

Bioactive Compounds of Mediterranean Cooked Tomato Sauce (Sofrito) Modulate Intestinal Epithelial Cancer Cell Growth Through Oxidative Stress/Arachidonic Acid Cascade Regulation

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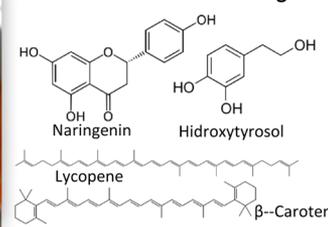
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ABSTRACT: Mediterranean diet (MD) is associated with a low incidence of colorectal cancer, but the specific dietary constituents involved and mechanisms related to these beneficial effects are still sparse. Sofrito, a traditional MD preparation, is a mix of foods characteristics of MD such as tomato, onion, garlic, and extra virgin olive oil, which contains many bioactive phenolic compounds and carotenoids. The aim of the present study was to determine the action of these components of sofrito on reactive oxygen species and eicosanoid production as well as the cell growth/cell cycle in adenocarcinoma cell cultures. We observed that hydroxytyrosol, naringenin, naringenin glucuronide, and to a lesser extent lycopene and β -carotene modulate these events in Caco-2 cell cultures. Interestingly, we also found an additive action of these bioactive compounds that could explain these biological actions on concentrations reached after the consumption of a traditional MD.



Olive oil + tomato + onion + garlic



INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in developed countries and is the second cause of world's cancer-related deaths.¹ The traditional Mediterranean diet (MD) is one of the healthiest in the world, and it is likely that the adoption of this diet would lead to a marked impairment in the incidence of high-prevalence chronic disorders.² This metaanalysis of prospective cohort studies considered that the MD is responsible for a significant decrease of death that included the impairment of neoplastic processes. In this sense, an updated report from a large cohort such as the European Prospective Investigation into Cancer and Nutrition (EPIC) has found a lower overall cancer risk among individuals with higher adherence to MD,³ and similar findings were reported in a large cohort belonging to the National Institutes of Health.⁴ Recently, several clinical studies reported a protective effect of MD adherence on CRC risk in Mediterranean^{5–7} and non-Mediterranean population.^{8,9} Beneficial effects that appear to be most attributable to fruit, vegetable, and whole grain intake, through multiple mechanisms.¹⁰

Much attention has been focused on the relationship between tomatoes and/or lycopene intake and cancer risk impairment. In 2004, Food and Drug Administration (FDA) received two petitions for qualified health claims regarding this above relationship. In response to both questions, FDA studied evidence for associations between lycopene (as a food component) and tomatoes (as food) and each type of cancer.

The FDA considered no credible evidence to support an association between tomato consumption or lycopene consumption and a reduced risk of several cancers including CRC.¹¹ Interestingly, Urquiza-Salvat *et al.* (2018)¹² recently reported that a high consumption of fruit, vegetables, and cooked tomato sauce in Mediterranean style (sofrito) was related to low prostate cancer aggressiveness.

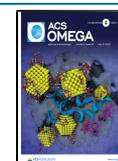
Sofrito is prepared by mixing tomato, onion, garlic, and olive oil, which contains many bioactive phenols and carotenoids.¹³ Naringenin is the main flavonoid in tomatoes and tomato-based sauces,¹⁴ whereas lycopene and β -carotene are the main characteristic carotenoids,¹³ and hydroxytyrosol is a representative phenol from olives and olive products that is presented in sofrito and tomato sauces prepared with olive oil.¹³ Furthermore, sofrito has higher concentrations of bioactive compounds than tomatoes. Thus, 120 g of sofrito added to different dishes and culinary preparations daily lead to a total phenolic consumption of 15–25 and 5–10 mg of carotenoids.¹³

An increased number of cycling cells leading to the enhancement of the cell proliferation zone and increased

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crypt height as well as impaired apoptosis are risk factors to CRC development and progression. Polyphenols could modulate oxidative stress and eicosanoid synthesis,¹⁵ involved in chronic processes with an important inflammatory component such as cancer. Considering these aspects, we aimed to study the effect of several polyphenols and carotenoids representative of sofrito on reactive oxygen species (ROS) and eicosanoid synthesis as well as the cell growth/cell cycle in adenocarcinoma cell cultures. Furthermore, we proposed to study the likely evidence of additive and/or synergistic actions of bioactive compounds of sofrito.

RESULTS

Table 1 shows that hydroxytyrosol is the most effective bioactive component present in the traditional cooked tomato

Table 1. Antioxidant Effect of Sofrito Components^a

| compound | antioxidant activity (equivalents μM quercetin) | reducing power (equivalents μM trolox) |
|--|--|---|
| naringenin (100 μM) | 43.5 \pm 1.6 | 42.1 \pm 3.6 |
| naringenin (300 μM) | 128.3 \pm 11.2 | 139.3 \pm 2.1 |
| naringenin 7-O- β -D-gluc. (100 μM) | 27.8 \pm 2.4 | 34.1 \pm 2.7 |
| Naringenin 7-O- β -D-gluc. (300 μM) | 56.1 \pm 1.2 | ND |
| hydroxytyrosol (10 μM) | 57.2 \pm 1.3 | 68.5 \pm 2.1 |
| hydroxytyrosol (50 μM) | 148.2 \pm 13.6 | 172.3 \pm 6.8 |
| hydroxytyrosol (100 μM) | 412.6 \pm 21.8 | 234.2 \pm 4.9 |
| lycopene (100 μM) | 74.3 \pm 3.5 | ND |
| lycopene (300 μM) | 125.2 \pm 6.7 | 63.2 \pm 3.7 |
| β carotene (100 μM) | 14.2 \pm 1.3 | ND |
| β carotene (300 μM) | 28.7 \pm 1.6 | 31.2 \pm 2.1 |
| naringenin (100 μM) + hydroxytyrosol (10 μM) + lycopene (100 μM) + β carotene (100 μM) | 228.1 \pm 8.6 | 235.2 \pm 3.7 |

^aData are the mean \pm SEM of three experiments performed in duplicate. ND, not determined.

sauce sofrito as the ROS scavenger *in vitro*. It presented a higher antioxidant and reducing power than trolox and quercetin, used as controls, respectively. Naringenin and lycopene show an antioxidant activity and reducing power lower than these control compounds, and finally, β -carotene shows a little antioxidant activity. These results are in agreement with those of previous studies that analyzed the antioxidant activity of these compounds.^{16–18} Naringenin is

mainly metabolized to 7- and/or 4'-glucuronides by UDP glucuronosyltransferase by losing hydroxyl groups,¹⁹ and consequently it is detected in plasma at low concentrations as aglycon.²⁰ The question is whether these changes modify its antioxidant and other biological activities. Our results show that naringenin 7-O- β -D-glucuronide shows a minor antioxidant activity than that of the aglycon (Table 1) as well as a low capacity to modulate Caco-2 cells' ROS production (Table 2). We also demonstrated an interesting additive antioxidant effect of hydroxytyrosol, naringenin, and lycopene to prevent 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) oxidation and an increase in the reducing power (Table 1). These findings were further corroborated by the modulation of ROS production in Caco-2 cell cultures by hydroxytyrosol and to a lesser extent by naringenin, naringenin 7-O- β -D-glucuronide, and lycopene (Table 2).

ROS production is among the underlying pathophysiological mechanisms involved in the development of cancer,²¹ and also modulated cell-signaling pathways that regulate the cell cycle and cell growth,²² and the activation of the arachidonic acid (AA) cascade²³ that controls Caco-2 cell growth.^{24–26} Thus, we studied the effects of their representative compounds on the cell growth/cell cycle and AA cascade using Caco-2 cells, for better understanding the mechanisms underlying the beneficial effects of sofrito consumption.

To study these effects, we incubated nondifferentiated Caco-2 cells in media supplemented with FBS 10% and polyphenols or carotenoids. As shown in Figure 1A, both polyphenols and carotenoids inhibited Caco-2 cell growth in a concentration-dependent manner. The sequence of potency was: hydroxytyrosol > naringenin > lycopene > β -carotene. The effects of these compounds on nondifferentiated Caco-2 cell DNA synthesis were also studied. At 10 μM , naringenin and hydroxytyrosol inhibited DNA synthesis around 40 and 80%, respectively, whereas carotenoids only inhibited around 20% after 48 h exposure (Figure 1B). Furthermore, we observed that naringenin 7-O- β -glucuronide shows a similar effect on Caco-2 cell growth and DNA synthesis to that of naringenin (Figure 2). Interestingly, we also observed an additive effect of these compounds on the impairment of Caco-2 cell growth and DNA synthesis (Figure 3) using concentrations near to physiological conditions, which can be related to their induced changes in the Caco-2 cell cycle. Thus, we observed that naringenin, naringenin 7-O- β -D-glucuronide, lycopene, and β -carotene increased the percentage of cells in the G₀/G₁ phase, whereas hydroxytyrosol enhanced the number of cells in the S

Table 2. Effects of Cooked Tomato Sauce (Sofrito) Bioactive Compounds on Caco-2 Cell ROS and Eicosanoid Production^a

| | DCF fluorescence (fluorescence units \times 1000) | PGE ₂ (ng/mL) | 5-HETE (ng/mL) | 12-HETE (ng/mL) | 15-HETE (ng/mL) |
|-------------------------|---|------------------------------|------------------------------|------------------------------|------------------------------|
| control | 0.053 \pm 0.02 ^a | 0.33 \pm 0.04 ^a | 2.38 \pm 0.05 ^a | 2.78 \pm 0.07 ^a | 2.98 \pm 0.04 ^a |
| FBS | 2.456 \pm 0.12 ^b | 1.89 \pm 0.23 ^b | 7.45 \pm 0.27 ^b | 5.13 \pm 0.36 ^b | 9.78 \pm 0.33 ^b |
| FBS + naringenin | 1.108 \pm 0.09 ^c | 1.22 \pm 0.11 ^c | 6.13 \pm 0.12 ^c | 3.95 \pm 0.11 ^c | 5.12 \pm 0.07 ^c |
| FBS + naringenin gluc | 1.379 \pm 0.11 ^c | 1.43 \pm 0.15 ^c | 6.28 \pm 0.11 ^c | 4.12 \pm 0.09 ^c | 5.32 \pm 0.05 ^c |
| FBS + hydroxytyrosol | 0.447 \pm 0.07 ^d | 0.77 \pm 0.03 ^d | 4.11 \pm 0.16 ^d | 3.26 \pm 0.03 ^d | 4.27 \pm 0.06 ^d |
| FBS + lycopene | 0.854 \pm 0.06 ^e | 0.95 \pm 0.03 ^e | 5.23 \pm 0.15 ^e | 3.68 \pm 0.04 ^e | 4.78 \pm 0.05 ^e |
| FBS + β -carotene | 2.021 \pm 0.13 ^b | 1.62 \pm 0.22 ^b | 7.27 \pm 0.21 ^b | 5.02 \pm 0.17 ^b | 8.54 \pm 0.11 ^b |
| FBS + mix components | 0.231 \pm 0.02 ^f | 0.54 \pm 0.07 ^f | 3.63 \pm 0.07 ^f | 3.01 \pm 0.03 ^f | 3.37 \pm 0.03 ^f |

^aNondifferentiated Caco-2 cells were cultured with FBS (10%) in the presence of naringenin (10 μM), naringenin 7-O- β -D-glucuronide (10 μM), hydroxytyrosol (10 μM), lycopene (25 μM), β -carotene (25 μM), or the mix of both polyphenols (10 μM) plus both carotenenes (25 μM). Data are the mean \pm SEM of three experiments performed in duplicate. Values that do not share a common letter (a, b, c, d, e, and f) were significantly different ($p < 0.05$).

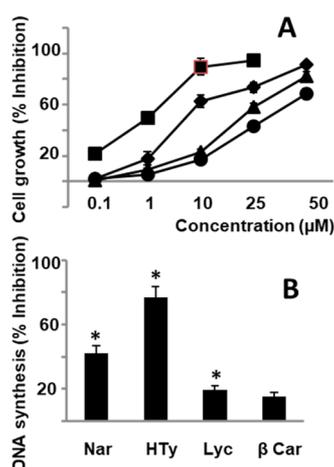


Figure 1. Effects of bioactive compounds of Mediterranean cooked tomato sauce (sofrito) on Caco-2 cell growth (A) and Caco-2 DNA synthesis (B) in the presence of fetal bovine serum (FBS). Caco-2 cells were incubated with hydroxytyrosol (HTy, squares, 10 μM), naringenin (Nar, rhombs, 10 μM), lycopene (Lyc, triangles, 10 μM), and β -carotene (β Car, circles, 10 μM) in a medium containing 10% FBS for 48 h and cells were then counted ($n = 8-12$). DNA synthesis ($n = 4-6$) was assessed by measuring BrdU incorporation. Results are expressed as mean \pm SEM. * Significantly different with respect to control (10% FBS) ($p < 0.05$).

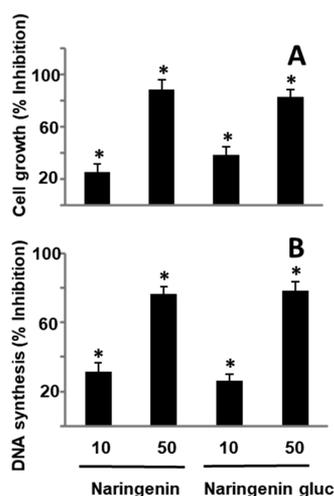


Figure 2. Effect of naringenin and naringenin 7-*O*- β -D-glucuronide on Caco-2 cell growth (A) and Caco-2 DNA synthesis (B) in the presence of FBS. Caco-2 cells were incubated with naringenin and naringenin glucuronide (10 and 50 μM) in a medium containing 10% FBS for 48 h and cells were then counted ($n = 9$) and DNA synthesis ($n = 4-6$) was assessed by measuring BrdU incorporation. Results are expressed as mean \pm SEM. * Significantly different with respect to control (10% FBS) ($p < 0.05$).

phase (Table 3). Interestingly, the mix of bioactive compounds that inhibits Caco-2 cell growth did not change the cell cycle distribution.

Ethidium bromide/acridine orange staining and the morphological study revealed that these compounds did not affect the cellular structure and cell viability at concentrations used. Moreover, we did not observe a hypodiploid DNA peak, characteristic of apoptosis, under our experimental conditions (data not shown). To confirm these data, we measured DNA fragmentation as an index of apoptosis. None of the bioactive compounds at the concentrations assayed induced marked

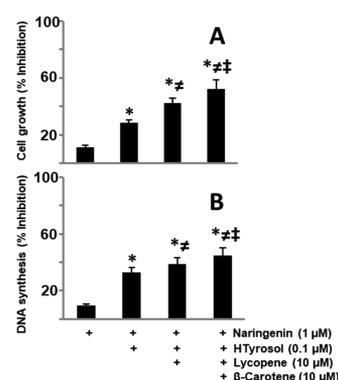


Figure 3. Effect of bioactive compounds of Mediterranean cooked tomato sauce (sofrito) on Caco-2 cell growth (A) and Caco-2 DNA synthesis (B) in the presence of FBS. Caco-2 cells were incubated with naringenin (1 μM) or naringenin (1 μM) plus hydroxytyrosol (0.1 μM), lycopene (10 μM), and β -carotene (10 μM) for 48 h and cells were then counted ($n = 9$) and DNA synthesis ($n = 4-6$) was assessed by measuring BrdU incorporation. Results are expressed as mean \pm SEM. * Significantly different with respect to control (10% FBS) ($p < 0.05$); \ddagger significantly different with respect to naringenin treatment ($p < 0.05$); $\#$ significantly different with respect to naringenin plus hydroxytyrosol treatment ($p < 0.05$).

Table 3. Effect of Cooked Tomato Sauce (Sofrito) Bioactive Compounds on the Caco-2 Cell Cycle^a

| compound | G ₀ /G ₁ (%) | S (%) | G ₂ /M (%) |
|---------------------------------------|------------------------------------|-------------|-----------------------|
| control | 70 \pm 2 | 20 \pm 2 | 10 \pm 1 |
| naringenin (10 μM) | 78 \pm 3 | 16 \pm 2 | 6 \pm 2 |
| hydroxytyrosol (10 μM) | 65 \pm 2* | 29 \pm 2* | 5 \pm 1* |
| lycopene (25 μM) | 77 \pm 4 | 9 \pm 1* | 14 \pm 2 |
| β -carotene (25 μM) | 75 \pm 3 | 13 \pm 2* | 12 \pm 3 |
| mix of four compounds | 68 \pm 3 | 22 \pm 3 | 10 \pm 2 |

^aMix of compounds contains naringenin (1 μM), hydroxytyrosol (0.1 μM), lycopene (10 μM), and β -carotene (10 μM). Data are the mean \pm SEM of three experiments performed in duplicate. * Significantly different with respect to control ($p < 0.05$).

DNA fragmentation under our experimental conditions (data not shown).

Recently, we reported that Caco-2 cells can synthesize COX and LOX pathway metabolites from AA such as prostaglandin E₂ (PGE₂), 5-hydroxyeicosatetraenoic acid (5-HETE), 12-HETE or 15-HETE,²⁷ eicosanoids that play a central role in the regulation of Caco-2 cell growth.²⁴⁻²⁶ Considering these antecedents, we studied whether these eicosanoids are involved, at least partly, in the modulation of Caco-2 cell growth by sofrito compounds. Here, we observed, for the first time in the same study that these polyphenols and carotenoids were able to inhibit eicosanoid production (Table 2). Interestingly, hydroxytyrosol was the more potent compound on COX and LOX pathways and we detected an additive effect of both polyphenols and carotenoids on eicosanoid biosynthesis.

DISCUSSION

In human, nutritional food choices depend on a wide range of factors such as personal preferences, culture, tradition, ethics, consumption patterns, environment, and so forth. In recent years, attention has been increasingly paid to the nutritional characteristics of traditional dishes and culinary preparations,²⁸ in order to provide dietary information and to accurately

estimate dietary intake of the population, prevent diseases of high prevalence such as cancer, and preserve some cultural elements. Spain is famous for its extremely rich traditional foods, dishes, and culinary preparations, which have been transferred from generation to generation, playing a key role within local culture. The interactions between single food components and/or between ingredients of dishes play a central role in understanding the “food synergy” concept,²⁹ which can also be applied to the study of the beneficial effects of polyphenols and polyphenol-rich recipes.³⁰ Cooked foods and composite dishes constitute the main part of diet, but there is a significant gap in the composition information and nutritional effects. Nutrient contents and energy values of the selected dishes vary widely because of the nature, variety, and composition of the preparations that defined different daily nutrient intakes and their association with health effects. All dishes prepared with plant origin ingredients exhibited appreciable antioxidant properties, at least in part, related to the total polyphenol and carotenoid content.

In this context, the study of antioxidant properties of the components of a food or a culinary preparation and their possible synergistic interactions could represent the first step in the investigation of potential benefits of culinary preparations, and as an indicator of playing a “possible beneficial role”, sofrito is a good example to perform this evaluation.

Polyphenols’ health-promoting properties have been mainly assayed *in vitro*, and the bioavailability of polyphenols is poor because they are rapidly metabolized.³⁰ Consequently, a great controversy exists as to whether polyphenols are active molecules *in vivo*. Considering that naringenin concentrations may be too low to play an effective biological role has raised the question whether naringenin metabolites are able to elicit biological responses to the concentrations reached in plasma or tissues. To help resolve this question, the present study demonstrates that the main naringenin metabolite, naringenin 7-*O*- β -D-glucuronide, has a lower antioxidant capacity than the aglycon. Probably, as consequence of the loss of hydroxyl. In this way, we recently reported the role of hydroxyls in the antioxidant action of polyphenols.³¹ These findings agree with the previous results that demonstrated the biological effects of resveratrol metabolites,³² but that unfortunately cannot explain the beneficial effects of sofrito. However, the sofrito bioactive compounds might have an additive/synergistic antioxidant action. These results might be a consequence of the ROS-scavenging capacity of these compounds as we reported here, but also as a consequence of the stimulation of antioxidant enzymatic systems that are pivotal elements of the redox homeostasis as we previously reported using other bioactive compounds such as β -sitosterol.³³

The cellular redox state may act as a molecular switch that controls the activity of many enzymes and genes. Thus, AA is released from cellular phospholipids by phospholipases in response to changes in the redox state²³ together with the modulation of AA cascade enzyme expression/activity,^{34,35} the rate-limiting steps being the subsequent metabolism to synthesize eicosanoids. Events that control Caco-2 cell growth.²⁴ Considering all together, we can relate the modulation of the redox state by sofrito components, and consequently their actions on eicosanoid biosynthesis and Caco-2 cell growth. It is likely that the more interesting finding of the present study is that we observed an additive effect of polyphenol and carotenoid components of sofrito on the biological actions studied. These findings are in agreement

with those of a recent study that demonstrated an additive action of sofrito components on oxidative stress and prostaglandin E₂/leukotriene B₄ biosynthesis by oxidized low-density lipoprotein-stimulated macrophages.³⁶ These additive/synergistic effects have been previously observed in the study of bioactive components of olive oil and wine.³⁷

Considering that hydroxytyrosol from extra virgin olive oil is the most potent bioactive compound of sofrito based on the parameters studied here, we must remark the importance to include olive oil, preferentially extra virgin olive oil (rich in hydroxytyrosol) in sofrito recipes as it is prepared using the traditional MD cooking procedures. Furthermore, we observed that these sofrito bioactive compounds have different mechanisms of action on Caco-2 cell growth. Thus, hydroxytyrosol increases the percentage of S phase cells whereas naringenin and both carotenoids elevate the percentage of cells in the G₀/G₁ phase.

CONCLUSIONS

Our findings suggest a novel molecular mechanism by which sofrito bioactive compounds might modulate the cellular redox state, AA cascade, and cell growth/apoptosis of intestinal epithelial cells, events involved in CRC development. Furthermore, we demonstrated that naringenin metabolites are active compounds, and that bioactive compounds of sofrito studied present additive actions, which can explain their beneficial effect at physiological concentrations. Thus, our results provide knowledge of the nutrient profile of a Spanish traditional dish. The availability of this new information will also facilitate further nutrition-related studies and will likely encourage the consumption of food products, dishes, and culinary preparations rich in bioactive molecules.

EXPERIMENTAL SECTION

Materials. Dulbecco’s modified Eagle’s medium (DMEM), trypsin/EDTA, penicillin, and streptomycin were supplied by GIBCO. Acridine orange, β -carotene, Dulbecco’s PBS, ethidium bromide, ferric chloride, FBS, hydroxytyrosol, lycopene, naringenin, nonessential amino acids, potassium ferricyanide, propidium iodide, quercetin, ribonuclease A from bovine pancreas, and Triton X-100 were supplied by Sigma-Aldrich (St. Louis, MO, USA). Naringenin 7-*O*- β -D-glucuronide, eicosanoids, and deuterated eicosanoid internal standards were from Cayman Chem. Co. (Ann Arbor, MI, USA). Chloromethyl dichlorofluorescein diacetate (CM-H₂DCF-DA) was obtained from Invitrogen (Carlsbad, CA, USA). Tissue culture supplies and sterile materials were supplied by Corning (Corning, NY, USA), Nirco S.L. (Barcelona, Spain) and Biosigma S.R.L. (Venice, Italy).

Total Antioxidant Activity and Reducing Power. We assayed different concentrations of bioactive compounds and used an Antioxidant Assay Kit (Cayman Chemical). This test is based on the capacity of antioxidants in the sample (products diluted in PBS) to decrease the oxidation of ABTS to ABTS⁺ by metmyoglobin.³⁸ The ability of the compounds in the sample to reduce ABTS oxidation is compared with that of trolox, to quantify the findings as molar trolox equivalents.

Total reducing power was measured according to the method of Oyaizu (1986).³⁹ First, 40 μ L of the sample was mixed with 200 μ L of phosphate buffer (0.2 M, pH 6.6) and 200 μ L of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, and 200 μ L of 10%

trichloroacetic acid was added. Finally, 60 μL of 0.167% ferric chloride was added and incubated at room temperature for 50 min and changes in absorbance at 690 nm indicated an increase in reducing power. These results were compared with a quercetin standard curve.

Cell Culture. Caco-2 cells from a moderately well-differentiated primary colon adenocarcinoma were supplied by American Type Culture Collection (HTB-37) (Manassas, VA, USA). Cells (passages 19–40) were grown in plastic flasks (25 or 75 cm^2) at a density of $2\text{--}2.5 \times 10^4$ cells/ cm^2 and cultured in DMEM with 4.5 g/L D-glucose and 2 mM L-glutamine, 1% (v/v) nonessential amino acids, 10% (v/v) heat-inactivated FBS, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. Cell cultures were maintained at 37 $^\circ\text{C}$ under a humidified atmosphere of 5% CO_2 in air. Cell cultures were released by trypsinization, and subcultured at a density of $1.5\text{--}2 \times 10^4$ cells/ cm^2 in 12 mm diameter plastic clusters and in 60 mm diameter plastic dishes, and the growth medium was replaced twice per week. The assays were performed in cultures maintained for 3 days (preconfluent cultures). All assayed bioactive compounds were diluted in DMSO (final concentration of DMSO was lesser than 0.1%).

Cell Growth and DNA Synthesis Assays. Caco-2 cell clusters in 24-well plates ($1.5\text{--}2 \times 10^4$ cells/ cm^2) were maintained for 96 h in the DMEM medium supplemented with 10% FBS. Then, cultures were incubated for 48 h in the presence of sofrito bioactive compounds. Finally, cultures were washed, trypsinized, and cells were microscopically counted using ethidium bromide/acridine orange staining to also assess cell viability.

DNA synthesis was determined by the measurement of BrdU incorporation during DNA synthesis (Cell proliferation ELISA, BrdU Kit, from Roche, Rotkreuz, Switzerland). Caco-2 cells were cultured at 1000–1500 cell/well in 96 well plates for 4 days in the DMEM medium supplemented with 10% FBS. Then, cultures were washed and incubated for 48 h in DMEM in the presence of the compounds, and cells were treated following the manufacturer's instructions and absorbance was measured at 450 nm.

Flow Cytometry Cell Cycle Analysis. Caco-2 cells were grown in 60 mm dishes ($1.5\text{--}2 \times 10^4$ cells/ cm^2) and maintained for 96 h. Cultures were then incubated for 48 h in 10% FBS DMEM containing the bioactive compounds. Cells were trypsinized, fixed with 70% ethanol, and stored at 4 $^\circ\text{C}$ for at least 2 h. Low-molecular-weight DNA was extracted from cells, which were stained with 20 $\mu\text{g}/\text{mL}$ propidium iodide solution (in PBS containing 0.1% Triton X-100 and 0.2 mg/mL DNase-free RNase A) for 1 h at room temperature. Finally, cells were counted on an Epics XL flow cytometer (Coulter Corporation, Philadelphia, PA, USA) and DNA was analyzed (ploidy analysis) on single fluorescence histograms using Multicycle software (Phoenix Flow Systems).

TUNEL Assay. DNA degradation was assayed by the TUNEL method using a MebStain Apoptosis Kit (MBL, Woburn, MA, USA). Caco-2 cell cultures were maintained in the 10% FBS DMEM medium containing compounds for 48 h. Then, cells were fixed with 4% paraformaldehyde and permeabilized using 70% ethanol. 3'-OH DNA ends generated by DNA fragmentation were labeled with fluorescein-dUTP using terminal deoxynucleotidyl transferase and were measured on an Epics XL flow cytometer (Coulter Corporation).

Intracellular ROS Generation. Intracellular ROS concentration was assayed by the rate of oxidation of 1 μM CM-

H₂DCF-DA to a fluorescent dichlorofluorescein (DCF) in the presence of sofrito compounds for 20 min at 37 $^\circ\text{C}$. The changes in the fluorescence intensity were determined using a FUOstar OPTIMA fluorometer (BMG Labtech, Ortenburg, Germany), with excitation at 495 nm and emission at 515 nm. These experiments were performed in the presence of superoxide dismutase and catalase to confirm that the CM-H₂DCF-DA probe can measure ROS.⁴⁰

Liquid Chromatography/Tandem Mass Spectrometry to Assay Eicosanoid Concentrations. Eicosanoids in the cell culture medium were extracted using a solid-phase method. Eicosanoids and deuterated internal standards were separated using a liquid chromatograph Perkin Elmer Series 200 system (Norwalk, CT, USA) equipped with a quaternary pump and a thermostatted autosampler. To identify and quantify eicosanoids, we used a triple quadrupole API3000 mass spectrometer (ABSciex, Concord, Ontario, Canada) equipped with a TurboIonSpray source operating in the negative ion mode, as we described previously.²⁷

Data Analysis. The results are expressed as the mean \pm standard error of the mean. Differences between groups were tested using Student's *t*-test or one-way ANOVA and the LSD multiple comparison test for posthoc analysis. Differences of *p* < 0.05 were considered significant. All statistical analyses were performed using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA).

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Author Contributions

R.M.L.-R. and J.J.M. designed the research; C.E.S., I.S., and J.J.M. performed the experiments; C.E.S., I.S., and J.J.M. analyzed the data; J.J.M. wrote the manuscript and R.M.L.-R. corrected and discussed the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AA, arachidonic acid; ABTS, 2,2'-azino-di[3-ethylbenzthiazoline sulfonate]; CRC, colorectal cancer; DCF, dichlorofluorescein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; HETE, hydroxyeicosatetraenoic acid; MD, Mediterranean diet; PG, prostaglandin; ROS, reactive oxygen species

REFERENCES

- (1) Bhandari, A.; Woodhouse, M.; Gupta, S. Colorectal cancer is a leading cause of cancer incidence and mortality among adults younger than 50 years in the USA: a SEER-based analysis with comparison to other young-onset cancers. *J. Invest. Med.* **2017**, *65*, 311–315.
- (2) Sofi, F.; Abbate, R.; Gensini, G. F.; Casini, A.; Trichopoulou, A.; Bamia, C. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am. J. Clin. Nutr.* **2010**, *92*, 1189–1196.
- (3) Couto, E.; Boffetta, P.; Lagiou, P.; Ferrari, P.; Buckland, G.; Overvad, K.; Dahm, C. C.; Tjønneland, A.; Olsen, A.; Clavel-Chapelon, F.; Boutron-Ruault, M.-C.; Cottet, V.; Trichopoulos, D.; Naska, A.; Benetou, V.; Kaaks, R.; Rohrmann, S.; Boeing, H.; von Ruesten, A.; Panico, S.; Pala, V.; Vineis, P.; Palli, D.; Tumino, R.; May, A.; Peeters, P. H.; Bueno-de-Mesquita, H. B.; Büchner, F. L.; Lund, E.; Skeie, G.; Engeset, D.; Gonzalez, C. A.; Navarro, C.; Rodríguez, L.; Sánchez, M.-J.; Amiano, P.; Barricarte, A.; Hallmans, G.; Johansson, I.; Manjer, J.; Wirfält, E.; Allen, N. E.; Crowe, F.; Khaw, K.-T.; Wareham, N.; Moskal, A.; Slimani, N.; Jenab, M.; Romaguera, D.; Mouw, T.; Norat, T.; Riboli, E.; Trichopoulou, A. Mediterranean dietary pattern and cancer risk in the EPIC cohort. *Br. J. Cancer* **2011**, *104*, 1493–1499.
- (4) Reedy, J.; Mitrou, P. N.; Krebs-Smith, S. M.; Wirfält, E.; Flood, A.; Kipnis, V.; Leitzmann, M.; Mouw, T.; Hollenbeck, A.; Schatzkin, A.; Subar, A. F. Index-based dietary patterns and risk of colorectal cancer: the NIH-AARP Diet and Health Study. *Am. J. Epidemiol.* **2008**, *168*, 38–48.
- (5) Castelló, A.; Amiano, P.; Fernández de Larrea, N.; Martín, V.; Alonso, M. H.; Castaño-Vinyals, G.; Pérez-Gómez, B.; Olmedo-Requena, R.; Guevara, M.; Fernandez-Tardon, G.; Dierssen-Sotos, T.; Llorens-Ivorra, C.; Huerta, J. M.; Capelo, R.; Fernández-Villa, T.; Díez-Villanueva, A.; Urriaga, C.; Castilla, J.; Jiménez-Moleón, J. J.; Moreno, V.; Dávila-Batista, V.; Kogevinas, M.; Aragonés, N.; Pollán, M. MCC-Spain researchers. Low adherence to the western and high adherence to the Mediterranean dietary patterns could prevent colorectal cancer. *Eur. J. Nutr.* **2019**, *58*, 1495–1505.
- (6) Fliss-Isakov, N.; Kariv, R.; Webb, M.; Ivancovsky, D.; Margalit, D.; Zelber-Sagi, S. Mediterranean dietary components are inversely associated with advanced colorectal polyps: a case-control study. *World J. Gastroenterol.* **2018**, *24*, 2617–2627.
- (7) Lavalette, C.; Adjibade, M.; Srouf, B.; Sellem, L.; Fiolet, T.; Hercberg, S.; Latino-Martel, P.; Fassier, P.; Deschasaux, M.; Kesse-Guyot, E.; Touvier, M. Cancer-specific and general nutritional scores and cancer risk: results from the prospective NutriNet-Santé Cohort. *Cancer Res.* **2018**, *78*, 4427–4435.

- (8) Haslam, A.; Wagner Robb, S.; Hébert, J. R.; Huang, H.; Ebell, M. H. Association between dietary pattern scores and the prevalence of colorectal adenoma considering population subgroups. *Nutr. Diet.* **2018**, *75*, 167–175.

- (9) Petimar, J.; Smith-Warner, S. A.; Fung, T. T.; Rosner, B.; Chan, A. T.; Hu, F. B.; Giovannucci, E. L.; Tabung, F. K. Recommendation-based dietary indexes and risk of colorectal cancer in the Nurses Health Study and Health Professionals Follow-up Study. *Am. J. Clin. Nutr.* **2018**, *108*, 1092–1103.

- (10) Donovan, M. G.; Selmin, O. I.; Doetschman, T. C.; Romagnolo, D. F. Mediterranean diet: prevention of colorectal cancer. *Frontiers Nutr.* **2017**, *4*, 59.

- (11) Kavanaugh, C. J.; Trumbo, P. R.; Ellwood, K. C. The U.S. Food and Drug Administration's evidence-based review for qualified health claims: tomatoes, lycopene, and cancer. *J. Natl. Cancer Inst.* **2007**, *99*, 1074–1085.

- (12) Urquiza-Salvat, N.; Pascual-Geler, M.; Lopez-Guarnido, O.; Rodrigo, L.; Martinez-Burgos, A.; Cozr, J. M.; Ocana-Peinado, F. M.; Alvarez-Cubero, M. J.; Rivas, A. Adherence to Mediterranean diet and risk of prostate cancer. *Aging Male* **2019**, *22*, 102.

- (13) Vallverdú-Queralt, A.; de Alvarenga, J. F. R.; Estruch, R.; Lamuela-Raventós, R. M. Bioactive compounds present in the Mediterranean sofrito. *Food Chem.* **2013**, *141*, 3365–3372.

- (14) Martínez-Huélamo, M.; Tulipani, S.; Estruch, R.; Escribano, E.; Illán, M.; Corella, D.; Lamuela-Raventós, R. M. The tomato sauce making process affect the bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study. *Food Chem.* **2015**, *173*, 864–872.

- (15) Mitjavila, M. T.; Moreno, J. J. The effect of polyphenols on oxidative stress and the arachidonic acid cascade. Implications for the prevention/treatment of high prevalence diseases. *Biochem. Pharmacol.* **2012**, *84*, 1113–1122.

- (16) Gallardo, E.; Palma-Valdés, R.; Sarriá, B.; Gallardo, I.; de la Cruz, J. P.; Bravo, L.; Mateos, R.; Espartero, J. L. Synthesis and antioxidant activity of alkyl nitroderivatives of hydroxytyrosol. *Molecules* **2016**, *18*, 656.

- (17) Chanput, W.; Krueyos, N.; Ritthiruangdej, P. Anti-oxidative assays as markers for anti-inflammatory activity of flavonoids. *Int. Immunopharmacol.* **2016**, *40*, 170–175.

- (18) Bangalore, D. V.; McGlynn, W.; Scott, D. D. Effect of beta-cyclodextrin in improving the correlation between lycopene concentration and ORAC values. *J. Agric. Food Chem.* **2005**, *53*, 1878–1883.

- (19) Bredsdorff, L.; Nielsen, I. L. F.; Rasmussen, S. E.; Cornett, C.; Barron, D.; Bouisset, F.; Offord, E.; Williamson, G. Absorption, conjugation and excretion of the flavanones, naringenin and hesperidin from α -rhamnosidase-treated orange juice in human subjects. *Br. J. Nutr.* **2010**, *103*, 1602–1609.

- (20) Erlund, I.; Meririnne, E.; Alfthan, G.; Aro, A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperidin in humans after ingestion of orange juice and grapefruit juice. *J. Nutr.* **2001**, *131*, 235–241.

- (21) Sabhanwal, S. S.; Schumacker, P. T. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nat. Rev. Cancer* **2014**, *14*, 709–721.

- (22) Mariani, F.; Sena, P.; Roncucci, L. Inflammatory pathways in the early steps of colorectal cancer development. *World J. Gastroenterol.* **2014**, *20*, 9716–9731.

- (23) Martinez, J.; Moreno, J. J. Role of Ca^{2+} -independent phospholipase A_2 on arachidonic acid release induced by reactive oxygen species. *Arch. Biochem. Biophys.* **2001**, *392*, 257–262.

- (24) Cabral, M.; Martín-Venegas, R.; Moreno, J. J. Role of arachidonic acid metabolites on the control of non-differentiated intestinal epithelial cell growth. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 1620–1628.

- (25) Cabral, M.; Martín-Venegas, R.; Moreno, J. J. Differential cell growth/apoptosis behavior of 13-hydroxyoctadecadienoic acid enantiomers in a colorectal cancer cell line. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2014**, *307*, G664–G671.

(26) Cabral, M.; Martin-Venegas, R.; Moreno, J. J. Leukotriene D₄-induced Caco-2 cell proliferation is mediated by prostaglandin E₂ synthesis. *Physiol. Rep.* **2015**, *3*, No. e12417.

(27) Martin-Venegas, R.; Jáuregui, O.; Moreno, J. J. Liquid chromatography-tandem mass spectrometry analysis of eicosanoids and related compounds in cell models. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2014**, *964*, 41–49.

(28) Costa, H. S.; Vasilopoulou, E.; Trichopoulou, A.; Finglas, P. New nutritional data on traditional foods for European food composition databases on behalf of the participants of the EuroFIR traditional Foods Work Package. *Eur. J. Clin. Nutr.* **2010**, *64*, S73–S81.

(29) Jacobs, D. R.; Tapsell, L. C. Food, not nutrients, is the fundamental unit in nutrition. *Nutr. Rev.* **2007**, *65*, 439–450.

(30) Tresserra-Rimbau, A.; Lamuela-Raventos, R. M.; Moreno, J. J. Polyphenols, food and pharma. Current knowledge and directions for future research. *Biochem. Pharmacol.* **2018**, *156*, 186–195.

(31) Storniolo, C. E.; Moreno, J. J. Resveratrol Analogs with Antioxidant Activity Inhibit Intestinal Epithelial Cancer Caco-2 Cell Growth by Modulating Arachidonic Acid Cascade. *J. Agric. Food Chem.* **2019**, *67*, 819–828.

(32) Storniolo, C. E.; Moreno, J. J. Resveratrol metabolites have an antiproliferative effect on intestinal epithelial cancer cells. *Food Chem.* **2012**, *134*, 1385–1391.

(33) Vivancos, M.; Moreno, J. β -Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. *Free Radicals Biol. Med.* **2005**, *39*, 91–97.

(34) Martinez, J.; Sanchez, T.; Moreno, J. J. Regulation of prostaglandin E₂ production by the superoxide radical and nitric oxide in mouse peritoneal macrophages. *Free Rad. Res.* **2000**, *32*, 303–311.

(35) Vivancos, M.; Moreno, J. J. Role of Ca²⁺-independent phospholipase A₂ and cyclooxygenase/lipoxygenase pathways in the nitric oxide production by murine macrophages stimulated by lipopolysaccharides. *Nitric Oxide* **2002**, *6*, 255–262.

(36) Storniolo, C. E.; Sacanella, I.; Mitjavila, M. T.; Lamuela-Raventos, R. M.; Moreno, J. J. Bioactive compounds of cooked tomato sauce modulate oxidative stress and arachidonic acid cascade induced by oxidized LDL in macrophage cultures. *Nutrients* **2019**, *11*, 1880.

(37) Vivancos, M.; Moreno, J. J. Effect of resveratrol, tyrosol and beta-sitosterol on oxidized low-density lipoprotein-stimulated oxidative stress, arachidonic acid release and prostaglandin E₂ synthesis by RAW 264.7 macrophages. *Br. J. Nutr.* **2008**, *99*, 1199–1207.

(38) Miller, N. J.; Rice-Evans, C. A. Factors influencing the antioxidant activity determined by the ABTS⁺ radical cation assay. *Free Rad. Res.* **1997**, *26*, 195–199.

(39) Oyaizu, M. Studies on products of the browning reaction. Antioxidative activities of browning reactions products prepared from glucosamine. *Japan J. Nutr.* **1986**, *44*, 307–315.

(40) Kalyanaraman, B.; Darley-Usmar, V.; Davies, K. J. A.; Dennery, P. A.; Forman, H. J.; Grisham, M. B.; Mann, G. E.; Moore, K.; Roberts, L. J.; Ischiropoulos, H. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radicals Biol. Med.* **2012**, *52*, 1–6.