 102% SA b) 2.9% FA 5.9% CA 45% SA c) 2.5% FA 6.0% CA 27% SA d) 2.9% FA 6.9% CA 33% SA e) 3.4% FA 9.2% CA 36% SA f) 3.1% FA 13% CA 25% SA g) 3.0% FA 12% CA 40% SA h) 3.0% FA 16% CA 20% SA i) 5.1% FA 15% CA 60% SA
Mandak &	Wheat	a) Whole grain bread	- Steryl ferulates (SF)	µg/g dw SF a) 51.2	n/a	µg/g *(calculated)*	a) 0.01%

Nystrom (2013)[77] (Static)		b) Whole grain bread with xylanase c) Whole grain bread with cellulase d) Whole grain bread with xylanase and cellulase e) Baking flour based bread f) Baking flour based bread with xylanase g) Baking flour based bread with cellulase h) Baking flour based bread with xylanase and cellulase		b) 53.0 c) 52.7 d) 52.1 e) 21.7 f) 18.3 g) 19.6 h) 17.0		a) 0.005 b) 0.016 c) 0.016 d) 0.130 e) 0.020 f) 0.004 g) 0.010 h) 0.017	b) 0.03% c) 0.03% d) 0.25% e) 0.09% f) 0.02% g) 0.05% h) 0.10%
Koistinen *et al.* (2017)[76] (Static)	Wheat, rye	a) Bread with native rye bran b) Bread with bioprocessed (enzymatic treatment and fermentation) rye bran	- FA - CA - SA	mg/g a) 1.082 FA 0.037 CA 0.242 SA b) 1.188 FA 0.036 CA 0.258 SA	mg/g a) 0.016 FA 0.001 CA 0.008 SA b) 0.162 FA 0.004 CA 0.029 SA	mg/g absorbed a) 0.549 FA 0.031 CA 0.146 SA b) 1.051 FA 0.034 CA 0.236 SA	a) 51% FA 84% CA 60% SA b) 88% FA 94% CA 91%SA

CA, *p*-coumaric acid; CAF: caffeic acid; FA, ferulic acid; GAE, gallic acid equivalents; PA, phenolic acid; SA, sinapic acid; TPC, total phenolic acid content; dw: dry weight.

ł as measured in the bread pre-digestion, unless otherwise indicated

* % of bioaccessibility was calculated as the percentage of phenolic compounds in the residue after *in vitro* digestion compared to the initial amount of total PAs/TPC in bread

Table 3. Human studies investigating the bioavailability* and the recovery of bread-derived polyphenols

Reference	Test Samples	Type of study	Subjects	Analysis	Findings
Single-dose dietary intervention					
Bresciani *et al*. (2016)[94]	- WGB: 94 g of wholegrain bread, 0.926 mg/g total FA; - AB-94: 94 g of a commercial wheat bread enriched in aleurone fraction (6% w/w), 0.458 mg/g total FA; - AB-190: 190 g of a commercial bread enriched in aleurone fraction (6% w/w), ~ 0.458 mg/g total FA.	Randomized, crossover, single-dose, single-blind, intervention, at least 1-week washout period.	15 healthy subjects, mean age 26 ± 4 y, mean BMI 21 ± 3 kg/m^2.	- Plasma ferulic acid- 4'-O-sulfate, dihydroferulic acid-4'-O-sulfate: 0, 0.5, 1, 2, 4, 7 and 24 h; - Urinary ferulic acid-4'-O-sulfate, dihydroferulic acid-4'-O-sulfate, and dihydroferulic acid-O-glucuronide, feruloylglycine, dihydrocaffeic acid sulfate, sinapic acid sulfate, vanillic acid-4-O-sulfate and hydroxybenzoic acid sulfate : 0-3, 3-6, 6-10, 10-14, 14-24, 24-28, 28-34 and 34-48 h.	Plasma phenolic acid metabolites: - Ferulic acid- 4'-O-sulfate C_{max} - WGB 84.3 nM; - AB-94 55.5 nM; - AB-190 76.6 nM. - Dihydroferulic acid-4'-O-sulfate C_{max} - WGB 9.2 nM; - AB-94 9.5 nM; - AB-190 11.9 nM. - No significantly differences in C_{max} among the tested bread for ferulic acid- 4'-O-sulfate and dihydroferulic acid-4'-O-sulfate. Urine metabolites: - Cumulative 48 h excretion - Dihydrocaffeic acid sulfate: - AB-94: ~2 µmol; - AB-190: ~2 µmol; - WGB: ~0.8 µmol. - Sinapic acid sulfate: - AB-94: ~2 µmol; - AB-190: ~1 µmol; - WGB: ~1 µmol. - Significantly higher ($p< 0.05$) cumulative 48 h excretion of dihydrocaffeic acid sulfate in AB-94 and AB-190 compared to WGB; no statistical differences between AB breads; - Significantly higher ($p< 0.05$) cumulative excretion of sinapic acid sulfate in AB-190 compared to AB-90 and WGB; no statistical differences between AB-90 and WGB breads. - % Bioavailability: - AB-94 +8%; - AB-190: +4%;

					- WGB: +4%. - 2-fold higher (p< 0.05) bioavailability of the sum of FA in AB-94 compared to WGB and AB-190 WGB. - ~2-fold higher bioavailability of the sum of FA in AB-190 compared to WGB (not significant).
Lappi *et al.* (2013)[95]	- R bread: 123g commercial wholegrain rye bread (100% rye flour), 0.602 mg/g FA; - WW bread: 109 g white wheat bread, 0.606 mg/g FA; - RB + WW bread: 164 g white wheat bread fortified with native rye bran (35% replacement), 0.713 mg/g FA; - BRB +WW bread: 166 g white wheat bread fortified with bioprocessed rye bran (35% replacement), 0.811 mg/g FA;	Randomized, cross-over, single-dose, intervention, at least 3-day washout period.	15 healthy subjects, mean age 57 y, mean BMI 26 kg/m^2.	- Urinary FA, SA and PA equivalents: the 0–4, 4–12, and 12–24 h.	FA equivalents bioavailability: - BRB+WW: 1%; - RB+WW: 0.4%; - R: 0.4%; - WW: 3.2%. SA equivalent bioavailability: - BRB+WW: 0.6%; - RB+WW: 0.4%; - R: 0.3%; - WW: 2.8%. PA equivalent bioavailability: - BRB+WW: 0.3%; - RB+WW: 0.3%; - R: 0.3%, 0.07; - WW: 3.8%.

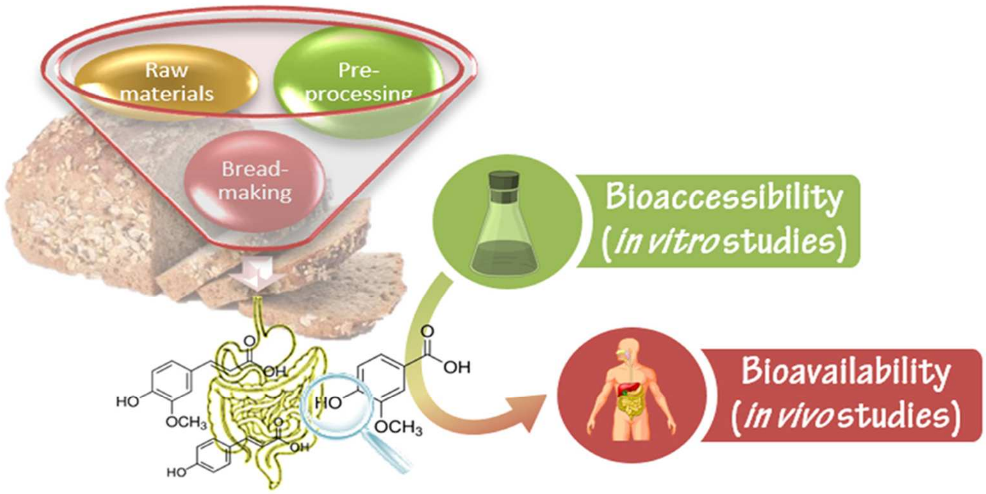
Mateo Anson *et al.* (2011)[49]	- Control Bread: 300 g whole wheat bread containing native bran, 0.767 mg/g FA, 0.057 mg/g sinapic acid, 0.018 mg/g *p*-coumaric acid, 0.017 mg/g vanillic acid;
	- Bioprocessed bread: 300 g bioprocessed bran, 0.733 mg/g FA, 0.057 mg/g, sinapic acid, 0.015 mg/g *p*-coumaric acid, 0.018 mg/g vanillic acid;
		Randomized, single-blind, single dose, cross-over intervention, at least 1-week washout period.	8 healthy men, range age 21-55 y, range BMI 20-30 kg/m^2.	- Plasma ferulic, vanillic and 3,4-dimethoxybenzoic acids relative bioavailability (AUC_{0-t}): 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 9, 12 and 24 h.
				- Urinary FA, SA, CA, VA, and their secondary metabolites: 0 and 24 h.
					Plasma metabolites:
					- FA relative bioavailability (AUC_{0-t}):
					  - Control Bread: 240 µmol*min/L;
					  - Bioprocessed Bread: 640 µmol*min/L.
					- VA relative bioavailability (AUC_{0-t}):
					  - Control Bread: 39 µmol*min/L;
					  - Bioprocessed Bread: 70 µmol*min/L.
					- 3,4-dimethoxybenzoic acid relative bioavailability (AUC_{0-t}):
					  - Control Bread: 5.4 µmol*min/L
					  - Bioprocessed Bread: 9.9 µmol*min/L
					- Significantly higher (p< 0.05) relative bioavailability (AUC_{0-t}) of ferulic acid (2.7-fold), vanillic and 3,4-dimethoxybenzoic acid (1.8-fold each) from bioprocessed bread compared to the control bread.
					
					Urine metabolites:
					- % Recovery FA:
					  - Control Bread: 4%;

					- Bioprocessed Bread: 10%. - % Recovery SA: - Control Bread: 7%; - Bioprocessed Bread: 15%. - % Recovery CA: - Control Bread: 2%; - Bioprocessed Bread: 2%. - % Recovery VA: - Control Bread: 104%; - Bioprocessed Bread: 160%. - 2-fold significantly higher ($p< 0.05$) urinary bioavailability of FA, SA, VA, from bioprocessed bread compared to control bread. - No differences in urinary bioavailability of CA from the tested breads.
Chronic dietary intervention					
Harder *et al.* (2004)[96]	- 250 g control wheat products (Vitacell®), 0 mg FA); - 250 g rye bran enriched products, 0.041 mg/g FA from rye bran - Both the categories included bread, muffin and crisp bread products.	Randomized, crossover intervention, two 6-week interventions, 4-week washout period.	18 healthy postmenopausal women, mean age 63.3±1.2 y, mean BMI 25.1±0.9 kg/m^2	- Urinary FA equivalents: 0-48 h.	- Urinary FA equivalents 24 h-excretion: - Baseline:1.92 mg; - Control wheat: 1.94 mg; - Rye Bran: 4.82 mg; - 1.5-fold higher urinary FA equivalents excretion from rye bran enriched products compared to the baseline (+39.8%, $p= 0.002$) and Vitacell® (+40.2%, $p= 0.001$); - Not significant difference in FA equivalents urinary excretion from Vitacell® products compared to baseline (+1%).
Juntunen *et al.* (2000)[97]	-Wheat bread consumption (Lignans: 0.109 µg/g); -Rye bread consumption (Lignans, 0.888 µg/g). A minimum of 4-5 slices of bread consumption per day was required, no maximum intake indicated.	Randomized, crossover, 2-week run-in, two 4-week interventions, 4-week wash-out.	43 healthy volunteers, mean age 43±2 y, range BMI 20-32 kg/m^2	- Serum ENL concentration: 0 and 4 weeks; - 24 h urinary ENL excretion:0 and 4 weeks. - 24 h urinary ENL concentration:0 and 4 weeks.	- Serum ENL concentration - Baseline: - Men: 28.1 nM; - Women: 39.3 nM. - Wheat bread: - Men: 12.5 nM; - Women: 14.8 nM. - Rye bread: - Men: 25.6 nM; - Women: 39.7 nM. - Significant higher serum ENL concentrations at the end of rye-brad intervention compared to wheat bread one (+51.2% for men, +62.7% for woman, $p <0.05$).

					- Not significant differences in serum ENL concentration at the end of rye- and wheat-brad compared to baseline. - Urinary ENL 24 h-excretion - Wheat bread: - Men: 4.0 µmol; - Women: 3.7 µmol. - Rye bread: - Men: 6.8 µmol; - Women: 7.8 µmol. - Significantly higher (p< 0.05) ENL 24 h-excretion in rye- compared to wheat-bread periods in both men and women. - No correlation between urine ENL and rye bread intake.

AUC_{0-t}: area under the curve; C_{max}: maximum plasma concentration; CA, *p*-coumaric acid; ENL: enterolactones; FA, ferulic acid; SA, sinapic acid; VA, vanillic acid.

[*] % of bioavailability was calculated as % ratio between the amount of the compound in the biological fluid on the amount of the ingested compound.


Raw materials
Pre-processing
Bread-making
Bioaccessibility (in vitro studies)
Bioavailability (in vivo studies)
HO
OCH3
OH
O
HO
OCH3
OH
O
HO
OH
O