Chapter 6

Foods or Bioactive Constituents of Foods as Chemopreventives in Cell Lines After Simulated Gastrointestinal Digestion: A Review

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1. Introduction

Epidemiological studies on the relationship between dietary habits and disease risk have shown that food has a direct impact on health. Indeed, our diet plays a significant role in health and well-being, since unbalanced nutrition or an inadequate diet is known to be a key risk factor for chronic age-related diseases [1]. An example that illustrates this fact is the protective effect of the so-called Mediterranean diet. The lower occurrence of cancer and cardiovascular disease in the population located around the Mediterranean sea has been linked to the dietary habits of the region, in which the components of the diet contain a wide array of molecules with antioxidant and antiinflammatory actions [2].

Many diseases with a strong dietary influence include oxidative damage as an initial event or in an early stage of disease progression [3]. In fact, Western diets (typically dense in fat and energy and low in fiber) are associated with disease risk [4]. Therefore, dietary modification, with a major focus on chronic age-related disease prevention through antioxidant intervention, could be a good and cost-effective strategy [5]. The intake of whole foods and/or new brand developed functional foods rich in antioxidants would be suitable for this purpose. In this sense, dietary antioxidants such as polyphenols, carotenoids and peptides, as well as other bioactive chemopreventive components such as fiber and phytosterols have been regarded to have low potency as bioactive compounds when compared to pharmaceutical drugs, but since they are ingested regularly and in significant amounts as part of the diet, they may have noticeable long-term physiological effects [6].

For decades, the beneficial role of antioxidants was related to the reduction of unwanted and uncontrolled production of reactive oxygen species (ROS), leading to a situation referred to as oxidative stress [7]. Nowadays, the term "antioxidant" has become ambiguous, since it has different connotations for distinct audiences. For instance, for biochemists and nutritionists, the term is related to the scavenging of metabolically generated ROS, while for food scientists the term implies use in retarding food oxidation or for the categorization of foods or substances according to *in vitro* assays of antioxidant capacity, such as the ORAC and TEAC tests [8]. The antioxidant values provided by these assays sometimes have been misinterpreted by both food producers and consumers due to the fact that health claims advertised on the package labeling are directly associated with benefits that include slowing of the aging process and decreasing the risk of chronic disease. Nevertheless, contemporary scientific evidence indicates that total antioxidant capacity measured by currently popular chemical assays may not reflect the actual activity *in vivo,* since none of them take biological processes such as bioavailability, uptake and metabolism into account [9]. Therefore, no *in vitro* assay that determines the antioxidant capacity of a nutritional product describes *in vivo* outcomes, and such testing should not be used to suggest such a connection. In this sense, it is currently recognized that the mechanisms of action of antioxidants *in vivo* might be far more complex than mere radical scavenging - involving interactions with specific proteins central to intracellular signaling cascades [10], and in the specific case of cancer cells there might be a direct antioxidant effect, antiproliferation and anti-survival action, the induction of cell cycle arrest, the induction of apoptosis, antiinflammatory effects and the inhibition of angiogenesis and metastasis [11].

In order to determine and verify the action of these bioactive compounds, it is clear that data from human intervention studies offer the reference standard and the highest scientific evidence considering the bioavailability and bioactivity of a food component, while *in vitro* methods are used as surrogates for prediction [12]. From a physiological perspective, food after consumption undergoes a gastrointestinal digestion process that may affect the native antioxidant potential of the complex mixture of bioactive compounds present in the food matrix before reaching the proximal intestine. *In vitro* methods which apply human simulated digestion models (including or not including colonic fermentation) are considered valuable and useful tools for the estimation of pre-absorptive events (i.e., stability, bioaccessibility) of different food components from distinct food sources, and also for determining the effect which processing may have upon food components bioavailability [13]. In addition, *in vitro* assays combining a simulated gastrointestinal digestion process and cell cultures as pre-clinical models can be useful for unraveling mechanisms of action and for projecting further *in vivo* assays [9]. Nevertheless, in most cases these *in vitro* studies are unrealistic, because they involve single compounds used at high concentrations (pharmacological and not dietary concentrations) far from the low micromolar or nanomolar concentrations detected *in vivo*, or use the bioactive compounds "as they are in food" versus the metabolites or derivatives considered to be the true bioactive compounds, over an extended period of time (up to 120 h). As a result, biological activity may be overestimated, since no account is taken of the possible transformation of these compounds during gastrointestinal digestion with or without colonic fermentation [6]. Likewise, the use of single or

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crude compounds instead of whole foods impedes the detection of synergistic and/or antagonistic actions among bioactive chemopreventive compounds [14, 15].

Taking this background together, and in order to obtain a more precise view of the *in vivo* situation, we propose the use of whole foods or related target bioactive constituents subjected to a human simulated gastrointestinal digestion including or not including colonic fermentation, depending on the nature of the studied compounds, in order to gain better insight from a nutritional/functional point of view of the chemopreventive action derived from foods and bioactive compounds in cell models of disease.

This review introduces the main features of the different *in vitro* gastrointestinal digestion (solubility and dialysis) and colonic fermentation procedures (batch, continuous and continuous with immobilized feces) for studying the bioaccessibility and further bioavailability and bioactivity of nutrients and bioactive compounds. It also includes a definition of the terms: bioavailability including bioaccessibility and bioactivity. Likewise, the main advantages and disadvantages of these *in vitro* methods versus *in vivo* approaches, the improvement of these models with the inclusion of cell lines, and a short comment on the main effects that digestion and/or fermentation have on bioactive compounds are included. On the other hand, a short description is provided of the studies involving the use of human simulated gastrointestinal digestion and/or colonic fermentation procedures, and of the subsequent bioactivity-guided assays with cell line models.

2. Simulated gastrointestinal digestion assays

Bioavailability is a key concept for nutritional effectiveness, irrespective of the type of food considered (functional or otherwise). Only certain amounts of all nutrients or bioactive compounds are available for use in physiological functions or for storage.

The term bioavailability has several working conditions. From the nutritional point of view, bioavailability is defined as the proportion of a nutrient or bioactive compound can be used for normal physiological functions [16]. This term in turn includes two additional terms: bioaccessibility and bioactivity. Bioaccessibility has been defined as the fraction of a compound that is released from its food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption. Bioaccessibility includes the sequence of events that take place during food digestion for transformation into potentially bioaccessible material, absorption/assimilation through epithelial tissue and pre-systemic metabolism. Bioactivity in turn includes events linked to how the bioactive compound is transported and reaches the target tissue, how it interacts with biomolecules, the metabolism or biotransformation it may undergo, and the generation of biomarkers and the physiologic responses it causes [12]. Depending on the *in vitro* method used, evaluation is made of bioaccessibility and/or bioactivity.

In vitro methods have been developed to simulate the physiological conditions and the sequence of events that occur during digestion in the human gastrointestinal tract. In a first step, simulated gastrointestinal digestion (gastric and intestinal stages, and in some cases a salivary stage) is applied to homogenized foods or isolated bioactive compounds in a closed

system, with determination of the soluble component fraction obtained by centrifugation or dialysis of soluble components across a semipermeable membrane (bioaccessible fraction). Simulated gastrointestinal digestion can be performed with static models where the products of digestion remain largely immobile and do not mimic physical processes such as shear, mixing, hydration. Dynamic models can also be used, with gradual modifications in pH and enzymes, and removal of the dialyzed components – thereby better simulating the actual *in vivo* situation. All these systems evaluate the aforementioned term "bioaccessibility", and can be used to establish trends in relative bioaccessibility.

The principal requirement for successfully conducting experimental studies of this kind is to achieve conditions which are similar to the *in vivo* conditions. Temperature, shaking or agitation, and the chemical and enzymatic composition of saliva, gastric juice, duodenal and bile juice are all relevant aspects in these studies. Interactions with other food components must also be taken into account, since they can influence the efficiency of digestion [12, 17]. A recent overview of the different *in vitro* digestion models, sample conditions and enzymes used has been published by Hur et al. [13]. En lipophilic compounds such as carotenoids and phytosterols, it is necessary to form mixed micelles in the duodenal stage through the action of bile salts, phospholipases and colipase. This allows the compounds to form part of the micelles, where they remain until uptake by the enterocytes [18]. In the case of lycopene, during digestion isomerization of trans-lycopene may occur with the disadvantage that trans-isomers are less soluble in bile acid micelles [19]. Salivary and gastric digestion exert no substantial effect on major phenolic compounds. However, polyphenols are highly sensitivity to the mild alkaline conditions in pancreatic digestion, and a good proportion of these compounds can be transformed into other unknown and/or undetected forms [20].

Bioactive compounds such as dietary fiber, carotenoids, polyphenols and phytosterols undergo very limited absorption, and may experience important modifications as a result of actions on the part of the intestinal microbiota. Small intestine *in vitro* models are devoid of intestinal microbes, and are designed to only replicate digestion and absorption processes; as a result, they are unable to provide information on intestinal fermentation processes. The incorporation of colonic/large intestine fermentation offers a better approximation to the *in vivo* situation, and allows us to study the effect/interaction between these compounds and the intestinal microbiota.

In vitro colonic fermentation models are characterized by the inoculation of single or multiple chemostats with fecal microbiota (of rat or human origin) and operated under physiological temperature, pH and anaerobic conditions. There are two types of colonic fermentation models: batch culture and continuous cultures. Batch culture describes the growth of pure or mixed bacterial suspensions in a carefully selected medium without the further addition of nutrients in closed systems using sealed bottles or reactors containing suspensions of fecal material under anaerobic conditions. The advantages of batch fermentation are that the technique is inexpensive, easy to set up, and allows large number of substrates of fecal samples to be tested. However, these models have their weakness in microbiological control and the need to be of short duration in order to avoid the selection of non-representative microbial populations. The technique is useful for fermentation studies, for the investigation of metabolic profiles of short chain

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fatty acids arising from the active metabolism of dietary compounds by the gut microbiota, and especially for substrate digestion evaluation studies [21, 22]. Several of the publications in this field are based on a European interlaboratory study for estimation of the fermentability of dietary fiber *in vitro* [23].

Continuous cultures allow us to control the rate and composition of nutrient feed, bacterial metabolism and the environmental conditions. These models simulate proximal (single-state models) or proximal, transverse and distal colonic regions (multistage models). Continuous cultures are used for performing long-term studies, and substrate replenishment and toxic product removal are facilitated - thereby mimicking the conditions found *in vivo*. The most variable factor in these models is the technique used for fecal inoculation. The use of liquid fecal suspension as inoculum, where the bacterial populations are in the free-cell state, produces rapid washout of less competitive bacteria; as a result, the operation time is less than four weeks. The formation of fecal beads from the immobilization of fecal microbiota in a porous polysaccharide matrix allows release of the microbiota into the culture medium, with better reproduction of the *in vivo* flora and longer fermentation times [21, 22].

Artificial continuous models including host functions/human digestive functions have been developed. Models of this kind control peristaltic movement, pH and gastrointestinal secretions. The SHIME model (Simulated Human Intestinal Microbial Ecosystem) comprises a 5-step multi-chamber reactor simulating the duodenum and jejunum, ileum, cecum and the ascending colon, transverse colon and descending colon [24]. In turn, TIM-1 is an intestinal model of the stomach and small intestine, while TIM-2 is a proximal colon simulator model developed by TNO (*Netherlands Organization for Applied Scientific Research)*. These models have been validated based on human and animal data [25]. They incorporate some host functions; however, they do not reproduce immune modulating and neuroendocrine responses. A remaining challenge is the difficulty of establishing a representative human gut microbiota *in vitro*. Other difficulties are the availability of the system, its cost, the prolonged time involved, its laboriousness, the use of large working volumes, and long residence times.

Combined systems that include the fractions obtained from simulated human digestion (gastrointestinal and/or colonic fermentation) and the incorporation of cell culture-based models allow us to evaluate bioaccessibility (estimate the amount of bioactive compounds assimilated from the bioaccessible fraction by cell culture) and to conduct bioactivity studies. The Caco-2 cell model is the most widely used and validated intestinal epithelium or human colon carcinoma cell model. Although colonic in origin, Caco-2 cells undergo spontaneous differentiation in cell culture to form a monolayer of well-polarized cells at confluence, showing many of the functional and morphological properties of mature human enterocytes (with the formation of microvilli on the brush border membrane, tight intercellular junctions and the excretion of brush border-associated enzymes) [26]. However it must be mentioned that this cell line differs in some aspects from *in vivo* conditions. For example, it does not reproduce the different populations of cells in the gut, such as goblet, Paneth and crypt cells, which are less organized and therefore leakier. Likewise, the model lacks regulatory control by neuroendocrine cells and through the blood [27].

The advantage of these systems versus those which only evaluate the influence of digestion is their greater similarity to the *in vivo* conditions. The combination of *in vitro* human intestinal cell models with *in vitro* digestion models in turn creates an advanced *in vitro* model system where samples obtained from host responses lacking in *in vitro* digestion models can be directly applied to monolayer cell models for host function studies [21].

3. Bioactivity of digested/fermented foods or related target bioactive compounds in cell lines

The chemopreventive properties of bioactive compounds have been investigated in cultured cells exposed to individual compounds. However, gut epithelial cells are more likely to be exposed to complex food matrixes containing mixtures of bioactive and antioxidant *in vivo* compounds [6]. In addition, food matrixes undergo a digestion process that may affect the structure and properties of the bioactive compounds. Therefore, the *in vitro* protective effects of antioxidant bioactive compounds do not necessarily reflect *in vivo* chemoprotection, which is more likely due to the combined effects of all the bioactive components present in the food [28].

A potential cell culture model for cancer or cardiovascular chemoprevention research involving dietary antioxidants (polyphenols, carotenoids and peptides) and other bioactive chemopreventive components such as phytosterols, should include some of the proposed mechanisms of action: inhibition of cell proliferation, induction of tumor suppressor gene expression, induction of cell cycle arrest, induction of apoptosis, antioxidant enzyme induction, and enhanced detoxification, antiinflammatory activities and the inhibition of cholesterol absorption [9, 15, 29, 30]. In addition, other mechanisms of chemoprevention could involve protection against genotoxic compounds or reactive oxygen species [31].

It recently has been stated that the measurement of cellular bioactivity of food samples coupled to *in vitro* digestion can provide information close to the real-life physiological situation [32]. In this sense, we surveyed more than 30 studies conducted in the past 10 years, involving human simulated gastrointestinal digestion and/or colonic fermentation procedures and subsequent bioactivity-guided assays with cell line models. These studies are presented in Tables 1, 2 and 3, which correspond to the mechanism of action related to chemoprevention of digested, fermented or digested plus fermented foods or bioactive constituents in cell lines, respectively.

The chemopreventive effect of digested foods or bioactive constituents in cell lines is summarized in Table 1. From the 22 studies surveyed, and according to the digestion method used, it can be seen that most of them involve solubility (n = 17) versus dialysis (n = 5). Samples used are preferably of vegetal origin (n = 15), the target compounds responsible for the chemopreventive action being polyphenols, antioxidants (in general), antioxidant peptides, lycopene and phytosterols. Furthermore, these compounds are mainly studied in colon-derived cells (as a cancer model when not differentiated, or as an intestinal epithelial model when differentiated). Concentrations tested are physiologically ach-

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ievable in colon cells, since the bioaccessible fractions obtained after digestion are considered to be fractions that can pass through the stomach and small intestine reaching the colon, where they can exert antioxidant activity *in situ* [33]. In addition, polyphenols are studied in neuronal cells, liver-derived cells and lymphocytes. In the case of neuronal cells, the concentrations used (0-6 μM polyphenols) are similar to those reported for dietary polyphenolic-derived metabolites found in plasma (0-4 μM) [34], but for lymphocytes and liver, the concentrations are unknown or higher than expected *in vivo*, respectively. Another aspect to bear in mind is the time of cell exposure to the digested food or bioactive constituents. The range found in these studies is from 30 min to 120 h (this latter timepoint not being expectable from a physiological standpoint).

Bioactive compounds of digested foods present four different but in some cases complementary modes of action: (1) inhibition of cholesterol absorption (phytosterols), and (2) antiproliferative, (3) cytoprotective and (4) antiinflammatory activities (polyphenols and general antioxidants).

1. The inhibition of cholesterol absorption has been reported to be mainly due to competition between phytosterols and cholesterol for incorporation to the micelles as a previous step before absorption by the intestinal epithelial cells [35].
2. Antiproliferative activity has been linked to cell growth inhibition associated to polyphenols [28, 32, 36-38] and lycopene [39], which is mainly regulated by two mechanisms: cell-cycle arrest and apoptosis induction. The cell cycle can be halted at different phases: G_0/G_1 with down-regulation of cyclin D_1 [39], S with down-regulation of cyclins D_1 and B_1 [28, 37] and G_2/M [36]. Apoptosis induction in turn occurs as a result of caspase-3 induction and down-regulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL [39].
3. The cytoprotective effect of polyphenols, peptides and antioxidants against induced oxidative stress is related to the preservation of cell viability [40-47], an increase in the activity of antioxidant enzymes (such as catalase, glutathione reductase or glutathione peroxidase) [41, 43, 47, 48], the prevention of reduced glutathione (GSH) depletion [46, 47, 49], a decrease in intracellular ROS content [46, 50, 51], the maintenance of correct cell cycle progression [41, 43, 47, 52], the prevention of apoptosis [43], and the prevention of DNA damage [42, 51, 52].
4. The antiinflammatory action of peptides and polyphenols is derived from the decrease in the release of proinflammatory cytokines such as IL-8 when cells are stimulated with stressors such as H_20_2 or TNFα [53, 54].

Studies on the chemopreventive effect of foods or isolated bioactive constituents following colonic fermentation or gastrointestinal digestion plus colonic fermentation in cell lines are shown in Tables 2 and 3, respectively. The colonic fermentation procedure used in these assays has always been a batch model, except for one study combining batch and dynamic fermentation. In turn, when gastrointestinal digestion is involved, dialysis has been the method used. Foods of plant origin rich in fiber, and short chain fatty acids (mainly butyrate) and polyphenols as the target compounds have been used in such studies. The use of colon-derived cell lines is common in these assays, which have been performed using phys-

iologically relevant concentrations and time periods of exposure of samples to cells ranging between 24 h and 72 h.

The mechanism of action underlying the treatment of cells with colonic fermented foods or isolated bioactive constituents (see Table 2) mainly comprises antiproliferative activity (i) and/or cytoprotective action (ii). In the first case, antiproliferative activity (i) has been attributed to cell growth inhibition [55-59], mainly due to apoptosis induction [58-59] and/or the up-regulation of genes involved in cell cycle arrest (*p21*) and apoptosis (*WNT2B*) [59]. Studies referred to a cytoprotective effect against oxidative damage (ii) in turn have been linked to the prevention of DNA damage [55, 56] and to the induction of antioxidant enzymes such as glutathione-S-transferase (GST) [56].

The bioactivity observed with the incubation of cells lines with foods or isolated bioactive constituents following gastrointestinal digestion plus colonic fermentation (see Table 3) is derived from antiproliferative activity (i) regulated by cell growth inhibition [60-62], cell cycle arrest [60] and/or apoptosis induction [60, 62], or by a cytoprotective effect against induced oxidative stress (ii) as a result of preservation of cell viability [63], protection against DNA damage [31, 61, 63] and/or induction of antioxidant enzymes such as CAT, GST and sulfotransferase (SULT2B1) [31].

4. Conclusions and future perspectives

From the data here reviewed in disease cell models, it can be concluded that gastrointestinal digestion/colonic fermentation applied to whole foods or isolated bioactive constituents may have potential health benefits derived from cell growth inhibition through the induction of cell-cycle arrest and/or apoptosis, cytoprotection against induced oxidative stress, antiinflammatory activity and the reduction of cholesterol absorption.

Studies conducted with single bioactive compounds are unrealistic from a nutritional and physiological point of view, since they do not take into account physicochemical changes during digestion and possible synergistic activities. Thus, a combined model of human simulated digestion including or not including colonic fermentation (depending on the nature of the studied compounds) with cell lines should be carried out if *in vitro* bioactivity assays with whole foods or bioactive chemopreventive compounds for the prevention of oxidative stress-related diseases are planned.

Although digested/fermented bioactive compounds appear as promising chemopreventive agents, our understanding of the molecular and biochemical pathways behind their mechanism of action is still limited, and further studies are warranted. In addition, the need for harmonization of the *in vitro* methods: (i) conditions of the gastrointestinal procedure, (ii) cell line used, (iii) concentrations of bioactive compounds used (usually much higher than those achievable in the human body when the digestion process is not considered), and (iv) time of cell exposure to the bioactive compounds (more than 24 h is unlikely to occur *in vivo*), should be considered for improved study designs more similar to the *in vivo* situation

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and for allowing comparisons of results among laboratories. This task is currently being carried out at European level within the project "Improving health properties of food by sharing our knowledge on the digestive process (INFOGEST) (2011-2015) (FAO COST Action FA 1005) (http://www.cost-infogest.eu/ABOUT-Infogest)".

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
		Gastrointestinal digestion (dialysis)		
(Polyphenols)				
Chokeberry juice	Caco-2 (human colon carcinoma)	85 to 220 (μM total polyphenols) 2 h a day for a 4-day period	Cell growth inhibition Viability decrease Cell cycle arrest at G_2/M phase Up-regulation of tumor suppression gene *CEACAM1*	Bermúdez-Soto et al. (2007) [36]
Raspberries	HT29, Caco-2 and HT115 (human colon carcinoma)	3.125 to 50 (μg/mL) 24 h	Prevention of H_2O_2 (75μM/5min)-induced DNA damage and decrease in G_1 phase of cell cycle (HT29 cells) No effect on epithelial integrity (Caco-2 cells) Inhibition of colon cancer cell invasion (HT115 cells)	Coates et al. (2007) [52]
Green tea	Differentiated PC12 (model of neuronal cells)	0.3-10 μg/mL (for H_2O_2) and 0.03-0.125 μg/mL (for $A\beta_{(1-42)}$) Pretreatment 24 h and stressed 24 h	Protection against H_2O_2 and $A\beta_{(1-42)}$ induced cytotoxicity (only at low concentrations)	Okello et al. (2011) [44]
Blackberry (*Rubus sp.*)	SK-N-MC (neuroblastoma cells)	1.5-6 μM total polyphenols 24 h	Preservation of cell viability against H_2O_2 (300 μM- 24 h) –induced oxidative stress (not related to modulation of ROS nor GSH levels)	Tavares et al. (2012a) [45]

CECAM1: Carcinoembryonic antigen-related cell adhesion molecule 1. Aβ(1-42): β-amyloid peptide 1-42. ROS: reactive oxygen species. GSH: reduced glutathione.

Table 1. Mechanisms involved in the chemopreventive effect of *in vitro* digested foods or bioactive constituents in cell lines.

The *in vitro* simulation of the conditions of gastrointestinal digestion represents an alternative to *in vivo* studies for evaluating the bioavailability and/or functionality of bioactive components of foods. *In vitro* studies do not replace *in vivo* studies; rather, both complement

each other. *In vitro* methods need to be improved and validated with more *in vivo* studies. Thus, caution is mandatory when attempting to extrapolate observations obtained *in vitro* in cell line studies to humans.

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
Wild blackberry species	SK-N-MC (neuroblastoma cells)	0-6 μM total polyphenols 24 h	Preservation of cell viability and mitochondrial membrane potential against H_2O_2 (300 μM -24 h)-induced oxidative stress Decrease of intracellular ROS against H_2O_2 (200 μM -1 h)-induced oxidative stress (only *R. brigantines*) Prevention of GSH depletion against H_2O_2 (300 μM -24 h)-induced oxidative stress Induction of caspase 3/7 activity against H_2O_2 (300 μM -24 h)-induced oxidative stress (preconditioning effect)	Tavares et al. (2012b) [46]
			Gastrointestinal digestion (solubility)	
(Polyphenols)				
Fruit beverages with/without milk and/or iron	Caco-2 (human colon carcinoma)	2%, 5% and 7.5% (v/v) in culture medium (3.4-22.7 mg/mL total polyphenols) 4 hours-4 days or 24 h	Cell growth inhibition (no clear dose-response) Cell cycle arrest at S phase (7.5%) Down-regulation of cyclins D_1 and B1 No apoptosis (cytostatic effect)	Cilla et al. (2009) [28]
Zinc-fortified fruit beverages with/without iron and/or milk	Caco-2 and HT-29 (human colon carcinoma)	7.5% (v/v) in culture medium (~50 μM total polyphenols) 24 h	Cell growth inhibition (without citotoxicity) Cell cycle arrest at S phase No apoptosis and resumption of cell cycle after digest removal (cytostatic effect)	Cilla et al. (2010) [37]
Fruit juices enriched with pine bark extract	Caco-2 (human colon carcinoma)	4% (v/v) in culture medium 24-120 h	Cell growth inhibition	Frontela-Saseta et al. (2011) [38]

ROS: reactive oxygen species. GSH: Reduced glutathione.

Table 1. (continued-I).

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
Feijoada-traditional Brazilian meal	HepG2 (human liver cancer cells)	10-100 mg/mL 72 h (antiproliferation) and 1 h (antioxidant)	Antiproliferative activity ("/ 80 mg/mL) Increase in cellular antioxidant activity (0.6 µM quercetin equivalents)	Kremer-Faller et al. (2012) [32]
Culinary herbs: rosemary, sage and thyme	PBL (peripheral blood lymphocytes) and Differentitated Caco-2 (model of intestinal epithelium)	1:10 (v/v) in culture medium. Stressors (H_2O_2 2 mM and TNFα 100 µg/mL) Co-incubation 24 h or pre-incubation 3h then stress 24 h	PBL: significant decrease in IL-8 release when co-incubation with H_2O_2 and pre-incubation prior H_20_2 and TNFα Caco-2: significant decrease in IL-8 release only when co-incubation with TNFα	Chohan et al. (2012) [54]
(Antioxidants)				
Fruit beverages with/without milk and/or iron/zinc	Differentiated Caco-2 (model of intestinal epithelia)	1:1 (v/v) in culture medium	Preservation of cell viability No alteration of SOD	Cilla et al. (2008) [40]
Fruit beverages with/without milk or CPPs	Differentiated Caco-2 (model of intestinal epithelia)	1:1 (v/v) in culture medium or CPPs (1.4 mg/mL)	Preservation of cell viability (only fruit beverages)	Laparra et al. (2008) [41]
Beef patties enriched with sage and oregano	Caco-2 (human colon carcinoma)	10-100% (v/v) 24 h	Increase in cell viability at low concentrations (20-40%) but slight decrease at high concentrations (80-100%) Increase in GSH (only sage-enriched samples at 10%) Protection against H_20_2 (200 µM/1h)-induced GSH depletion (at 10%)	Ryan et al. (2009) [49]

IL-8: Proinflammatory interleukin-8. TNFα: tumor necrosis factor α. SOD: Superoxide dismutase. CPPs: caseinophosphopeptides. GSH-Rd. glutathione reductase. GSH: reduced glutathione.

Table 1. (continued-II).

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
Ellagic acid-, lutein- or sesamol-enriched meat patties	Caco-2 (human colon carcinoma)	0-20% (v/v) in culture medium 24 h	Viability maintenance against H_20_2 (500 μM/1h)-induced stress Prevention of H_20_2 (50 μM/30 min)-induced DNA damage	Daly et al. (2010) [42]
Pacific hake fish protein hydrolysates	Caco-2 (human colon carcinoma)	0.625-5 mg/mL 2 h	Inhibition (at non cytotoxic doses) of intracellular oxidation induced by AAPH (50 μM/1-2 h)	Samaranayaka et al. (2010) [50]
Human breast milk	Co-culture of Caco-2 BBE and HT29-MTX (model of human intestinal mucosa)	1:3 (v/v) in culture medium 30 min	Decrease of H_20_2 (1 mM/30 min)-induced ROS Prevention of H_20_2 (500 μM/30 min)-induced DNA damage	Yao et al. (2010) [51]
Fruit beverages with/without milk and/or iron/zinc	Differentiated Caco-2 (model of intestinal epithelia)	1:1 (v/v) in culture medium Pre-incubation 24 h then stressed 2h with H_20_2 5 mM	Preservation of cell viability Increase in GSH-Rd activity (only Fe or Zn with/without milk samples) Prevention of G_1 cell cycle phase decrease induced by H_202 Prevention of apoptosis (caspase-3) induced by H202	Cilla et al. (2011) [43]
Purified milk hydrolysate peptide fraction from digested human milk	Caco-2 and FHs 74 int (human colon carcinoma and primary fetal enterocytes)	0.31-1.25 g/L (peptide) and 150 μM (tryptophan) 2 h (peptide) and 1-12 h (tryptophan)	Exacerbation of AAPH (50 μM/1-2 h)-induced oxidative stress (peptide) Up-regulation of Nrf-2 and subsequent up-regulation of GSH-Px2 gene as adaptive response to stress (tryptophan)	Elisia et al. (2011) [48]

AAPH: 2,2′-azobis (2-amidinopropane) dihydrochloride. ROS: reactive oxygen species. GSH-Rd: glutathione reductase. Nrf-2: nuclear response factor 2. GSH-Px2: glutathione peroxidase.

Table 1. (continued-III).

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
CPPs from digested cow's skimmed milk	Differentiated Caco-2 (model of intestinal epithelia)	1, 2 and 3 mg/mL Pre-incubation 24 h then stressed 2h with H_20_2 5 mM	Preservation of cell viability Increase in GSH content and induction of CAT activity Decrease in lipid peroxidation Maintenance of correct cell cycle progression	García-Nebot et al. (2011) [47]
Purified hen egg yolk-derived phosvitin phosphopeptides	Differentiated Caco-2 (model of intestinal epithelia)	0.05-0.5 mg/mL 2 h	Reduced IL-8 secretion in H_20_2 (1 mM/6 h)-induced oxidative stress	Young et al. (2011) [53]
(Lycopene)				
Tomatoes	HT29 and HCT-116 (human colon carcinoma)	20-100 mL/L 24 h	Cell growth inhibition Cell cycle arrest at G_0-G_1 phase and apoptosis induction (caspase-3) Down-regulation of cyclin D_1 and anti-apoptotic proteins Bcl-2 and Bcl-xL	Palozza et al. (2011) [39]
(Phytosterols)				
Orange juice enriched with fat-free phytosterols	Differentiated Caco-2 (model of intestinal epithelia)	2 mL test medium/well 4 h	Reduced micellarization of cholesterol Decrease in cholesterol accumulation by Caco-2 cells	Bohn et al. (2007) [35]

GSH: reduced glutathione. CAT: catalase. IL-8: proinflammatory interleukin-8.

Table 1. (continued-IV).

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
(SCFA)				
Fibre sources: linseed, watercress, kale, tomato.soya	HT29 (human colon carcinoma)	2.5-25% (v/v) in culture medium 72 h	Cell growth inhibition (all samples except watercress) Prevention of HNE (150μM/30 min)-induced DNA damage (only soya flour)	Beyer-Sehlmeyer et al. (2003) [55]

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
flour, chicory inulin and wheat				
Wheat bran-derived arabinoxylans	HT29 (human colon carcinoma)	0.01-50% (v/v) in culture medium 24-72 h	Cell growth inhibition Prevention of HNE (200μM/30 min)-induced DNA damage (at 25-50%) Induction of GST activity (at 10%)	Glei et al. (2006) [56]
Inulin-type fructans	LT97 and HT29 (human colon adenoma and carcinoma)	1.25-20% (v/v) in culture medium 24-72 h	Cell growth inhibition (at 5-10%) Apoptosis induction (cleavage of PARP) only in LT97 cells (at 5-10%)	Munjal et al. (2009) [58]
Wheat aleurone	LT97 and HT29 (human colon adenoma and carcinoma)	5-10% (v/v) in culture medium 24-72 h	Cell growth inhibition Apoptosis induction (caspase-3) Up-regulation of genes *p21* (cell cycle arrest) and *WNT2B* (apoptosis)	Borowicki et al. (2010a) [59]
(polyphenols)				
Apples	LT97 and HT29 (human colon adenoma and carcinoma)	100-900 μg/mL 24-48 h	Cell growth inhibition (LT97 more sensitive than HT29 cells)	Veeriah et al. (2007) [57]

SCFA: short chain fatty acids. GST: Glutathione-S-Transferase. HNE: 4-Hydroxynonenal. PARP: Poly (ADP-ribose) polymerase. WNT2B: Wingless-type MMTV integration site family member 2.

Table 2. Mechanisms involved in the chemopreventive effect of *in vitro* colonic fermented (in batch) of foods or bioactive constituents in cell lines.

Sample (Target compound/s)	Cell type	Cell treatment (Concentrations and time)	Cellular mechanism	References
(SCFA)				
Resistant starches	Differentiated Caco-2 (model of intestinal epithelia)	10% (v/v) in culture medium 24 h	Preservation of cell viability Prevention of H_20_2 (75 μM/5 min)-induced DNA damage Maintenance of barrier function integrity (TEER)	Fässler et al. (2007) [63]
Wheat aleurone	HT29 (human colon carcinoma)	10% (v/v) in culture medium 24-72 h	Cell growth inhibition Cell cycle arrest in G_0-G_1 phase Apoptosis induction (caspase-3)	Borowicki et al. (2010b) [60]
Wheat aleurone	HT29 (human colon carcinoma)	5-10% (v/v) in culture medium 24-72 h	Induction of antioxidant enzymes (CAT and GST) Up-regulation of genes *CAT*, *GSTP1* and *SULT2B1* Prevention of H_20_2 (75 μM/5 min)-induced DNA damage	Stein et al. (2010) [31]
(SCFA and polyphenols)				
Bread	HT29 (human colon carcinoma)	2.5-5% (v/v) in culture medium 24-72 h	Cell growth inhibition Prevention of H_20_2 (75 μM/5 min)-induced DNA damage	Lux et al. (2011) [61]
(butyrate)				
Bread	LT97 (human colon adenoma)	5-20% (v/v) in culture medium 24-72 h	Up-regulation of genes from DNA repair, biotransformation, differentiation and apoptosis Increase in GST activity, GSH content and AP activity (differentiation) Cell growth inhibition Apoptosis induction (caspase-3)	Schölrmann et al. (2011) [62]

SCFA: Shot chain fatty acids. TEER: Trans Epithelial Electrical Resistance. GST: Glutathione-S-Transferase. GSH: Glutathione. CAT: catalase. SULT: Sulfotransferase. AP: Alkaline phosphatase.

Table 3. Mechanisms involved in the chemopreventive effect of *in vitro* digested (dialysis) plus colonic fermented (batch) foods or bioactive constituents in cell lines.

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