

Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity

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Processed fruits and vegetables have been long considered to have lower nutritional value than their fresh commodities due to the loss of vitamin C during processing. This research group found vitamin C in apples contributed <0.4% of total antioxidant activity, indicating most of the activity comes from the natural combination of phytochemicals. This suggests that processed fruits and vegetables may retain their antioxidant activity despite the loss of vitamin C. Here it is shown that thermal processing elevated total antioxidant activity and bioaccessible lycopene content in tomatoes and produced no significant changes in the total phenolics and total flavonoids content, although loss of vitamin C was observed. The raw tomato had 0.76 ± 0.03 μmol of vitamin C/g of tomato. After 2, 15, and 30 min of heating at 88 °C, the vitamin C content significantly dropped to 0.68 ± 0.02 , 0.64 ± 0.01 , and 0.54 ± 0.02 μmol of vitamin C/g of tomato, respectively ($p < 0.01$). The raw tomato had 2.01 ± 0.04 mg of *trans*-lycopene/g of tomato. After 2, 15, and 30 min of heating at 88 °C, the *trans*-lycopene content had increased to 3.11 ± 0.04 , 5.45 ± 0.02 , and 5.32 ± 0.05 mg of *trans*-lycopene/g of tomato ($p < 0.01$). The antioxidant activity of raw tomatoes was 4.13 ± 0.36 μmol of vitamin C equiv/g of tomato. With heat treatment at 88 °C for 2, 15, and 30 min, the total antioxidant activity significantly increased to 5.29 ± 0.26 , 5.53 ± 0.24 , and 6.70 ± 0.25 μmol of vitamin C equiv/g of tomato, respectively ($p < 0.01$). There were no significant changes in either total phenolics or total flavonoids. These findings indicate thermal processing enhanced the nutritional value of tomatoes by increasing the bioaccessible lycopene content and total antioxidant activity and are against the notion that processed fruits and vegetables have lower nutritional value than fresh produce. This information may have a significant impact on consumers' food selection by increasing their consumption of fruits and vegetables to reduce the risks of chronic diseases.

KEYWORDS: Phenolics; antioxidant; phytochemicals; fruits; vegetables; processing; tomato

INTRODUCTION

Cardiovascular disease and cancer are ranked first and second, respectively, as the leading causes of death in the United States. Regular consumption of fruits and vegetables is associated with reduced risks of chronic diseases such as cancer, coronary heart disease, diabetes, Alzheimer's disease, cataracts, and age-related functional decline (1). Approximately 35% of deaths due to cancer in the United States are related to diet (2). Therefore, dietary modification is a practical strategy for the prevention of chronic diseases. The original aim of Recommended Dietary Allowances (RDA) to prevent clinical deficiencies has been shifted, and it is now focused on the prevention of diseases such as cancer, coronary heart disease, and birth defects (3). The National Research Council (NRC) has recommended eating five

or more servings of fruits and vegetables to increase public awareness of the health benefits of fruit and vegetable consumption.

Both the growth in consumers' awareness of the health benefits of fruits and vegetables and the emerging need for convenience due to a fast-paced lifestyle have increased the demand for ready-to-use (RTU) fruit and vegetable products. In recent years, product manufacturers have come up with various vegetable products to bring convenience to consumers. However, processed fruits and vegetables have been considered to have a lower nutritional value than their respective fresh commodities due to the loss of vitamin C content during the processing (4–7). Our research group found vitamin C in apples contributed <0.4% of total antioxidant activity, indicating most of the activity comes from the natural combination of phytochemicals (8). This suggests that processed fruits and vegetables may retain their antioxidant activity despite the loss of vitamin C. Therefore, the objective of this study is to evaluate the effect of thermal processing on the nutritional quality of tomatoes by assessing the total phenolics content, total fla-

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vonoids content, vitamin C content, lycopene content, and total antioxidant activity of raw and heat-treated tomatoes.

MATERIALS AND METHODS

Chemicals. Sodium nitrite, (+)-catechin, Folin–Ciocalteu reagent, hydrochloric acid, and α -keto- γ -methiolbutyric acid (KMBA) were obtained from Sigma Chemical Co. (St. Louis, MO). Aluminum chloride, sodium hydroxide, methyl *tert*-butyl ether, methanol, and acetone were purchased from Fisher Scientific (Pittsburgh, PA). Gallic acid, *all-trans*-lycopene, and metaphosphoric acid were purchased from ICN Biomedical Inc. (Costa Mesa, CA). Chloroform and hexane were purchased from Mallinckrodt (Paris, KY). 2,2'-Azobis(amidinopropane) (ABAP) was obtained from the Wako Chemicals (Richmond, VA). All reagents used were of analytical grade.

Sample Preparation. The tomatoes used were regular red tomatoes grown in village farm greenhouses and purchased from the local supermarket. Ten kilograms of tomatoes were sliced and placed into the Stephan cooker in the pilot plant of the Department of Food Science, Cornell University. The sliced tomatoes were blended with the vertical cutter mixer of the Stephan cooker for 5 min to obtain a homogeneous tomato slurry before being subjected to the four different treatments: raw, cooked at 88 °C for 2 min (commercial processing condition for canned tomato), cooked at 88 °C for 15 min, and cooked at 88 °C for 30 min. The raw treatment consisted of storing the homogeneous tomato slurry without any thermal treatment. The samples were subjected to heat treatment in a well-sealed pressure Stephan cooker to prevent water loss during the thermal processing. After the heating time for each treatment was reached, the samples were poured into the cans that were sealed immediately. All samples in each treatment were packaged in 8.8 cm (H) by 6.2 cm (D) cans with eight cans for each treatment and stored at -40 °C until use.

Extraction. One hundred grams of tomato slurry were weighed and homogenized with 80% acetone (1:2 w/v) using a chilled Waring blender for 5 min. The sample was then further homogenized using a Polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. The homogenates were filtered through No. 2 Whatman paper on a Büchner funnel under vacuum. The filtrate was recovered, and the acetone was evaporated by a rotary evaporator at 45 °C until ~90% of the filtrate had evaporated. The tomato extract was frozen at -40 °C until the time of analysis.

Determination of L-Ascorbic Acid Content. Total L-ascorbic acid content was determined using a 2,6-dichlorophenol (DIP) titrimetric method adapted from the *Official Methods of Analysis of the Association of Official Analytical Chemists* (9). All values were expressed as mean \pm SD μ mol of ascorbic acid equivalents per gram of tomato for six replications, each from a different can.

Determination of Total Phenolics Content. Samples were analyzed spectrophotometrically for the content of total phenolics using a modified Folin–Ciocalteu colorimetric method (8, 10). All tomato extracts were diluted 1:5 with distilled water to obtain readings within the standard curve ranges of 0.0–600.0 μ g of gallic acid/mL. Then 125 μ L of the standard gallic acid solution or 1:5 diluted tomato extract was mixed with 0.5 mL of distilled water in a test tube followed by addition of 125 μ L of Folin–Ciocalteu reagent (FCR). The samples were mixed well and then allowed to stand for 6 min before 1.25 mL of a 7% sodium carbonate aqueous solution was added. Water was added to adjust the final volume to 3 mL. Samples were allowed to stand for 90 min at room temperature before measurement at 760 nm versus the blank using an MRX II DYNEX spectrophotometer (DYNEX Technologies, Inc., Chantilly, VA) in comparison with the standards prepared similarly with known gallic acid concentrations. All values were expressed as mean (micrograms of gallic acid equivalents per gram of tomato) \pm SD for eight replications.

Determination of Total Flavonoid Content. Total flavonoid content was determined by using a colorimetric method described previously (8, 11). Briefly, 0.25 mL of the tomato extract or (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube followed by addition of 75 μ L of a 5% NaNO₂ solution. After 6 min, 150 μ L of a 10% AlCl₃·6H₂O solution was added and allowed to stand for another 5 min before 0.5 mL of 1 M NaOH was added. The mixture

was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm using an MRX II DYNEX spectrophotometer (DYNEX Technologies, Inc.) in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results are expressed as mean (micrograms of catechin equivalents per gram of tomato) \pm SD for eight replications.

HPLC Analysis of Lycopene. Approximately 10 g of tomato slurry was extracted using a mixture of 5 mL of chloroform, 3 mL of acetone, and 15 mL of hexane followed by vigorous shaking for 5 min. The mixture was centrifuged at 2000 rpm, and the organic solvent fraction was removed. The sample was further extracted three times with 5 mL of acetone and 15 mL of hexane. After the extractions, the sample was devoid of red pigments, indicating that lycopene had been completely extracted. All hexane fractions were combined and evaporated under a stream of nitrogen at 37 °C until the volume fell below 15 mL. The extract was dried over anhydrous sodium sulfate. Hexane was added to the dried extract up to a volume of 50 mL. Direct exposure of samples to light was avoided during the extraction. Separation, analysis, and quantification of *all-trans*-lycopene were accomplished by HPLC using a Carotenoid C₃₀ column, 250 \times 4.6 mm, 3 μ m (YMC, Inc., Wilmington, NC). An isocratic mobile phase was used for the separation and was composed of methyl *tert*-butyl ether and methanol in a ratio of 7:3 at a flow rate of 1 mL/min. Lycopene was detected using a Waters 484 UV-visible detector (Waters Corp., Milford, MA) at a wavelength of 471 nm. Detector signals were acquired and integrated by Waters Millennium32 software (Waters Corp.) on a personal computer. The lycopene calibration curve was generated from peak heights of calibration standards and gave an R² value of 0.996. The recovery of spiked lycopene after extraction and chromatographic procedures was 95.56 \pm 7.58% (*n* = 6). Lycopene concentrations in samples were calculated by extrapolation on the calibration curve. Peak identification of *all-trans*-lycopene in sample extracts was based on retention time and cochromatography of authentic lycopene standard (ICN Biomedical Inc., Costa Mesa, CA) (*all-trans*, 92.9% purity). The total *cis*-lycopene isomers were combined and reported as *trans*-lycopene equivalents (12).

Quantification of Total Antioxidant Activity. The total antioxidant activity of the phytochemical extracts from tomato was measured by total oxyradical scavenging capacity (TOSC) assay (8, 13). Antioxidant activity was assessed at four different time points (15, 30, 45, and 60 min) and six different concentrations to determine the TOSC value. The TOSC value for each concentration of tomato sample was calculated using the integration of the area under the kinetic curve. The TOSC value for each concentration was quantified according to the equation

$$\text{TOSC} = 100 - \left(\frac{\int SA}{\int CA} \times 100 \right)$$

where $\int SA$ is the integrated area from the sample reaction and $\int CA$ is the integrated area from the control reaction.

For the tomato extract of each treatment, the median effective dose (EC₅₀) was determined from the dose–response curve of concentration of tomato versus TOSC. Using the median effective dose for each treatment, the TOSC value was expressed as micromoles of vitamin C equivalents for 1 g of tomato. All TOSC values are presented as mean \pm SD for six replicates.

Statistical Analysis. Statistical analyses were conducted using SigmaStat version 1.0 (Jandel Corp., San Raphael, CA). Differences among treatments were determined using a *t* test. For relationship plots significance of the relationship was determined by regression analysis of variance.

RESULTS

The vitamin C content declined with increased heating time at 88 °C, which is consistent with previous results (4–7). The raw tomato had the highest vitamin C content (0.76 \pm 0.03 μ mol of vitamin C/g of tomato). After 2, 15, and 30 min of heating at 88 °C, the vitamin C content dropped to 0.68 \pm 0.02,

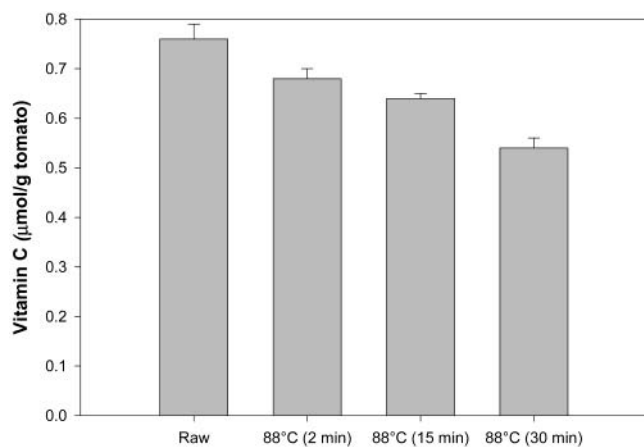


Figure 1. Effect of thermal processing at 88 °C on vitamin C content in tomatoes (mean \pm SD, $n = 6$).

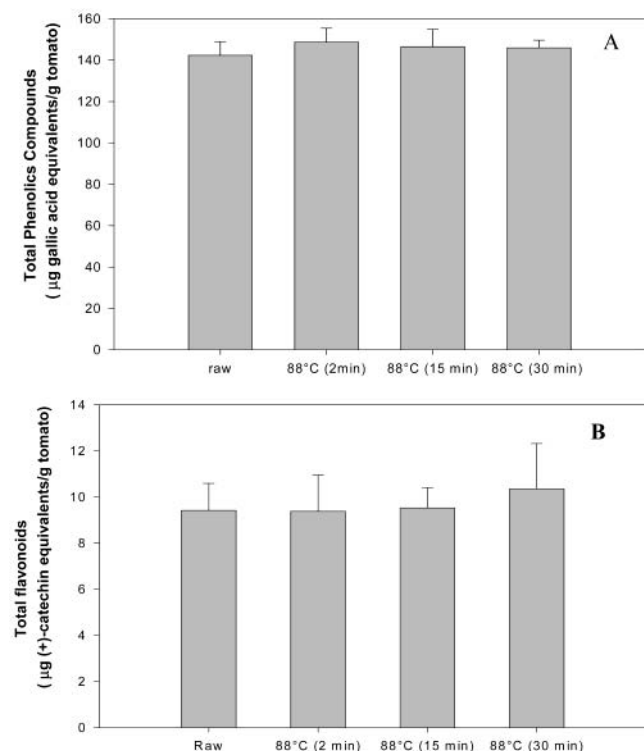


Figure 2. Effect of thermal processing at 88 °C on total phenolic content (A) and total flavonoid content (B) in tomatoes (mean \pm SD, $n = 8$).

0.64 ± 0.01 , and 0.54 ± 0.02 μmol of vitamin C/g of tomato (decreases of 10.53, 15.79, and 28.95%), respectively (**Figure 1**). The decline in vitamin C content was statistically significant between the raw tomato sample and the heat-treated tomato samples (t test, $n = 6$, $p < 0.01$).

The total phenolics content of the raw tomato sample was 142.4 ± 6.5 $\mu\text{g/g}$ of tomato. With thermal processing at 88 °C, the total phenolics contents in the 2, 15, and 30 min heat-treated tomato samples were 148.7 ± 6.5 , 146.5 ± 8.4 , and 145.9 ± 3.7 $\mu\text{g/g}$ of tomato, respectively (**Figure 2A**). The total flavonoids content of the raw tomato sample was 9.42 ± 1.17 $\mu\text{g/g}$ of tomato. With thermal treatment at 88 °C, the flavonoids contents in the 2, 15, and 30 min treated tomato samples were 9.38 ± 1.58 , 9.53 ± 0.88 , and 10.35 ± 1.97 $\mu\text{g/g}$ of tomato, respectively (**Figure 2B**). Although the heat-treated samples had slightly higher contents of total phenolics and flavonoids, there were no significant differences among all treatments (t test, $n = 8$, $p > 0.05$). Therefore, there was no loss or gain in content

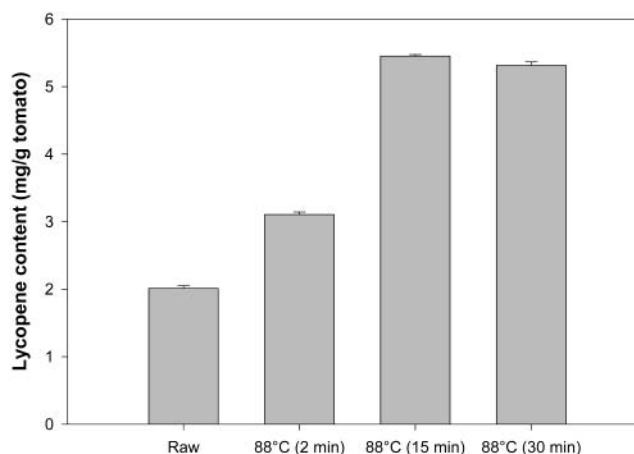


Figure 3. Effect of thermal processing at 88 °C on lycopene content in tomatoes (mean \pm SD, $n = 3$).

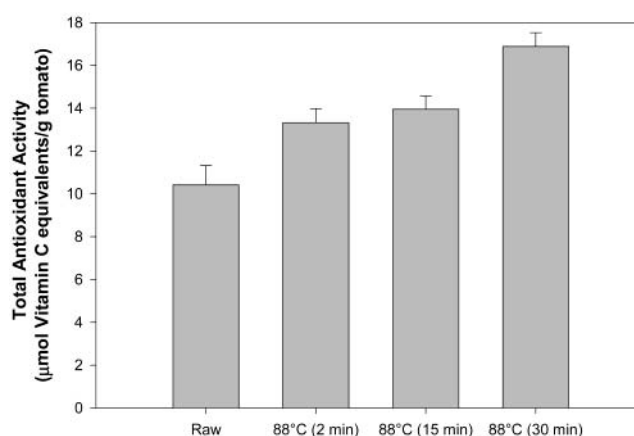


Figure 4. Effect of thermal processing at 88 °C on total antioxidant activity in tomatoes (mean \pm SD, $n = 6$).

of both total phenolics and flavonoids in tomatoes with thermal processing at 88 °C for 2, 15, and 30 min.

Total *trans*-lycopene content in the tomatoes increased with increased heating time at 88 °C compared to the constant amount of total phenolics and flavonoids and the decline in vitamin C content. The raw tomato had the lowest *trans*-lycopene content (2.01 ± 0.04 mg of *trans*-lycopene/g of tomato). After 2, 15, and 30 min of heating at 88 °C, the *trans*-lycopene content had increased to 3.11 ± 0.04 , 5.45 ± 0.02 , and 5.32 ± 0.05 mg of *trans*-lycopene/g of tomato (increases of 54.39, 171.11, and 164.26%), respectively (**Figure 3**). The increase in total *trans*-lycopene content was statistically significant between the raw tomato sample and the heat-treated tomato samples (t test, $n = 3$, $p < 0.01$).

Total *cis*-lycopene content in the tomatoes also increased with increased heating time at 88 °C. The raw tomato had the lowest total *cis*-lycopene content (0.86 ± 0.09 mg/100 g of tomato). After 2, 15, and 30 min of heating at 88 °C, the *cis*-lycopene content had increased to 0.91 ± 0.15 , 1.01 ± 0.13 , and 1.16 ± 0.21 mg/100 g of tomato (increases of 5.81, 17.44, and 34.88%), respectively. The increase in total *cis*-lycopene content was statistically significant between the raw tomato sample and the heat-treated tomato samples (t test, $n = 3$, $p < 0.05$).

Thermal treatment at 88 °C increased the total antioxidant activity of the tomatoes. The raw tomato sample was found to have a total antioxidant activity of 10.42 ± 0.91 μmol of vitamin C equiv/g of tomato (**Figure 4**). With thermal treatment at 88 °C, the total antioxidant activity in the 2, 15, and 30 min treated

Table 1. Percent Changes of Vitamin C Content, Lycopene Content, and Total Antioxidant Activity in Processed Tomatoes in Comparison to Those in Unprocessed Tomatoes

	processing time at 88 °C		
	2 min	15 min	30 min
vitamin C	-10.2	-15.5	-29.4
lycopene	54.4	171.1	164.3
total antioxidant activity	28.1	33.9	62.2

tomato samples increased to 13.33 ± 0.65 , 13.95 ± 0.62 , and 16.89 ± 0.64 μmol of vitamin C equiv/g of tomato, respectively. These were increases of 27.93, 33.88, and 62.09% in total antioxidant activity in the 2, 15, and 30 min samples in comparison to the raw samples, respectively. The increase in the total antioxidant activity between the raw and the heat-processed tomatoes was found to be statistically significant (*t* test, *n* = 6, *p* < 0.01).

DISCUSSION

Processed fruits and vegetables have long been perceived to have lower nutritional value than the fresh commodities because of the decline in vitamin C (4–7). We also observed the loss of vitamin C in heat-processed tomatoes at 88 °C with an estimated $D_{88^\circ\text{C}}$ value (the time taken for 90% reduction of the initial vitamin C content at 88 °C) of 276 min (Table 1). This value is consistent with the kinetic study of the loss of vitamin C in canned peas with a $D_{121^\circ\text{C}}$ value of 246 min (5). Loss of vitamin C occurs primarily by chemical degradation that involves oxidation of ascorbic acid to dehydroascorbic acid (DHAA), followed by hydrolysis to 2,3-diketogulonic acid and further polymerization to form other nutritionally inactive products (14). Because heat is known to speed the oxidation process of ascorbic acid, thermal processing results in loss of vitamin C content in fruits and vegetables (14). Our group had found that vitamin C contributed <0.4% of the total antioxidant activity in apples (8). This suggested the heat-processed tomatoes might retain their total phenolics, flavonoids, and total antioxidant activity despite the loss of vitamin C. The results supported our hypothesis, as there were no changes in total phenolics and total flavonoids content in tomatoes with thermal treatment at 88 °C. Phenolic acids occur in plants as metabolic intermediates, and they also accumulate in the vacuoles (15). Thermal processing may release more bound phenolic acids from the breakdown of cellular constituents. Although disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (15), thermal processing at 88 °C deactivates these enzymes to avoid the loss of phenolic acids.

Thermal processing significantly increased the content of bioaccessible lycopene in tomatoes. We believe that the increase in bioaccessible lycopene content is primarily due to the increased release of phytochemicals from the matrix to make it more accessible in the extraction. The amounts of *trans*-lycopene in 15 and 30 min heat-processed tomato samples were similar because the complete release of lycopene from the cell matrix by thermal processing had been reached. The amount of *cis*-lycopene had also increased with heating time. *cis*-Lycopene isomers were found to have higher antioxidant potential with an estimated twice the activity of *all-trans*- β -carotene (16). It was reported that food processing such as cooking or grinding might improve lycopene bioavailability by breaking down cell walls (17, 18). In humans, lycopene bioavailability was greater from heat-processed tomatoes compared to fresh tomatoes (17,

19). This suggests that heat-processed tomatoes had higher bioaccessible lycopene because of the elevated release of lycopene from the cell matrix. Most lycopene is located in the outer pericarp and the skin attached to the insoluble fiber portion of the tomatoes (18). Thermal processing disrupts the cell membranes and cell walls and releases lycopene from the insoluble portion of the tomatoes, which increases the pool of bioaccessible lycopene and improves lycopene absorption. The increase in *trans*-lycopene content reported in this paper was higher than expected for the heat-treated samples, but the values in raw tomatoes were within a similar range as those reported (18). It was also reported that lycopene was relatively stable to survive the commercial thermal processing conditions (12). Of all the carotenoid pigments, lycopene is the most efficient singlet oxygen quencher (20). The quenching constant of lycopene was found to be more than twice that of β -carotene and 10 times more than that of α -tocopherol, thus making its presence in the diet important (18).

Our results clearly showed lycopene content and total antioxidant activity significantly increased with thermal processing despite the nonsignificant changes in the total phenolic and total flavonoid content and the decline in vitamin C content in the heat-processed tomatoes. The increase in total antioxidant activity of the heat-processed tomatoes could be explained by the increased amount of lycopene, a major phytochemical in tomatoes, and other bound phytochemicals released from the matrix with thermal processing at 88 °C. Another reason could be the additive and synergy effects of other phytochemicals such as phenolics and flavonoids (8).

Thermal processing enhances the nutritional value of tomatoes by increasing the content of bioaccessible lycopene and total antioxidant activity (Table 1). The findings are against the notion that processed fruits and vegetables have lower nutritional value and may create a new image for processed fruits and vegetables. This will have a direct impact on consumers' food selection and will increase their awareness of the health benefits of processed fruits and vegetables in the prevention of chronic diseases. Processed fruits and vegetables satisfy consumers' need for convenience as they are readily available, and easy to use, and, in some instances, better nutritional and economical values. Consequently, this may help consumers increase their intake of fruits and vegetables to reduce the risk of chronic diseases. This work will also open up more opportunities for the food-processing industry.

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