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Effect of *In Vitro* Gastrointestinal Digestion on Phytochemicals and Antioxidant Activities in Cherry Tomatoes (*Solanum lycopersicum* var. *cerasiforme*)

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ABSTRACT: We investigated the impact of simulated *in vitro* gastrointestinal digestion on the levels of total polyphenols, total flavonoids, carotenoids, and antioxidant capacity in cherry tomatoes. The initial total polyphenol content of fresh tomatoes was 220.51 μg GAE/g, which decreased to 203.24 μg GAE/g after 120 min of stomach treatment and further decreased to 138.23 μg GAE/g after 120 min of small intestine treatment. Similarly, the initial total flavonoid content in fresh tomatoes was 43.28 μg QE/g, but after 120 min of small intestine digestion, it decreased by approximately 50.72% to 21.33 μg QE/g. Lycopene, lutein, and β-carotene also experienced a decrease of 69.71～78.38% during the digestion process compared to fresh tomatoes. The antioxidant activity exhibited a reduction of 34.95～37.67% compared to fresh tomatoes after digestion in the stomach and intestines. The bioactive compounds present in tomatoes undergo decomposition and conversion into other substances during digestion, and these degradation products are believed to inhibit the growth of SK-Hep1 human hepatoma cells while enhancing antioxidant activity within the intracellular environment.

Keywords: antioxidant activity, cherry tomato, digestion, phytochemical

INTRODUCTION

The cherry tomato (*Solanum lycopersicum* var. *cerasiforme*), a smaller variant of the general tomato, is one of the most popular fruits worldwide, with consumption increasing due to the convenience of eating them in one bite without any additional processing, as opposed to regular tomatoes, which are large in size (Aldrich et al., 2010; Tian et al., 2016). Cherry tomatoes have a high nutritive value since they are abundant in sugars, vitamins, and minerals such as calcium, phosphorus, folic acid, and iron, as well as antioxidants such as carotenoids, polyphenols, flavonoids, and tocopherols (Aldrich et al., 2010; Coyago-Cruz et al., 2018). Tomato carotenoids, particularly lycopene, have a significant antioxidant impact, preventing illness and providing health benefits. Tomato intake has been shown to lower the risk of various malignancies, including prostate, colon, and breast cancers, as well as the risk of heart disease, cholesterol, and diabetes (Bhowmik et al., 2012; Rodrigues et al., 2022).

When there is a deficiency of antioxidant molecules to eliminate accumulated reactive oxygen species (ROS) in the body, oxidative stress develops (Keane et al., 2015). ROS is a compound composed of singlet oxygen, superoxide anions, hydroxyl radicals, nitric oxide, nitrogen peroxide, peroxide anion, and hydrogen peroxide (Görlach et al., 2015). It degrades proteins, lipids, and DNA in the body, resulting in chronic disorders such as cancer, cardiovascular disease, and neurological disease (Juan et al., 2021).

In vitro digestion is a technique for simulating the digestion of food or medicines that comprises the oral, stomach, and small intestine phases (Bornhorst and Paul Singh, 2014; Li et al., 2022). By displaying digestive enzymes and their concentrations, pH, digesting duration, and temperature, among other parameters, these systems seek to imitate *in vivo* physiological circumstances (Sams et al., 2016). Microogranis and enzymes present in the gastrointestinal system, as well as pH conditions, all impact the digestion of phenolic compounds in food (Li et al., 2022). Furthermore, bioavailability differs depending on the plant matrix and the structural properties of phenolic compounds (Pinto et al., 2017). Because physiologically active components in food are not entirely digested and absorbed by the body, the *in vitro* digestion technique may predict the digestion, absorption, and bioaccessibility

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of bioactive compounds in meals. *In vitro* digestion models have been used to investigate the bioaccessibility of antioxidants found in fruits and vegetables such as blackberries, green jujube, spinach, and kale (Pavan et al., 2014; Eriksen et al., 2017; Sánchez-Velázquez et al., 2021; Li et al., 2022; Bas-Bellver et al., 2023). A few investigations, however, have been undertaken to investigate the antioxidant constituents and antioxidant potential of the cherry tomato before and after *in vitro* digestion.

Therefore, we analyzed total polyphenols, total flavonoids, carotenoids, and antioxidant capacity in the cherry tomato before and during *in vitro* digestion in the mouth, stomach, and intestine.

MATERIALS AND METHODS

Chemicals and tomato samples

Mucin, bile extract, pancreatin, carotenoid standards (lutein, lycopene, β-carotene), gallic acid, catechin, Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), fetal bovine serum (FBS), and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma-Aldrich Chemical Co.. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Flunk. Pepsin and α-amylase were purchased from MP Biomedicals. Acetonitrile, methanol, ethyl acetate, sodium chloride, potassium chloride, and sodium bicarbonate were obtained from Fisher Scientific, and all chemicals used were of analytical grade. Cherry tomatoes (*Lycopersicon esculentum*) were purchased from a local market in Korea and stored at 4°C until the experiment. The *in vitro* digestion study was conducted using fresh cherry tomatoes.

Simulated *in vitro* gastrointestinal digestion

Fresh cherry tomatoes were subjected to *in vitro* gastric and intestinal digestion using the reported procedures (Hwang et al., 2019) with some modifications. Simulated saliva was prepared by dissolving 1 g/L of mucin, 2.10 g/L of $NaHCO_3$, 0.117 g/L of NaCl, 0.149 g/L of KCl, and 1.18 g/L of α-amylase in deionized water at pH 7.0. Simulated gastric juice was prepared by dissolving 1.50 g/L of mucin, 8.78 g/L of NaCl, and 1 g/L of pepsin in deionized water at pH 1.8. Simulated intestinal juice was prepared by dissolving 10.0 g/L of bile extract, 16.8 g/L of $NaHCO_3$, and 1,000 U/mL of pancreatin in deionized water at pH 6.7.

Fresh cherry tomatoes (100 g) were finely chopped in a food processor (Stanley Black and Decker) at low speed for 60 s (15 s. 4 times). The chopped tomatoes were combined with 30 mL of amylase-containing saliva and digested for 1 min in a 37°C shaking incubator. The oral digesta was combined with 200 mL of pepsin-containing gastric solution and digested for 2 h in a 37°C shaking incubator at 50 *g*. 50 g of gastric digesta was collected every 30 min to study the physicochemical parameters and antioxidant activity in relation to digesting time in the stomach. After 2 h of stomach digestion, 54 mL of intestinal juice was added to 80 g of gastric digesta, and the intestine was digested for 2 h in a 37°C shaking incubator at 50 *g*. 30 g of intestinal digesta were collected every 1 h to investigate the physicochemical characteristics and antioxidant activity in relation to digesting time in the gut. The obtained samples at various time points were kept at 4°C to inhibit enzymatic digestion and utilized for subsequent analysis.

Moisture content, pH and total acidity of fresh and digested cherry tomato

The moisture content of the samples before and throughout digestion was measured using a drying oven (EYELA) at 105°C. Each sample's pH was determined using a pH meter (420 Benchtop, Orion Research). Total acidity was estimated by adding 0.1 N NaOH to the sample until the pH reached 8.20, and it was expressed as the amount of citric acid in the sample.

Determination of total polyphenol, total flavonoid and carotenoid contents

The total polyphenol content was assessed using the Vasco et al. (2008) technique, and the total polyphenol content was represented as gallic acid equivalent (GAE). The total flavonoid content was calculated using Sakanaka et al. (2005)'s approach and represented as catechin equivalent.

Carotenoids present in fresh and digested tomatoes were extracted using the method described by Hwang et al. (2012). The carotenoids were then analyzed using an HPLC system (Shimadzu) with a mobile phase consisting of methanol:acetonitrile:tetrahydrofuran (50:45:5) on a C18 Novapak column (3.9×150 mm, 5 μm particle size, Waters Corp.). The analysis was performed at a flow rate of 1 mL/min at 30°C. Each peak was confirmed using a Waters 490 Programmable Multiwavelength Detector (Shimadzu).

Determination of antioxidant activity

DPPH and ABTS radical scavenging activities and reducing power were used to investigate the antioxidant activities of fresh and digested tomatoes throughout the digestion stages (Oyaizu et al., 1986). The DPPH radical scavenging activity of fresh and digested tomato extract was measured by combining equal parts sample and 0.2 mM DPPH solution, reacting at 37°C for 30 min, and determining the absorbance at 515 nm using the Cheung et al. (2003) technique.

One day before the experiment, the ABTS solution was

produced in preparation. In the dark, 7.0 mM of ABTS reagent and 2.45 mM of potassium persulfate were reacted to create ABTS cations, which were then diluted with ethanol to get an absorbance value of 0.73±0.03 at 735 nm. The ABTS radical scavenging activity of fresh and digested tomato extracts was evaluated by combining equal parts sample and ABTS solution, reacting at 37°C for 30 min, and measuring the absorbance at 732 nm using the Re et al. (1999) technique.

By adding 200 mM phosphate buffer (pH 6.6) and 1% potassium ferricyanide to 1 mL of sample extract, the reducing power was reacted for 20 min in a constant temperature water bath at 50°C. After the reaction was finished, 1 mL of 10% TCA solution was added, and the supernatant was centrifuged at 13,500 *g* for 15 min (Mega 17R, Hanil Co.). One milliliter of distilled water and one milliliter of ferric chloride were added to 1 mL of supernatant, stirred, and the absorbance at 720 nm was measured. The resulting number was denoted as the reduction power.

Cell culture and cell viability assay

The Korean Cell Line Bank provided SK-Hep1 human hepatoma cells, which were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS and penicillin/streptomycin in a humidified incubator at 37°C and 5% CO_2.

For the cell viability assay, SK-Hep1 cells were seeded at a density of 2×10^4 cells per well in 96-well plates and cultured in DMEM supplemented with 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Following cell seeding, the media were replaced with fresh, gastric, or intestinal digesta at the appropriate concentration, and the cells were then incubated for 24 h at 37°C under 5% CO_2 conditions. After the 24 h incubation period, 10 μL of MTT solution (10 mg/mL) was added to each well and incubated for 4 h. Subsequently, the media were removed, and 100 μL of dimethyl sulfoxide was added to dissolve the water-insoluble formazan dye. The mixture was dissolved and shaken at 72 *g* for 20 min at room temperature. The content of blue formazan formed in reaction with the MTT reagent was measured at 570 nm, and the percentage of surviving cells in the sample was expressed relative to the untreated control cells.

Cellular oxidative stress measured by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) assay

Oxidative stress in SK-Hep1 cells was evaluated using the DCFH-DA assay (Jang et al., 2010). After culturing the SK-Hep1 cells in a 96-well microplate until they were confluent, the growth media were removed, and each well was washed twice with phosphate-buffered saline (PBS) buffer. Then, 100 μM of DCFH-DA reagent and each sample at the appropriate concentration were added to the wells, followed by a 30 min incubation. After the incubation, the wells were washed again with PBS buffer. Next, 100 μM of H_2O_2 was added, and the fluorescence increase ratio was measured by absorbance at 0 and 30 min (ex/em: 485/530 nm) using a microplate reader. Positive control wells contained cells treated with DCFH-DA and H_2O_2. For the negative control wells, 100 μL of HBSS without H_2O_2 was added to the blank wells.

Statistical analysis

Statistical analysis was conducted using the statistical analysis system R-Studio (version 3.5.1). The data were compared using one-way analysis of variance, with a significance level of $P<0.05$ considered statistically significant.

RESULTS

Measurement of moisture content, pH and total acidity

Table 1 shows the moisture content, pH, and total acidity, which indicate the quantity of citric acid measured in fresh and digested cherry tomatoes. Fresh cherry toma-

Table 1. Moisture content, pH, and total acidity in fresh and digested cherry tomatoes

	Moisture content (%)	pH	Total acidity
Fresh cherry tomato	91.64±1.47^{c}	4.14±0.00^{c}	0.17±0.00^{e}
Oral digestion	94.21±0.25^{b}	4.13±0.00^{c}	0.17±0.00^{e}
Gastric digestion (min)			
30	95.22±0.00^{a}	3.18±0.00^{e}	0.41±0.00^{a}
60	95.02±0.12^{a}	3.15±0.00^{f}	0.41±0.00^{a}
90	95.93±0.17^{a}	3.18±0.01^{e}	0.38±0.00^{b}
120	95.48±0.89^{a}	3.20±0.00^{d}	0.34±0.02^{c}
Intestinal digestion (min)			
60	95.40±0.55^{a}	6.57±0.00^{b}	0.22±0.00^{d}
120	95.12±0.87^{a}	6.66±0.01^{a}	0.22±0.01^{d}

Values are presented as mean±SD of triplicate experiment.
Means with different superscripts (a-f) in the same column are significantly different at $P<0.05$.

toes had a moisture percentage of 91.64%, while oral, gastric, and intestinal digesta had moisture values ranging from 94.21% to 95.93%. Because they mingled with each digestive juice, moisture content rose in all samples throughout *in vitro* oral, gastric, and intestinal digestion processes.

The pH of a fresh cherry tomato was 4.14 and fell to 3.15~3.20 throughout the stomach's digestive process. The pH of the intestine rose by 6.57~6.66 after digestion. The total acidity of fresh cherry tomato was 0.17%, however, during gastric digestion, the total acidity rose to 0.34% to 0.41% before decreasing by 0.22% during the intestinal digestion stage. The pH of the digestive fluids at each step of digestion is thought to be intimately connected to changes in pH and overall acidity.

Total polyphenol and total flavonoid contents

Table 2 demonstrates the total polyphenol and total flavonoid content of fresh and digested cherry tomato extracts at various stages of digestion. The overall polyphenol content of an undigested cherry tomato was 220.51 μg GAE/g; after a 1-min oral digestion phase, the total polyphenol content of tomatoes was 218.01 μg GAE/g. The tomato digestates had 214.65 μg GAE/g during the gastric digestion phase at 30 min, with no significant difference (P> 0.05) when compared to the matching gastric digestion phase at 60 min for the digestates (211.14 μg GAE/g). The total polyphenol content of digestates obtained after 90 and 120 min of gastric digestion was 208.57 μg GAE/g and 203.24 μg GAE/g, respectively, and was not substantially (P>0.05) different. The total polyphenol levels of cherry tomatoes decreased throughout the intestine digestion phase compared to the stomach digestion phase, with values of 151.08 and 138.23 μg GAE/g, respectively, with no significant differences (P>0.05) between the intestinal phase at 60 and 120 min. Total polyphenol content decreased by 7.8% in the stomach phase and 37.31% in the intestinal phase. In comparison to undigested fresh tomato, the phenolic components digested in the colon for 2 h are somewhat less stable than those detected in the stomach for 2 h.

The overall flavonoid content of an undigested cherry tomato was 43.28 μg QE/g, however, after 1 min of oral digestion, the total flavonoid content of tomatoes reduced to 38.32 μg QE/g. At 30 min, the tomato digestates revealed values of 32.58~28.77 μg QE/g, a loss of 24.72% to 33.53% as compared to the undigested fresh tomato. The total flavonoid concentration of cherry tomatoes fell by 24.56 and 21.33 μg QE/g, respectively, during the intestinal digestion phase at 60 and 120 min. During the 2 h intestinal digestion phase, there was a fast loss of total polyphenols and total flavonoids. At the conclusion of the intestinal phase, only 37.31% of total polyphenols and 50.72% of total flavonoids were found.

Table 2. Total polyphenol and total flavonoid contents in fresh and digested cherry tomatoes

	Total polyphenol (μg GAE/g)	Total flavonoid (μg QE/g)
Fresh cherry tomato	220.51±4.58^{a}	43.28±1.37^{a}
Oral digestion	218.01±2.46^{a}	38.32±1.51^{b}
Gastric digestion (min)		
30	214.65±3.49^{b}	32.58±0.24^{c}
60	211.14±0.73^{b}	33.68±0.26^{c}
90	208.57±3.13^{c}	30.18±0.96^{d}
120	203.24±2.36^{c}	28.77±1.25^{d}
Intestinal digestion (min)		
60	151.08±4.25^{d}	24.56±0.77^{e}
120	138.23±2.43^{e}	21.33±0.20^{f}

Values are presented as mean±SD of triplicate experiment. Means with different superscripts (a-f) in the same column are significantly different at P<0.05.
GAE, gallic acid equivalent; QE, quercetin equivalent.

Determination of carotenoid contents

We identified three carotenoids, namely lutein, lycopene, and β-carotene, in both fresh and digested tomato samples using HPLC analysis with retention times of 2.1, 8.7, and 14.3 min, respectively (Fig. 1). Table 3 provides the carotenoid contents in fresh and digested cherry tomato extracts at different digestion stages. The lutein content in undigested cherry tomatoes was 28.68 μg/g. Following the oral digestion phase of 1 min, the lutein content in the oral digesta remained similar to the fresh sample, measuring 28.24 μg/g. During the gastric digestion process, there was a continuous decrease in lutein content. Specifically, it decreased from 20.59 μg/g at 30 min of digestion in the stomach to 11.47 μg/g at 120 min, representing a reduction of approximately 28.21% to 60.01% compared to the lutein content in fresh tomatoes. Further reductions in lutein content occurred during the 2-h digestion process in the intestinal phase. The lutein content in the small intestine digested after 60 and 120 min was 8.81 μg/g and 6.20 μg/g, respectively, which was approximately 69.28% to 78.38% lower than the lutein content in undigested fresh tomatoes.

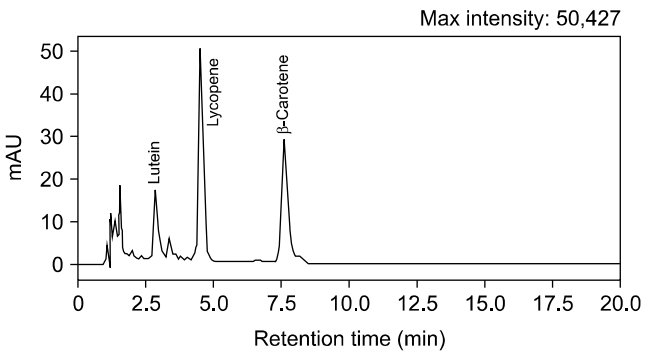

Fig. 1. Typical chromatogram of carotenoids detected in fresh and digested cherry tomato extract.

Table 3. Carotenoid concentration of fresh and digested cherry tomatoes (μg/g)

	Lutein	Lycopene	β-Carotene
Fresh cherry tomato	28.68±0.68[a]	86.12±1.92[a]	62.98±0.53[a]
Oral digestion	28.24±0.75[a]	82.02±0.56[a]	59.82±1.03[b]
Gastric digestion (min)			
30	20.59±1.84[b]	74.38±0.28[b]	43.67±0.85[c]
60	15.46±0.28[c]	66.88±3.68[c]	35.45±0.03[d]
90	14.02±0.27[c]	49.93±1.10[d]	28.89±1.08[e]
120	11.47±0.71[d]	40.12±1.22[e]	23.20±0.80[f]
Intestinal digestion (min)			
60	8.81±0.12[e]	31.63±2.21[f]	18.24±0.70[g]
120	6.20±0.40[f]	26.09±0.41[g]	14.57±1.49[h]

Values are presented as mean±SD of triplicate experiment.
Means with different superscripts (a-h) in the same column are significantly different at $P<0.05$.

In the undigested cherry tomato, the lycopene content was found to be 86.12 μg/g. Following the oral digestion phase of 1 min, the lycopene content in the oral digesta remained similar to the fresh sample, measuring 82.02 μg/g. During the gastric digestion process, there was a continuous decrease in lycopene content. Specifically, it decreased from 74.38 μg/g at 30 min of digestion in the stomach to 40.12 μg/g at 120 min, representing a reduction of approximately 13.63% to 53.41% compared to the lycopene content in fresh tomatoes. A further reduction in lycopene content occurred during the 2-h digestion process in the intestinal phase. The lycopene content in the small intestine digested after 60 and 120 min was 31.63 μg/g and 26.09 μg/g, respectively, which was approximately 63.27% to 69.71% lower than the lycopene content in undigested fresh tomatoes.

For undigested cherry tomatoes, β-carotene content was 62.98 μg/g. Following the oral digestion phase of 1 min, the β-carotene content in the oral digesta remained similar to the fresh sample, measuring 59.82 μg/g. During the gastric digestion process, the β-carotene content showed a continuous decrease. Specifically, it decreased from 43.67 μg/g at 30 min of digestion in the stomach to 23.20 μg/g at 120 min, representing a reduction of about 30.60% to 63.16% compared to the β-carotene content in fresh tomatoes. During the 2-h digestion process in the intestinal phase, the β-carotene content was further reduced. The small intestine digesta after 60 and 120 min contained 18.24 μg/g and 14.57 μg/g of β-carotene, respectively, which was approximately 71.04% to 76.87% lower than the β-carotene contained in undigested fresh tomato.

Measurement of antioxidant activities

DPPH and ABTS radical scavenging activities and reducing power tests were used to investigate the antioxidant potential of undigested and digested cherry tomatoes, and the results are reported in Table 4. Undigested fresh cherry tomatoes had a DPPH radical inhibition rate of 66.98%, whereas the rate after oral digestion fell to 65.29% and did not significantly vary from that of the undigested fresh tomato. Additionally, for the gastric phase fell to 64.53% at the 30 min mark, and subsequently fell to 60.64% at the 120 min mark. The DPPH radical scavenging activity was reduced by 48.82% after 60 min and by 41.75% after 120 min of intestinal digestion during the intestinal phase.

In the oral digesting phase, the inhibition rate of ABTS radicals reduced to 64.03%, and it was not statistically

Table 4. Antioxidant activity of fresh and digested cherry tomatoes

	DPPH radical scavenging activity (%)	ABTS radical scavenging activity (%)	Reducing power (absorbance at 720 nm)
Fresh cherry tomato	66.98±0.72[a]	64.78±0.78[a]	1.03±0.05[a]
Oral digestion	65.29±1.36[a]	64.03±0.74[a]	1.01±0.06[a]
Gastric digestion (min)			
30	64.53±1.44[b]	62.73±2.93[b]	0.91±0.04[b]
60	63.79±1.71[b]	63.57±2.60[b]	0.83±0.02[c]
90	59.35±0.84[c]	58.15±0.60[c]	0.82±0.04[c]
120	60.64±0.75[c]	54.21±0.33[cd]	0.81±0.04[cd]
Intestinal digestion (min)			
60	48.82±1.00[d]	43.22±1.22[d]	0.72±0.03[d]
120	41.75±1.35[e]	40.84±0.66[e]	0.67±0.05[e]

Values are presented as mean±SD of triplicate experiment.
Means with different superscripts (a-e) in the same column are significantly different at $P<0.05$.

different from the inhibition rate in the undigested fresh tomato. The inhibition rate in the undigested fresh cherry tomato was 64.78%. Consequently, for the gastric phase fell to 62.73% at the 30 min mark and subsequently fell to 54.21% at the 120 min mark. The ABTS radical scavenging activity was reduced by 43.22% after 60 min and by 40.84% after 120 min of intestinal digestion during the intestinal phase.

At 720 nm, absorbance was measured to evaluate the activity of reducing power. In the oral digesting phase, the inhibition rate reduced to 1.01 and was not statistically different from that of the undigested fresh tomato. The inhibition rate for reducing power in the undigested fresh cherry tomato was 1.03. Consequently, the value for the gastric phase declined to 0.91 at the 30 min mark and subsequently to 0.81 at the 120 min mark. The reducing power dropped by 0.72 after 60 min and by 0.67 at 120 min of intestinal digestion during the intestinal phase.

Cell cytotoxicity

SK-Hep1 human hepatoma cell viability was measured after supplementation with various concentrations of fresh and digested tomato extract for 24 h (Fig. 2). Compared with the control group, the number of viable cells showed a moderate decrease at low concentrations of tomato and tomato intestinal digesta, but a significant decrease at high concentrations. The viability of SK-Hep1 cells ranged from 101.15% to 72.24% within the concentration range of 1~1,000 μg/mL of fresh tomato extracts, indicating cell growth inhibition. Gastric digesta exhibited less cancer cell growth inhibition, ranging from 107.44% to 89.87% compared to other treatments. Intestinal digesta showed 98.27% to 51.89% inhibition of cancer cell growth. These results indicate that, compared to fresh tomatoes, the intestinal digesta of tomatoes effectively inhibits SK-Hep1 cell proliferation.

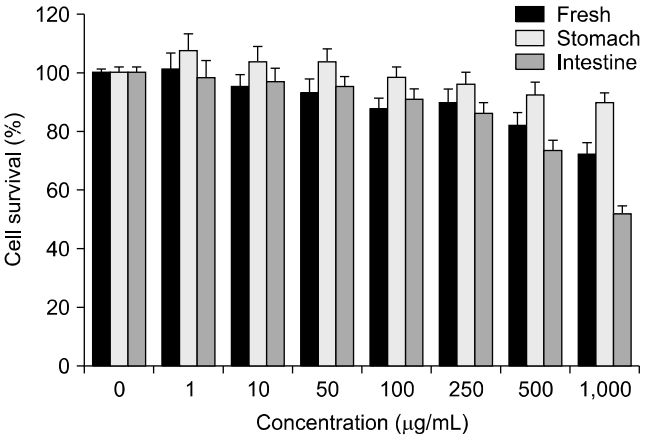

Fig. 2. Effect of fresh, stomach-digested, and intestinal-digested cherry tomato extract on the cytotoxicity of SK-Hep1 human hepatoma cells.

Determination of DCFH-DA

In the *in vitro* antioxidant measurements and cytotoxicity experiment, gastrointestinal digestive juice showed little effect compared to fresh tomato extract and small intestine digestive juice. Therefore, we used fresh tomato extracts and intestinal digesta for testing the *in vivo* antioxidant efficacy. SK-Hep1 cells were treated with different concentrations of fresh and intestinal digests of cherry tomatoes for 24 h. The treatment with both fresh and intestinal digesta significantly suppressed H_2O_2-induced ROS production (Fig. 3). Higher concentrations of the extracts resulted in lower DCFH-DA levels, indicating a dose-dependent inhibition of *in vivo* antioxidant activity. The addition of H_2O_2 increased intracellular ROS levels by approximately 1.41-fold, but the presence of undigested tomato or intestinal digesta clearly reduced ROS production. Treatment with fresh and intestinal digesta at concentrations of 100~1,000 μg/mL led to a significant decrease *in vitro* ROS production induced by H_2O_2. Intestinal digesta exhibited a higher inhibitory effect on ROS levels compared to fresh tomato at the same concentration. The treatment of 100, 500, and 1,000 μg/mL of tomato resulted in a reduction of intracellular ROS levels by 94.00, 86.74, and 77.28%, respectively. Similarly, the production of superoxide anion induced by H_2O_2 was dose-dependently inhibited by intestinal digesta, demonstrating its stronger antioxidant activity compared to fresh tomato. As shown in Fig. 3, the increase in intracellular ROS levels induced by H_2O_2 was reduced by 89.81, 83.39, and 76.62% with the treatment of 100, 500, and 1,000 μg/mL of intestinal digesta of cherry tomatoes, respectively.

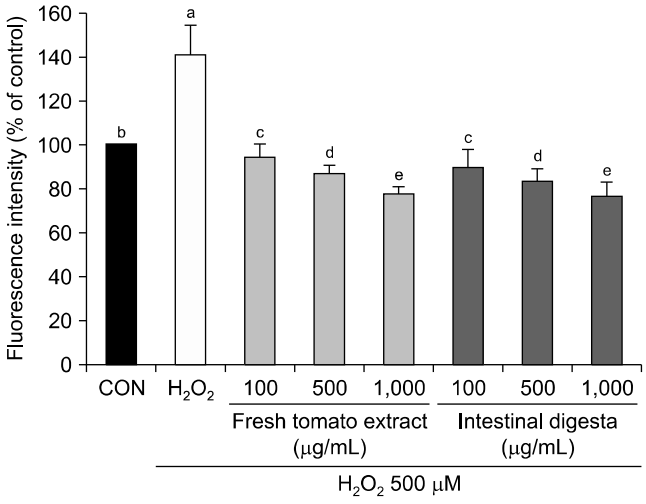

Fig. 3. Effect of fresh and intestinal-digested cherry tomato extract on DCFH-DA levels in SK-Hep1 human hepatoma cells. Values with different letters (a-e) indicate a significant difference (P<0.05). CON, control.

DISCUSSION

In vitro measurements of cherry tomato physicochemical parameters revealed the inhibitory effects of small intestine digestion products on SK-Hep1 human hepatoma cell proliferation and cellular oxidation. When the moisture content of cherry tomatoes was compared before and after digestion, it was discovered that the moisture content increased because the digestive fluids mixed with the food while it was being broken down and because the food's surface area grew owing to the action of the digestive enzymes. In accordance with our findings, it was discovered that the moisture content of kale, red beets, and apples increased as the *in vitro* digestion process progressed as compared to before digestion, showing a similar trend to the results of this study (Dalmau et al., 2017; Hwang et al., 2019; Mennah-Govela et al., 2020).

The pH of the oral cavity is typically slightly acidic at pH 6.0; however, the pH rapidly drops to pH 1.5∼2.0 in the stomach due to the release of hydrochloric acid and is then restored to slightly acidic in the small intestine by combining bile acid and sodium sulfate (Hwang et al., 2019; Diab et al., 2022). The environment in each digestive system influences the pH and acidity of food while it is being broken down throughout the digestive process (Bell et al., 2016). Meal digestion is crucial because it allows the body to release the nutrients that are present in the meal by breaking down the food via the chewing action of the tongue and digestive enzymes. Structure, texture, pH, and overall acidity of digestive fluids all have an impact on how quickly and completely food breaks down during digestion (Mennah-Govela et al., 2020).

The total polyphenol content did not show a significant reduction during gastric digestion, but a decrease was observed during small intestine digestion. It has been previously reported that phenolic compounds are highly sensitive to changes in pH. While they may not undergo significant changes in weak alkaline environments, they can degrade in strong alkaline conditions (Liu et al., 2007). Carotenoids, consequently, are known to be sensitive to various factors such as light, oxidation, and pH. They can undergo degradation during food processing, storage, and handling. Acidic pH conditions are particularly known to accelerate carotenoid decomposition, whereas relatively higher pH values in the range of 6.0 to 7.0 result in comparatively less degradation (Shao et al., 2017).

By demonstrating that there were no losses in polyphenol content during *in vitro* digestion of broccoli, Vallejo et al. (2004) provided evidence that polyphenols are really fairly stable at low pH levels and that pepsin digestion had no impact on polyphenol stability during stomach digestion. However, owing to digestion and pH changes, polyphenols in the digestive tract lost or changed structure for 2 h. According to studies, dietary polyphenols are particularly susceptible to circumstances comparable to those in the small intestine, and certain polyphenols are converted into matching breakdown products during intestinal digestion (Wong et al., 2014).

The stomach's acidic pH conditions helped stabilize polyphenol components throughout digestion in the stomach, so the antioxidant activity did not dramatically diminish. The highest levels of antioxidant activity were seen in the undigested cherry tomatoes. The antioxidant activity is significantly influenced by the pH of the digestive system, where food is digested (Li et al., 2022). In that instance, it has been noted that the release of phenolic and flavonoid chemicals found in food is enhanced to boost antioxidant activity when the pH is altered from neutral or alkaline to acidic (Sollano-Mendieta et al., 2021).

Because of its capacity to pass past the cellular membrane and be enzymatically hydrolyzed by intracellular esterases to DCFH, DCFH diacetate may be used in cell investigations (Halliwell et al., 2004). The presence of cellular peroxidases is required for H_2O_2 to oxidize DCFH to DCF (Myhre et al., 2003). Fresh tomatoes and tomato digestion products inhibited intracellular oxidation in the same way. When compared to fresh tomatoes, total polyphenols, total flavonoids, and carotenoids in tomatoes and tomato digesta decreased during digestion in the stomach and small intestine. Except for gastrointestinal digestive juices, growth inhibition of SK-Hep1 human hepatocellular carcinoma cells was effectively suppressed in fresh tomatoes and small intestine digestive products, and the efficacy for inhibiting intracellular oxide production was comparable in fresh tomatoes and small intestine digestive products. As digestion progresses, physiologically active substances in tomatoes are decomposed and converted into other substances; however, these degradation products are thought to contribute to cancer cell growth and antioxidant efficacy, and further research on digestion degradation products is considered necessary in the future.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: ESH. Analysis and interpretation: ESH. Data collection: all authors. Writing the article: ESH. Critical revision of the article: ESH. Final approval of the article: all authors. Statistical analysis: SK. Obtained funding: ESH. Overall responsibility: ESH.

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