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Lycopene Bioaccessibility and Starch Digestibility for Extruded Snacks Enriched with Tomato Derivatives

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ABSTRACT: To improve the nutritional value of energy-dense extruded snacks, corn grits were replaced with tomato paste and/or tomato skin powder at ratios of 5, 10, and 20% and extruded to make expanded snack foodlike products. Using a model digestion system, lycopene bioaccessibility and uptake from the snacks into Caco-2 cells were determined. The digestibility of the starch, the main nutrient component of the snacks, was also investigated. While extrusion cooking reduced the lycopene content of the snacks, the proportion of bioaccessible lycopene increased. Lycopene uptake by the Caco-2 cells from the extruded snacks exceeded that of the control in which the lycopene was not extruded, by 5% ($p < 0.05$). The digestibility of starch in the snacks varied depending on the type of tomato derivative and its concentration. Optimization of the extrusion cooking process and the ingredients can yield functional extruded snack products that contain bioavailable lycopene.

KEYWORDS: Extrusion, lycopene bioaccessibility, starch digestibility, tomato enrichment

INTRODUCTION

Snacks produced by the extrusion cooking process are versatile, convenience foods that are shelf stable for up to 9 months. Cereal-based extruded snacks are made from a foamed matrix, largely comprised of gelatinized starch at low water activity ($a_w < 0.4$), and they usually have a crisp, crunchy texture.[1] These snacks are generally poor in nutritional components other than starch.[2,3] Fruit and vegetable derivatives that are rich in nutrients can be added to produce extruded snacks with improved nutritional value.

Tomatoes are of particular interest for this enrichment due to their attractive red color and high levels of fiber and lycopene. Lycopene is a carotenoid that is responsible for the red color of the tomatoes. It has been reported to reduce free radicals and have antioxidant properties. Thus, many health claims have been associated with its consumption including reduced risk of cancer and cardiovascular disease and the promotion of immune system functionality.[4,5] The incorporation of tomatoes that have lycopene, into the extruded snacks, introduces a functional ingredient into these foods, and along with the energy obtained from the starch, an extruded snack with improved nutritional value can be produced.[2,6]

For a nutrient to be beneficial to health, it has to be bioavailable. In other words, it needs to become bioaccessible or released from the food matrix during digestion in a state that allows uptake by cells, situated in the lining of the intestine. Upon absorption by these cells, the lycopene is delivered to the target tissue where it is used or stored.[7]

The bioavailability of nutrients is manipulated by many factors including the processing technique employed to prepare the food.[8,9] For instance, extrusion cooking of snack foods is a high shear, high temperature process, typically above 120 °C, that develops a plastic melt from the starch. The melt expands as it leaves the extruder through the die due to the sudden drop in pressure and vaporization of water at the high temperatures present in the melt. During processing in the extruder, starch is gelatinized, increasing its digestibility.

The extrusion process also mechanically disrupts cell walls, releasing the cell contents into the starchy matrix.[10,11] In tomatoes, lycopene is situated within the plastids scattered around the chromoplast of the cells,[10,12] and if the cells remain intact, lycopene may not be bioavailable during digestion. Disruption of the cell walls improves the bioavailability of lycopene; however, it can also expose the labile lycopene molecule to the high shear and temperatures during processing, resulting in its partial or complete loss.[6]

Apart from processing itself, the presence of other food components in the ingredients affects the bioavailability of nutrients. For example, soluble fibers such as pectin and hemicelluloses present in tomato products can reduce shear and processing temperatures, thus reducing disruption of cell walls.[1] Furthermore, soluble fiber at high concentrations may interfere with the activity of the digestive enzymes and the absorption of both starch and lycopene.[13−16] Starch also interacts with other ingredients, including lipids, during extrusion cooking to form less digestible structures such as amylose−lipid complexes.[9,17] Thus, the fate of both lycopene and starch during digestion should be investigated when determining the nutritional value of the extruded snacks enriched with tomato derivatives.

The bioavailability of nutrients in foods can be predicted using in vitro digestion methods when costly clinical trials are inappropriate. For lycopene to be absorbed, it must be released from the food matrix and partitioned into the micellar phase of the digesta in the intestinal lumen with the aid of bile salts.[8] For this

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Table 1. Formulation and Proximate Composition of the Ingredients[a]

formulation	total derivative (%)	tomato skin (%)	tomato paste (%)	moisture	protein	fat	ash	starch	fiber
control	0	0	0	11.77	6.74	2.33	0.57	78.31	1.10
1	5	100	0	11.18	7.11	2.36	0.88	74.71	4.97
2	5	75	25	11.18	7.03	2.34	0.82	74.72	4.23
3	5	50	50	11.18	6.96	2.32	0.76	74.72	3.50
4	5	25	75	11.18	6.88	2.29	0.70	74.72	2.76
5	5	0	100	11.18	6.81	2.27	0.64	74.73	2.03
6	10	100	0	10.60	7.48	2.39	1.19	71.11	8.84
7	10	75	25	10.60	7.33	2.35	1.07	71.12	7.37
8	10	50	50	10.60	7.18	2.30	0.95	71.13	5.90
9	10	25	75	10.60	7.02	2.26	0.83	71.14	4.42
10	10	0	100	10.60	6.87	2.21	0.71	71.15	2.95
11	20	100	0	9.42	8.22	2.46	1.80	63.91	16.58
12	20	75	25	9.42	7.92	2.37	1.57	63.93	13.64
13	20	50	50	9.43	7.61	2.28	1.33	63.95	10.69
14	20	25	75	9.43	7.31	2.19	1.09	63.97	7.75
15	20	0	100	9.43	7.00	2.10	0.86	63.99	4.80

[a] Calculated from the proximate composition of the raw ingredients. Data are reported as percentage values.

reason, lycopene bioaccessibility is measured as the fraction of lycopene in the food material that is transferred to the micellar phase of the digesta during simulated digestion. The proportion of micellar lycopene absorbed by cultured Caco-2 cells can be used as a model for uptake by human intestinal epithelial cells.[9,18,19] Similarly, for starch, in vitro methods that measure the starch digestibility during the simulated digestion of food have been shown to provide a good estimate of the bioavailability of starch.[20]

In the present study, the effect of varying the proportion of tomato skin and/or tomato paste in corn-based extruded snacks on the lycopene bioaccessibility and digestibility of starch was investigated. Furthermore, one snack type was used to investigate the uptake of lycopene by Caco-2 cell lines.

MATERIALS AND METHODS

Ingredients. Tomato processing waste, consisting of tomato skin and seeds, and tomato paste were donated by Heinz Watties (Heinz-Watties NZ, Ltd., Hastings, New Zealand). Tomato skin was separated from seeds by flotation on water. Both tomato skin and paste were freeze-dried (model FD18LT "ISLA", Cuddon, Blenheim, New Zealand) at −35 °C for 36 h and milled to a particle size of less than 710 μm (ZM200, Retsch, Germany). Preparations of tomato skin and paste powder were made by combining them at ratios of 0:100, 25:75, 50:50, 75:25, or 100:0. Corn grits (Specification 220) were supplied by Corson Grain Ltd. (Gladstone Rd, Gisborne, New Zealand). Blends of corn grits with the tomato derivative were prepared by substituting 5, 10, or 20% of corn with the five tomato product blends (100:0, 75:25, 50:50, 25:75, and 0:100 tomato skin:tomato paste ratio). Thus, a total of 15 formulations were used to make the extruded snacks. Extruded corn grits were also produced as the control without tomato enrichment.

For digestion, all chemicals including solvents and other reagents were purchased from Sigma Chemicals Co. (St. Louis, MO). The enzymes used were pepsin (from porcine stomach mucosa, Sigma product code P7125, 56 units/mg solids), pancreatin (from porcine pancreas, Sigma product code P7525, 8 × USP), invertase concentrate (Sigma product code E.C. 3.2.1.26), and amyloglucosidase (AMG-300 L, BrewQ) and were obtained from Novozymes (Australia). Porcine bile extract (Sigma product code B-8631) was used. The analytical standard for lycopene (L9879) was also purchased from Sigma Chemicals Co.

Proximate Composition. The proximate composition of the ingredients was determined using the appropriate AOAC method.[21] The moisture content of the raw ingredients and extruded products was determined by overnight oven drying at 105 °C. The ash content was determined from the percentage of uncombusted material following heating in a furnace at 550 °C. The crude fat proportion was determined following Soxhlet extraction with petroleum ether. The crude protein content was estimated from the Kjeldahl digestion method to determine nitrogen content and 6.25 as a factor to convert N to estimated protein content. The total dietary fiber and starch were determined using the Megazyme Total Dietary Fiber and Starch analysis kit (Megazyme International, Wexford, Ireland). The chemical composition of the raw ingredients, corn grits, tomato paste, and tomato skin powder, was determined and used to calculate the proximate composition of the formulations (Table 1).

Extrusion Processing. The extruded products were made using a laboratory scale twin-screw extruder (Clextral BC21 twin-screw Clextral, Firminy Cedex, France). The screw diameter was 24.7 mm, and the total working barrel length of 700 mm was divided into seven temperature - controlled working sections set to 40/60/80/100/140/140/140 °C from the ingredient inlet end of the machine to the die output. A single 3 mm diameter die was fitted. The feed rate of the dry ingredients was 10.5 kg/h, and the screw speed was 350 rpm

Extrusion processing parameters such as specific mechanical energy (SME), power consumption (kW), and torque values (M) showed that the energy used to extrude the product can be used to relate the extrusion cooking process with the product characteristics. The extrusion parameters are given in Table 2.

Simulated Digestion. The in vitro digestion method was carried out according to the method described previously.[22] Briefly, 4 g (dry basis) of the ground sample (<2 mm particle size) was weighed into glass jars, and 15 mL of distilled water was added. For the gastric phase of digestion, the pH was adjusted to 2.5 using 1 M HCl. Then, porcine pepsin in 0.1 M HCl was added to reach a final concentration of 3.3 mg/mL. After 30 min of incubation at 37 ± 0.5 °C, the pH of sample was increased to 5.2 by adding 0.5 M sodium acetate . The samples were further incubated for 15 min at high shaking speed. This was followed by the intestinal phase of digestion where the pH was adjusted to 6.5 by

Table 2. Extrusion Conditions of the Products[a]

	total derivative (%)	skin (%)	paste (%)	torque (N m)	power consumption (kW)	SME (kJ/kg)	expansion (%)
1	5	100	0	7.90 ± 0.00 a	1.90 ± 0.00 a	382 ± 1 a	475.59 ± 5.00 a
2	5	75	25	7.70 ± 0.40 ab	1.86 ± 0.08 ab	376 ± 16 ab	454.59 ± 8.30 b
3	5	50	50	7.53 ± 0.25 abc	1.74 ± 0.11 de	356 ± 18 bcd	447.90 ± 13.61 bc
4	5	25	75	6.90 ± 0.20 de	1.60 ± 0.06 f	341 ± 6 de	420.97 ± 4.93 de
5	5	0	100	5.73 ± 0.12 hi	1.42 ± 0.00 h	332 ± 3 de	370.92 ± 9.33 gh
6	10	100	0	7.50 ± 0.00 bc	1.82 ± 0.00 bc	378 ± 1 ab	434.00 ± 12.17 cd
7	10	75	25	7.27 ± 0.32 cd	1.76 ± 0.05 cde	357 ± 43 bcd	413.20 ± 8.03 ef
8	10	50	50	7.17 ± 0.06 cde	1.70 ± 0.03 e	341 ± 1 de	399.36 ± 6.97 f
9	10	25	75	6.80 ± 0.17 ef	1.62 ± 0.02 f	334 ± 5 de	363.05 ± 6.90 h
10	10	0	100	5.40 ± 0.17 i	1.29 ± 0.01 i	303 ± 4 f	301.03 ± 18.43 j
11	20	100	0	6.50 ± 0.00 fg	1.59 ± 0.00 f	371 ± 6 abc	380.00 ± 6.00 g
12	20	75	25	6.33 ± 0.06 g	1.56 ± 0.01 fg	355 ± 7 cd	343.11 ± 3.93 i
13	20	50	50	5.97 ± 0.15 h	1.50 ± 0.02 g	348 ± 4 cde	299.87 ± 7.81 j
14	20	25	75	5.68 ± 0.13 hi	1.41 ± 0.02 h	324 ± 2 ef	294.21 ± 3.99 j
15	20	0	100	4.60 ± 0.53 j	1.17 ± 0.02 j	256 ± 3 g	230.50 ± 6.22 k

[a] Different letters represent the significant differences between samples within each column ($p < 0.05$), $n = 3$.

adding sodium bicarbonate, after which pancreatin and bile extract were added to reach a final concentration of 4.8 and 10 mg/mL, respectively. Finally, invertase and amyloglucosidase were added to a final concentration of 0.012 and 0.008 mg/mL, respectively. The total volume of digesta was increased to 50 mL, and the incubation was carried out for 120 min at 37 ± 0.5 °C.

Starch digestibility was determined from 1.0 mL aliquots of the digesta removed at 0, 20, and 120 min after digestion commenced and added to 4 mL of ethanol (95% w/v) to stop the digestion process. To separate the micellar lycopene, the digesta were centrifuged at 5000*g* for 45 min at 4 °C (Multifuge 1S-R, Thermofisher Scientific, Germany) and filtered (0.2 μm; cellulose acetate) according to the method of Thakkar et al.[23] The filtrate was used to measure the micellar lycopene content of the digesta.

Cell Culture. Samples used for the Caco-2 cell model consisted of (1) digesta obtained from the extruded pellets containing 20% of tomato paste powder, (2) digesta from the extruded corn grits coated with unextruded tomato paste powder at 20% of the total weight (this was the positive control to compare the effect of extrusion on the bioavailability of tomato paste lycopene), and (3) digesta from the extruded corn pellets as the negative control. The data obtained from the extruded corn pellets were subtracted from the treatments 1 and 2, to eliminate the possible errors caused by the interference of other carotenoids present in corn with the test with lycopene.

The cell cultures were prepared according to the method of Garret et al.[19] after minor modifications as follows. Caco-2 cells at passage number 39 were grown in six-well cell culture plates (Beckton Dickinson Labware, Franklin Lakes, NJ) and used for experiments at day 14 after reaching confluency. The monolayers of Caco-2 cells were observed under a light microscope to determine if they maintained normal morphological appearance, ensuring that treatment did not have any adverse effect on the cells.

To obtain a similar lycopene concentrations throughout samples (1 μM), the test media were diluted with water. Monolayers were washed twice with 1 mL of phosphate-buffered saline (PBS) before the addition of 1 mL of the test media that was diluted 1:3 (v/v) with basal Dulbecco's modified Eagle's medium. Cultures were incubated at 37 °C and harvested at 0, 4, and 8 h after incubation.[24,19] Subsequently, the spent media were separated and stored. Monolayers were washed twice with ice cold PBS containing 2 g/L albumin to remove any residual carotenoids adhering to the surface of the cells before washing twice with cold PBS.[25] Cells were collected in 1 mL of ice-cold PBS containing 10% (v/v) ethanol. To assess the stability of micellar lycopene, the media were added to culture plates without cells and incubated under conditions similar to the remainder of the samples. Samples including the cells, spent, and control media were stored at −70 °C under a blanket of nitrogen before analysis.

Lycopene Extraction and Analysis of Samples. The extraction was performed as quickly as possible in dim light under constant nitrogen gas flushing. To extract lycopene from extruded products, a predigestion step was employed using the method described previously.[26] It was assumed that all reductions in lycopene concentration were due to uptake by the cells, as the lycopene was reasonably stable (>90%) during the incubation time that it was exposed to the Caco-2 cell cultures. The concentration of lycopene was determined by a mixture of petroleum ether:acetone (3:1 w/w) containing 0.1% (w/v) butylated hydroxytouluene (BHT). The total lycopene content of the extruded products and the digesta were determined spectrophotometrically at 472 nm (Helios Epsilon spectrophotometer, Thermo electron Corp.). To do this, a set of standards at different concentrations were prepared from pure lycopene in petroleum ether, and a calibration graph was obtained using the concentration versus the absorbance values. The lycopene concentration of the samples was determined directly from the graph. The percentage of bioaccessible lycopene was determined by dividing the amount of micellar lycopene in the digesta by the amount of lycopene present in the extruded product.

Spent media and cell samples were extracted twice, once using a mixture of petroleum ether:acetone (3:1 v/v) and once using petroleum ether. Subsequently, the organic phase was evaporated using nitrogen gas and then reconstituted in 200 μL of methanol as the mobile phase for the high-performance liquid chromatography (HPLC). The HPLC analysis was performed according to the method of Porrini et al.[27] after minor modifications. These included the use of SCL-10AVP liquid chromatograph HPLC apparatus (Shimadzu Scientific Instruments, Japan). Lycopene separation was achieved by reversed phase 5 μm Luna C_{18} column (150 mm × 4.6 mm, i.d.) with a guard column. The absorbance was measured at 472 nm using a UV−visible detector (SPD-10Avp, Shimadzu Scientific Instruments, Japan). The total analysis run was completed within 20 min.

Lycopene stock solutions were dried under a blanket of nitrogen and stored at −70 °C. Using a range of concentrations for the standards, the concentration range over which a linear response was obtained for the HPLC analysis was used as the standard range for the experiment.

Table 3. Lycopene Concentration in the Extruded Products and Digesta and Proportion of Lycopene Transferred from the Extruded Product to the Digesta[a]

	total derivative (%)	skin (%)	paste (%)	lycopene content of extruded products (ppm)	micellar lycopene in the digesta (ppm)	proportion of lycopene bioaccessibility
1	5	100	0	26.13 ± 1.04 j	27.34 ± 0.46 h	105.25 ± 2.31 a
2	5	75	25	55.74 ± 1.32 i	42.04 ± 1.02 def	75.44 ± 0.04 b
3	5	50	50	74.80 ± 0.33 h	31.79 ± 3.34 gh	42.48 ± 4.28 d
4	5	25	75	95.99 ± 3.72 g	37.13 ± 0.76 fg	38.77 ± 2.3 d
5	5	0	100	118.17 ± 0.59 f	39.4 ± 1.90 efg	37.31 ± 6.75 d
6	10	100	0	28.45 ± 1.40 j	19.19 ± 1.19 i	67.71 ± 7.51 b
7	10	75	25	96.90 ± 3.56 g	49.92 ± 0.79 d	51.57 ± 1.09 c
8	10	50	50	146.55 ± 6.24 e	61.55 ± 3.97 c	38.19 ± 4.14 d
9	10	25	75	166.31 ± 8.41 d	59.74 ± 4.29 c	36.06 ± 4.35 d
10	10	0	100	241.82 ± 24.64 b	104.24 ± 9.64 a	40.17 ± 5.2 d
11	20	100	0	33.78 ± 1.61 j	23.76 ± 1.61 hi	71.48 ± 9.53 b
12	20	75	25	136.59 ± 1.04 e	46.38 ± 0.42 de	33.93 ± 0.56 d
13	20	50	50	233.99 ± 3.72 b	49.53 ± 9.98 d	21.15 ± 4.11 e
14	20	25	75	209.77 ± 10.16 c	39.03 ± 9.44 def	20.26 ± 4.29 e
15	20	0	100	363.73 ± 18.02 a	70.51 ± 2.90 b	19.44 ± 1.65 e

[a] Different letters represent the significant differences between samples within each column ($p < 0.05$), $n = 3$.

On each experimental day, three standards were analyzed along with the test samples.

Glucose Analysis. The glucose concentration of the samples was determined using the dinitrosalicylic acid (DNS) colorimetric method described by Mishra et al.[28] To determine starch digestibility, the amount of glucose available at time zero was subtracted from the amount released after 20 and 120 min of digestion. The reported glucose values are based on the amount of starch available in the dry samples.

Statistical Design. The experimental design was a full factorial consisting of three replacement concentrations of tomato skin and paste powder at 5, 10, or 20% × 5 tomato skin to paste ratios of 100:0, 75:25, 50:50, 25:75, or 0:100. The effect of varying proportions of tomato skin and/or tomato paste on the lycopene bioaccessibility and starch digestibility in the tomato enriched snacks was investigated. At least three replicates were carried out for each treatment. A completely randomized experimental design was used, and the data were statistically evaluated using analysis of variance (ANOVA). When the F value was significant ($p < 0.05$), the comparison between the mean values was carried out using Duncan's multiple range test.

The statistical analysis for the Caco-2 cell experiment was carried out on only two samples; extruded snacks made from a blend of corn and tomato paste were compared with corn snacks coated with unextruded tomato paste powder (positive control). Extruded corn snacks alone (negative control) were also prepared. The data obtained from corn snacks alone were used to set base values and avoid interference from other carotenoids present in the corn.

Spent media and Caco-2 cells were collected at 0, 4, and 8 h from the start of incubation. Five replicates were taken for each treatment. A completely randomized design was used, and when $p < 0.05$, the comparison between the means was carried out using Duncan's multiple range test. All statistics were calculated using SAS software version 9.1 (SAS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Proximate Composition. With the addition of tomato derivatives, the starch content of the ingredient mix decreased by up to 18% as compared to the control. The amount of dietary fiber present in the formulations increased by more than 16 times (Table 1). Higher levels of fiber were associated with a greater proportion of tomato derivatives in the formulation. Adding tomato derivatives also increased the ash content by up to 3 times. Among the tomato derivatives, increasing the tomato skin to tomato paste ratio increased the amount of dietary fiber by up to four times, the protein content by up to 15%, and the ash content by up to 50% (Table 1).

Lycopene Bioaccessibility. The lycopene content of the snacks ranged from 26 to 364 ppm (Table 3). The torque and energy used to process the snacks was strongly and negatively correlated with the total lycopene content of the products (Table 4), showing a detrimental effect of extrusion cooking on lycopene. This corresponds with our previous findings.[6] On the other hand, extrusion cooking improved the bioaccessibility of lycopene. The bioaccessibility of lycopene in the raw ingredients varied from 16 to 56% (data not shown), while after the extrusion processing it increased to a range of 19−105% (Table 3). Furthermore, the proportion of bioaccessible lycopene increased as torque and power consumption increased (Table 4). In the tomato derivatives, lycopene is associated with the fibrous tissue of the cell walls. The extrusion cooking involves severe mechanical and heat processing treatments, which reduce the particle size of the insoluble fiber component and solubilize some of the cell wall nonstarch polysaccharides, also referred to as soluble fiber components. The breakdown of these resistant structures frees the cell components, increasing their release during digestion.[8,10,11,29]

As the concentration of tomato derivatives increased, the proportion of the lycopene present that was bioacessible decreased, but the absolute amount of bioacessible lycopene increased (Table 3). According to the data presented in Table 2, increases in the tomato derivative concentration reduced the torque, power consumption, and SME. It is clear that the shear force applied by the extruder to the melt was reduced as the proportion of tomato derivatives increased, thus reducing the proportion of cells that were ruptured. As a result, a lower proportion of the lycopene present was released.

When 50% of tomato paste was present in the ingredients, the proportion of bioaccessible lycopene was on average 39% of the

Table 4. Correlation Coefficients (R) between the Extrusion Parameters with G20, G120, and Lycopene Content of the Extruded Products in the Digesta and the Proportion of Lycopene Transferred to the Micellar Phase of the Digesta

	lycopene content			starch digestibility	
	extruded product	micellar phase of digesta	bioaccessibility (%)	G20	G120
power consumption (kW)	−0.84[b]	−0.64[a]	0.68[a]	0.63[a]	0.53[a]
torque (N m)	−0.83[b]	−0.60[a]	0.65[a]	0.66[a]	0.54[a]

[a] Significant at $P < 0.05$. [b] Significant at $P < 0.01$.

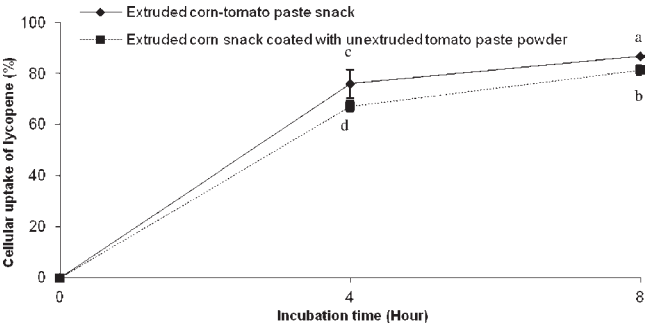

Figure 1. Relative uptake of micellar lycopene by Caco-2 cultures during 8 h of incubation; $n = 5$. Different letters above the error bars indicate significant differences between the values ($p < 0.05$). Data normalized to the proportion of lycopene at time zero. The values for extruded corn grits were used to remove the effect of other carotenoids present in corn.

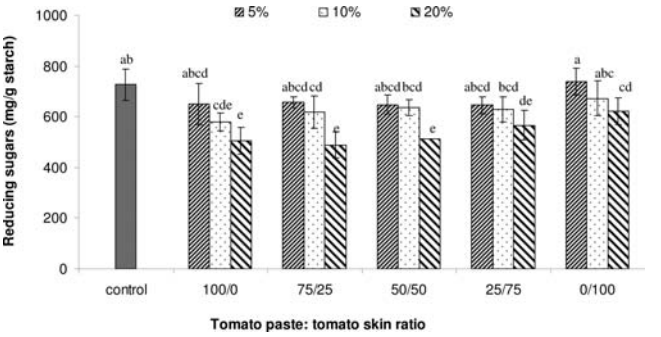

Figure 2. Amount of glucose released by the digestion of starch after 20 min of incubation. The control is extruded corn snack, while the enriched products contained various concentrations of tomato paste and tomato skin. Letters on top of the columns indicate the significant differences between the products ($p < 0.05$); $n = 3$.

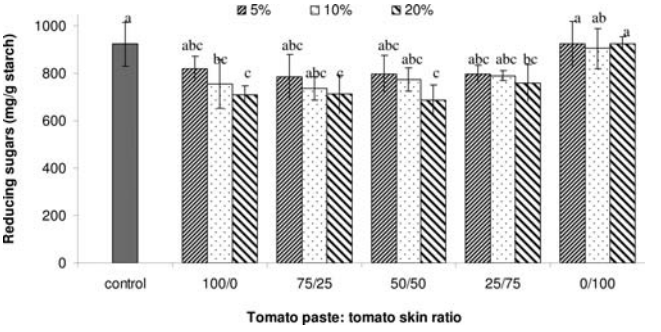

Figure 3. Amount of glucose released by the digestion of starch after 120 min of incubation. The control is extruded corn snacks, while the enriched products contained various concentrations of tomato paste and tomato skin. Letters on top of the columns indicate the significant differences between the products ($p < 0.05$); $n = 3$.

total lycopene for products containing 5 or 10% tomato derivative (Table 3). When the proportion of tomato derivatives was increased to 20%, the proportion of bioaccessible lycopene was reduced to 20%. The proportion of bioaccessible lycopene was lowest when the proportion of tomato paste was 50% of the tomato derivatives added. Further increases in the proportion of tomato paste did not change the proportion of bioaccessible lycopene, despite the increases in the total lycopene concentration in the product (Table 3).

The proportion of lycopene taken up by the Caco-2 cells after 8 h of incubation from the extruded corn− tomato paste snacks exceeded that of the extruded corn snacks coated with tomato paste powder (unextruded control) by up to 5%. The majority of uptake by the cells occurred in the first 4 h of incubation (Figure 1). This finding confirms that the exposure of tomato cells to temperature during extrusion cooking can result in a greater uptake of lycopene by the Caco-2 cells. This may be due to the presence of higher concentrations of *cis*-lycopene in the extruded products as compared to the unextruded tomato paste powder. It is suggested that the *cis*-isomers of lycopene are more soluble in oil; thus, they are transferred more easily from the aqueous component of the digestion system into the micellar phase of the digesta.[8,30] Heat processing has been shown to promote the isomerization of carotenoids.[30]

The amount of cellular uptake reported in the present study (Figure 1) was greater than previous reports, such as commercial baby food preparation and stir-fried vegetables containing tomato paste.[19,31] The differences between the simulated digestion and lycopene extraction method, specific attributes of the cell culture used, the nature of the food matrix, and the extrusion processing used may have caused this variation. However, these questions could not be resolved from the data presented, and clinical trials would be required to validate these hypotheses.

Starch Digestibility. The amount of glucose released from the unprocessed ingredients after 20 min of digestion varied from 100 to 184 mg/g and after 120 min from 208 to 345 mg/g (data not shown). For the extruded products, glucose released at G20 was much greater than for the unextruded product and varied from 488 to 780 mg/g and at G120 had increased to between 687 and 924 mg/g (Figures 2 and 3). This is in line with previous reports, which state that extrusion cooking results in the gelatinization of starch, thus improving its susceptibility to the digestive enzymes.[2,3]

The digestibility of the starch from samples containing 5% tomato derivatives did not differ from the extruded corn snacks ($p > 0.05$); however, with increases in the concentration of tomato paste powder to 10 and 20%, the rate and/or extent of starch digestibility was reduced (Figures 2 and 3). For the sample

containing 100% of tomato skin, digestibility of the starch was identical to the control sample. It therefore seems that components, perhaps pectin in the tomato paste, slow the rate of starch digestion by possibly forming starch−lipid−pectin complexes.[32]

As the data presented record the amount of starch digested as a proportion of starch present, the reductions in starch concentration associated with the substitution of corn grits by tomato derivatives were not the reason for lower starch digestibility values.

Samples containing high proportions of tomato paste powder were less expanded (Table 2) and had the lowest G20 and G120 values (Figures 2 and 3). It is possible that adding tomato paste reduces the starch concentration and increases sugars and other polysaccharides, thus reducing the shear force developed during processing (Table1), which in turn reduces the proportion of gelatinized starch and slows the rate of digestion.[1,6,32] The positive correlation between the power consumption and the torque values with the amount of starch digested at G20 and G120 support this hypothesis (Table 4).

Another reason for the reduced starch digestibility in these products could be due to competition for available water by the fiber components, limiting gelatinization of the starch, thus reducing the susceptibility of the starch to digestion.[33] Dietary fiber can also reduce the rate of amylolytic activity by directly binding to the amylolytic enzymes,[14] although this has only been reported at much higher fiber concentrations than those occurring in this work.

Altan et al.[32] have suggested that the reduction in starch digestibility in tomato pomace-enriched barley snacks may be due to the presence of amylose−lipid complexes. The formation of these complexes during extrusion cooking from the raw ingredients containing less than 4% of lipids has been previously reported.[17] However, in this work, the rate of starch digestion was independent of the proportion of lipid present in the samples (Table 1).

In conclusion, the present study investigated the effect of ingredients on the bioaccessibility of lycopene from the tomato-enriched expanded snacks. The study also measured the digestibility of starch in the snacks, which made it possible to simultaneously record the changes in these nutritional parameters with ingredient composition and processing.

The study showed that despite the exposure of lycopene to severe heat and shear during extrusion cooking, the pigment is not completely destroyed and is available for uptake by the cells. It was also shown that the mechanical treatment during extrusion cooking plays a major role on the release of lycopene from the matrix of tomato cells. Although extrusion processing results in a net loss of lycopene from the products, there is a net gain of bioacessible lycopene in the extruded snacks due to the breakdown of resistant cell structures. It can be suggested that by the optimization of the extrusion processing, tomato-enriched extruded snacks can be produced that contain significant amounts of potentially bioavailable lycopene.

On the other hand, starch digestibility of the snacks decreased with the proportion of tomato paste powder that they contained but not with the proportion of tomato skin powder. This indicates that by carefully proportioning these ingredients when designing a snack food, the rate of starch digestion from the snacks can be controlled to some degree.

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