horticulturae

Article

Antioxidant Properties of Tomato Fruit (*Lycopersicon esculentum* Mill.) as Affected by Cultivar and Processing Method

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Abstract: Tomatoes are the most consumed vegetables worldwide and a valuable source of several antioxidants. The consumption of tomato products from appropriate cultivars after suitable processing methods may significantly improve human diet. The purpose of this study was investigating the variations in the contents of the main antioxidants present in tomato fruits, in the new Cuban breeds and yellow varieties, as well as their changes during the processing to tomato puree and ketchup. The quality evaluation comprised the detection of lycopene, ascorbic acid and total phenolics and the analysis of their contribution to antioxidant capacity in selected tomato genotypes. Heating (90–100 °C/15 min) enhanced the content of lycopene and total phenolics in puree, resulting in an increment in antioxidant capacity, despite the reduction in ascorbic acid as a result of concentration processes. The conducted experiments revealed that cultivars 'Vyta' and 'Cima' are very suitable for industrial purposes due to their high dry-matter content of more than 9% fresh mass and high biological value. With respect to serving size, the best sources of antioxidants are fresh tomatoes, followed closely by tomato puree, irrespective of cultivar. However, the differences are mainly due to the edible portion size (200 g for fresh tomatoes and 60 mL for puree, respectively).

Keywords: antioxidant capacity; ascorbic acid; carotenoids; lycopene; phenolics; puree; ketchup; genetic potential

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Citation: Rivero, A.G.; Keutgen, A.J.; Pawelzik, E. Antioxidant Properties of Tomato Fruit (*Lycopersicon esculentum* Mill.) as Affected by Cultivar and Processing Method. *Horticulturae* **2022**, *8*, 547. https://doi.org/10.3390/horticulturae8060547

Academic Editor: Lucia Guidi

Received: 2 May 2022
Accepted: 10 June 2022
Published: 18 June 2022

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1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most frequently cultivated vegetables, with a cultivation area of 5.1 million ha yielding over 186.8 million tons per year in 2020, and belongs to the most important vegetables worldwide because of its large consumption and numerous uses [1,2]. The leading tomato producer is well-spaced China with 64.9 mln tons, followed by India with 20.6 mln tons, Turkey with 13.2 mln tons and USA with 12.2 mln tons [1]. The highest amounts of yield in tons per ha in 2020 were reached in the USA (110.72), Morocco (94.64), Turkey (72.60), Brazil (72.24), Italy (62.63) and China (58.36) [1]. This development shows the occurrence of new production centers in Africa. Tomato is highly appreciated due to its versatile usability and attractive color. The consumption of tomato is the highest of all vegetables and accounts for a mean value of 18.18 kg per capita and year of the world consumption [3]. The highest consumption in kg per capita and year was found in Turkey in 2012-13 (98.62), followed by Egypt (90.06), Greece (85.78), Armenia (84.12) and Tunisia (83.91). For Europe, the amount reached only 22.58 kg, with the highest amount in Mediterranean areas of 44.50 kg. In comparison, USA consumption was noted at the level of 37.74 kg [3]. Tomatoes are consumed to a considerable extent as processed products, e.g., ketchup and puree, with still-increasing tendency. In recent years, there has been a growing interest in the use of tomato by-products such as tomato seeds [4,5] to produce high-value oils with high contents of unsaturated fatty acids and further antioxidant compounds and peel as a partial substitution of wheat flour of up to 20% [6]. The valuable chemical composition of such by-products makes them beneficial, and they are already used in the food industry to enrich products with antioxidants. However, their contents in by-products are mainly affected by the cultivar and the growing year [7], while the processing method (cold and hot breaking processes) does not significantly affect the physicochemical properties of tomato seed oils [7]. Tomato is considered as an excellent source of bioactive compounds, especially for carotenoids such as lycopene and ß-carotene but also for phenolic substances, ascorbic acid, tocopherols and flavonoids, which possess high antioxidative capacities [2]. Due to the high contents of antioxidants present in tomato and tomato products, an increased interest in tomato products has risen due to the fact that their consumption is correlated with a reduced risk of some types of cancer, cardiovascular diseases (CVDs) or neurodegenerative diseases [2,8–12]. In addition to scavenging free radicals, the multiple activities of antioxidants include inactivating metal catalysts by chelation, reducing hydroperoxides into stable hydroxyl derivatives and interacting synergistically with other reducing compounds [13]. A higher consumption of tomato products increases the lycopene level in blood and tissues and acts against oxidative stress, which is responsible for the damage of lipids, proteins, enzymes and DNA, resulting in chronic diseases such as cancer or CVD [8–14]. To lower the risk of these diseases, the recommended daily intake ranges from 7 to more than 50 mg of lycopene, which corresponds approximately to 2–10 middle-sized tomatoes [15,16]; e.g., considering San Marzano Cirio (9.87 mg lycopene 100 g^{-1} FM) or San Marzano Antico (10.26 mg lycopene 100 g^{-1} FM) of sizes of 90–110 g, the consumption from 4.6 to 5.6 or from 4.4 to 5.4 tomatoes covers the RDI of lycopene (about 487–507 g) [17]. With the consumption of the large-sized variety Brandywine (250 g and a lycopene content of 20.16 mg 100 g^{-1} FM), only one fruit would be sufficient [17].

However, it was reported that food processing may impair the nutritional and biological value of products due to the decomposition or removal of important phytochemicals such as vitamins or phenolics [18]. Processing techniques such as heat treatment, homogenization, peeling or other preparation procedures, as well as the presence of oxidants, metal ions, dietary fiber or lipids, may considerably change the content of certain nutrients and their bioavailability. Changes in the biological activity of tomato products and the resulting antioxidative capacity and health-promoting value directly depend on the applied techniques, such as the industrial processing of tomatoes to peeled and canned tomatoes (cold breaking, evaporation, pasteurization and sauce production) vs. home processing to juice, concentrate and sauce [19–21]. Studies on tomato products have investigated the impact of processing (single-strength juice, concentrate and sauce) on the contents of carotenoids, their profile and isomerization [22]; few of them have dealt with changes in further bioactive compounds, such as ascorbic acid, tocopherols, total phenolics and total flavonoids, and antioxidant state or capacity [21,23,24]. However, available results are often conflicting owing to the investigated compounds, processing techniques and conditions [2,8,25]. Generally, water-soluble bioactive substances are susceptible to degradation during hydrothermal processing. Conversely, carotenoids are relatively stable, and technical processing can even enhance the rate of extractability or their content and availability as a result of cell wall disruption and carotenoids release or as a result of enzymatic degradation weakening protein–carotenoid aggregates [25] and the effect of moisture content reduction. However, when investigating the contents of bioactive compounds as influenced by processing, the selection of a broad range of tomato cultivars is of special significance, because not only the content of phytochemicals but also the sensitivity to processing and the stability of nutritive compounds is known to be cultivar-dependent. Still, the available information is very scarce [26].

The purpose of this study was to evaluate the influence of cultivar and processing method on the composition and variation in some bioactive compounds (ascorbic acid, lycopene and total phenolics) and antioxidant capacity of different tomato cultivars from Cuba and Germany, the former almost unknown in Europe. This included common tomato cultivars and new breeds as well as varieties not usually used for processing purposes such as yellow tomatoes.

2. Materials and Methods

2.1. Plant Materials

For this study, nine tomato (*Lycopersicon esculentum* Mill.) cultivars with certain levels of commercialization in Cuba ['Roma VF-p73' ('Ro'), 'Rilia' ('Ri'), 'Campbell-28' ('Ca'), 'Vyta' ('Vy'), 'CIMA' ('Ci')] and Germany ['Suso F1 Hybride RZ' ('Su'), 'Marmande' ('Ma'), 'Goldene Königin' ('GK'), 'Yellow Pearshaped' ('YP')] were selected. The varieties from Germany were selected because of their high rate of consumption and their differences in color, shape, size and uses, and thus the potential differences in their antioxidants' pools, as well as in their retention during processing. The red-colored tomatoes 'Marmande' and 'Suso F1' are used for fresh consumption, but also for processing, while yellow varieties such as 'Yellow Pearshaped' and 'Goldene Königin' mainly serve for salad preparation. The Cuban varieties are all red-colored, with the exception of 'Ci', which is orange-red colored, and are large to middle-sized tomatoes used fresh and for processing. Nevertheless, their shapes and commercial purposes are different. 'Roma' (pear-shaped, middle-sized) is used only for processing, 'Vyta' (round, middle-sized) for salad preparation (although nowadays it is being used also for industrial purposes), 'Campbell' (round, large to middle-sized) and 'Rilia' (heart-shaped-middle-sized) are used for both purposes. All Cuban varieties were selected because of their high consumption levels in Cuba. Cuban seeds were obtained from the National Investigation Institute "Liliana Dimitrova". German seeds were obtained from the SPERLI-Samen Company (Carl Sperling & Co. GmbH, Lüneburg, Germany). The experiments were carried out during two consecutive vegetative periods in Göttingen, Germany and all of the presented data are based on the combination of two years of investigations. At the end of February, tomato seeds were sown into the growth medium, a substrate–quartz sand mixture. The used substrate is a soil developed for tomato and vegetables production (Tomaten und Gemüseerde, Compo Sana, Germany), with a pH value ($CaCl_2$) of 5.9, a salt content of 1.9 mg L^{-1} (KCl), an organic matter of 28% and a water content of 55%. The soil contains 72% of peat moss (decay degree H2-H8), green compost, carbonate lime, NPK fertilizer, N-fertilizer, methylene urea, trace elements fertilizers; minor components: 380 mg N L^{-1} ($CaCl_2$), 320 mg P_2O_5 L^{-1} (CAL), 1050 mg K_2O L^{-1} (CAL), 160 mg Mg L^{-1} ($CaCl_2$), 500 mg S L^{-1} (total). The quartz sand is characterized by a grain size of 0.7–1.2 mm and the ratio substrate to quartz sand is 2:1 (w/w). The seedlings were cultivated for 3 weeks in a controlled environment chamber (day 25 °C/night 21 °C) and watered every third day, depending on soil moisture content. After 4 weeks, 44 seedlings of each cultivar of equal size were selected, transplanted to small pots (2 plants per pot) containing 3 kg of substrate (substrate to quartz sand ratio of 3:1 (*w*/*w*)) and kept in the controlled environment chamber for the next 4 weeks under the conditions described above, but with daily water supply. Then, the selected tomato plants were cultivated in Mitscherlich pots containing 6 kg of substrate (1 plant per pot) in the greenhouse until the end of the harvest, with a daily watering regime (200 mL in the morning). For both experiments, 22 plants per cultivar were used. During the first experiment, the soil was enriched with a modified Hoagland solution [27] at a rate of 400 mL per pot twice a week to cover the nutritional requirements of plants. During the replication of the experiment the plants were fertilized with Compo Tomaten Langzeit-Dünger, COMPO GmbH, Münster, Germany (9% N, 6% P_2O_5, 15% K_2O, 4% MgO and 30% of organic matter) at a rate of 3 g per pot. Against fungal diseases, the fungicide Funganil® with the active substance metalaxyl-M 480 g L^{-1} (Syngenta, Germany) was applied. The fruits were harvested at the full ripening stage, where over 98% of the fruit was deep red or intense yellow (from end of June to the end of August). Immediately after the harvest, fruit fresh mass was determined and cut fruits were frozen for further processing or investigations. The total number of fruits (about 300 per cultivar) were divided into 3 groups for each treatment with 4 replications (fresh, concentration to puree and ketchup preparation), where each replication consisted of the same number of fruits.

Fruits frozen immediately after the harvest were used as fresh tomatoes for the investigations. Tomato puree was prepared according to the method proposed by the FAO [28], where the fruits were washed and pulped in a pulping machine, pasteurized for 15 min at 90–100 °C and stored under freezing conditions at −25 °C until further analyses. Ketchup was prepared after Valencia et al. [29], where for 100 g of ketchup, 65% of tomato puree was used. After the addition of a pre-mixed combination of spices and ingredients (20% sugar, 9.5% vinegar, 2.9% salt, 5% onion pulp and 1% garlic puree) ketchup was homogenized manually and heated for 15 min at 90–100 °C under constant stirring. After cooling at room temperature, ketchup was stored under freezing conditions at −25 °C until further analyses. Part of the frozen material was lyophilized (Epsilon 2–40, Christ, Germany). Immediately after the drying process, the dry-matter content was determined. The experimental procedure is presented in the flowchart of the study (Figure 1).

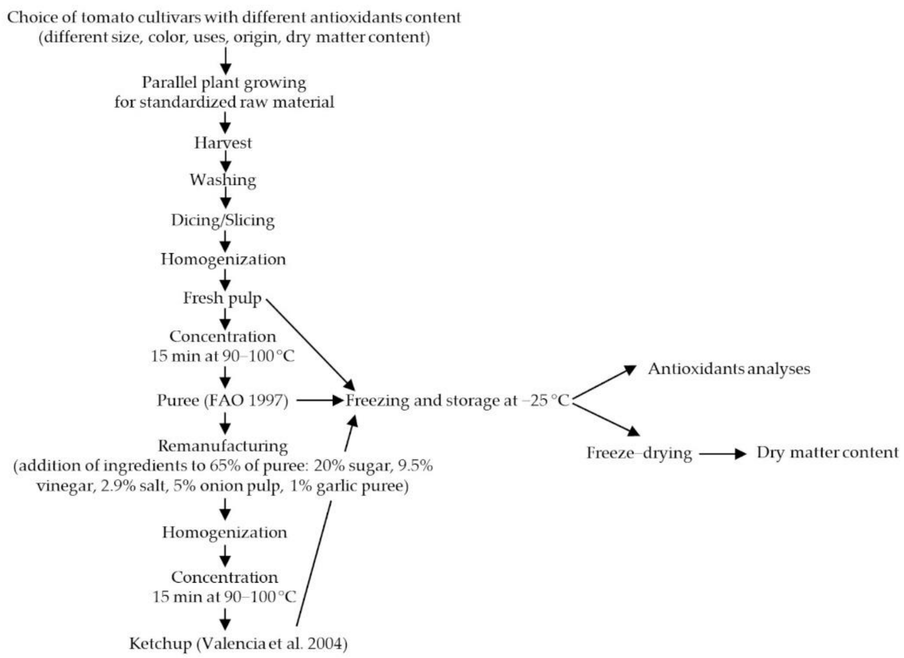

Figure 1. Flowchart of the experimental procedure from the choice of the tomato cultivar until final analyses.

2.2. Analyses of Fresh and Processed Tomato Fruits

All chemicals used in the analytical investigations were obtained from Sigma-Aldrich and Merck KGaA (both Darmstadt, Germany).

Ascorbic acid: sample preparation and measurement of L-ascorbic acid (LAA) was performed as described by Albrecht [30]. Frozen samples were extracted with meta-phosphoric acid (5%) at a ratio of 1 g per 4 mL and homogenized with an ultra turrax for 2 min, diluted with pure water (1:2.5; vol/vol) and filtered using filter paper no. 595 (Schleicher and Schuell BioScience GmbH, Dassel, Germany). The filtrate was titrated with 0.21% of 2.6 dichlorophenol-indophenol (DIP) solution until the titration end point (color change from light yellow to light pink) and the amount of LAA was calculated per product mass. The calibration curve was prepared with LAA in the range of 0–1 g L^{-1}.

Lycopene content was measured in freeze-dried samples spectrophotometrically according to Binoy et al. [31], where 0.5 g of samples was treated twice with 10 mL of extraction solution (hexane:methanol:acetone 2:1:1 (*vol*/*vol*/*vol*), containing 2.5% of butylated hydroxyl toluene), mixed and centrifuged at 3500 *s* for 20 min. Obtained supernatants were collected, combined and stored frozen until analyses. For the measurement, 1.5 mL of extraction solution and 1 mL of the sample were mixed and scanned using a spectrophotometer (HP Aglient 8453 UV/VIS with multi-cell sampler; Hewlett Packard, Böblingen, Germany) at 502 nm against hexane as the blank. The results were calculated per product mass. A standard solution was prepared with lycopene from tomato (≥90%) at a concentration of 0.04 g L^{-1} hexane with 2.5% of butylated hydroxytoluene (BHT).

Total phenolic compounds: The concentration of total phenolic compounds in tomato samples was determined spectrophotometrically using the colorimetric assay with Folin–Ciocalteu's phenol reagent [32]. Methanol extraction was performed as described by Iswari and Susanti [21] with 0.5 g of freeze-dried material and 10 mL of methanol (double extraction). The obtained supernatants were combined and stored frozen until analyses. The calibration curve was prepared with gallic acid (GA) in the range of 0–66.08 µg GA L^{-1}. After incubating the measuring solution containing 0.6 mL of the sample for 30 min at 37 °C, 1 mL of 0.5 mol L^{-1} NaOH, 0.1 mL of Folin–Ciocalteu reagent and 2.3 mL distilled water were added. The reaction was stopped by quick cooling and the content of total phenolics was determined spectrophotometrically at 735.8 nm (HP Agilent 8453 UV/VIS with multi-cell sampler; Hewlett Packard, Böblingen, Germany). The results were expressed in µg gallic acid equivalents (GAE) kg^{-1} FM (product mass).

Antioxidant capacity was measured spectrophotometrically using the ferric reducing antioxidant potential (FRAP) assay [27], where freeze-dried material (0.5 g for fresh tomatoes and 1 g for ketchup) was extracted twice for 20 min with 5 mL of methanol under continuous shaking and centrifuged for 15 min at 10,000× *g* at 4 °C. Supernatants were combined and the pellet was again dissolved twice with 4 mL of hexane for 30 min, mixed, centrifuged again as described above and the supernatants were immediately frozen. To perform the FRAP assay, 30 µL of the tomato extracts was added to 1 mL of FRAP reagent and mixed thoroughly. After incubation at 37 °C for 4 min, the absorbance was measured at 593 nm against water as the blank. The calibration curve was in the range of 0 to 1000 µmol L^{-1} ferrous ion and was perform with daily freshly prepared ammonium iron sulphate.

For the calculation of changes of bioactive compounds, the frozen fresh tomatoes were set as the base of the calculation. So, the changes (losses and concentration processes) are expressed in relation to the fresh tomatoes, which are always characterized by a zero value).

2.3. Statistical Analyses

The data were analyzed for both years together. All statistical analyses were performed with SPSS version 9.0 for Windows. After testing the data for homogeneity of variances, the mean values obtained in the different groups were compared by one-way ANOVA, at a significance level of 0.05. Duncan tests were used when equal variances were assumed, while in the case of non-equal variances, Tamhane's T2 tests were applied. Results are presented as mean values plus standard deviation. Correlation coefficients were determined between bioactive compounds and antioxidant capacity using the Pearson coefficient at $p \leq 0.01$, when results were normally distributed.

3. Results and Discussion

3.1. Dry-Matter Content

Dry-matter content varied from 5.9 to 9.5% for fresh tomato, from 13.8 to 18.3% for puree and from 24.7 to 30.0% for ketchup, respectively (Table 1). These results are in line with those of 5.0 to 7.5% dry matter for fresh and 29 to 90% for processed tomato reported in the literature [33,34]. Differences between cultivars were significant, with higher percentages in fresh fruits of cvs Vyta (Vy) and Cima (Ci), as well as in processed samples of cvs Vy, Roma (Ro), Suso (Su) and Goldene Königin (GK). Yellow cultivars showed generally low contents of dry matter in tomato products. However, all of the used cultivars were characterized by a dry-matter content higher than 5%, which is a minimum requirement for industrial processing. Contents of dry matter higher than 9%, as in the case of 'Ci' and 'Vy', favor these cultivars for technological use and enhance bioactive properties significantly (Tables 1 and 2). As expected, significant differences were also found between fresh and processed samples due to the reduction in water content during the concentration of the products, which caused an increase in the dry matter percentage. Ketchup, subjected to the most intense processing, was characterized by the highest content of dry matter irrespective of cultivar (Table 1).

Table 1. Dry-matter content and antioxidant properties of different tomato cultivars as a function of processing type. Data are expressed per 1 kg of fresh mass (FM) or in % of FM.

Cultivar	DM [%]	FRAP [mmol Fe^{2+}]	LAA [mg]	Lycopene [mg]	Total Phenolics [mg GAE]
			fresh fruits		
'Rilia'	6.06 ± 0.96 d	25.3 ± 8.1 d	192.3 ± 22.3 b	111.7 ± 35.3 c	234.5 ± 18.1 cd
'Roma'	6.80 ± 1.62 d	27.0 ± 10.8 d	179.6 ± 10.8 c	103.7 ± 42.0 cd	211.7 ± 17.3 d
'Campbell-28'	7.23 ± 0.07 cd	23.1 ± 6.3 de	165.6 ± 36.8 c	117.5 ± 45.8 c	217.5 ± 15.3 d
'Goldene Königin'	7.81 ± 0.27 c	33.7 ± 5.9 c	226.2 ± 23.8 ab	-	281.4 ± 18.0 c
'Marmande'	5.93 ± 0.03 e	21.9 ± 6.3 e	139.4 ± 48.0 d	96.0 ± 17.2 cd	212.6 ± 15.3 d
'Suso'	8.21 ± 0.35 bc	26.1 ± 2.8 d	156.0 ± 10.7 cd	89.7 ± 3.3 d	106.9 ± 1.1 e
'Yellow Pearshaped'	8.81 ± 0.33 b	24.8 ± 1.2 d	185.2 ± 15.7 bc	-	102.1 ± 8.3 e
'CIMA'	9.03 ± 0.21 ab	43.6 ± 0.4 b	204.7 ± 6.3 b	144.8 ± 4.6 b	483.7 ± 9.4 b
'Vyta'	9.53 ± 0.04 a	47.9 ± 0.3 a	255.7 ± 10.5 a	202.5 ± 3.8 a	567.6 ± 10.1 a
			tomato puree		
'Rilia'	16.07 ± 0.05 b	63.2 ± 17.7 b	164.6 ± 20.0 a	303.2 ± 57.9 ab	758.7 ± 384.9 ab
'Roma'	18.04 ± 0.03 a	60.3 ± 23.7 b	150.5 ± 36.7 ab	246.9 ± 116.4 c	765.4 ± 378.7 ab
'Campbell-28'	14.15 ± 0.11 c	47.8 ± 8.8 cd	146.8 ± 15.9 ab	315.0 ± 71.0 ab	634.5 ± 308.5 b
'Goldene Königin'	17.99 ± 0.38 ab	50.0 ± 8.1 d	172.6 ± 13.1 a	-	681.5 ± 293.2 b
'Marmande'	14.15 ± 0.15 c	41.5 ± 20.5 c	115.7 ± 16.4 c	238.4 ± 20.3 c	918.8 ± 442.6 ab
'Suso'	18.05 ± 0.42 a	43.8 ± 1.0 cd	124.5 ± 15.7 ab	291.6 ± 9.9 b	452.4 ± 47.4 c
'Yellow Pearshaped'	13.84 ± 0.17 c	43.1 ± 1.1 cd	168.1 ± 4.1 a	-	409.6 ± 5.4 c
'CIMA'	16.13 ± 0.73 b	67.9 ± 1.0 b	178.5 ± 3.5 a	363.4 ± 6.9 a	1062.0 ± 29.2 b
'Vyta'	18.30 ± 0.03 a	79.0 ± 0.7 a	134.8 ± 4.9 b	370.0 ± 2.7 a	1168.3 ± 35.3 a
			Ketchup		
'Rilia'	28.05 ± 0.27 b	92.1 ± 13.0 bc	136.0 ± 27.4 a	116.9 ± 45.6 cd	1389.2 ± 761.4 ab
'Roma'	29.42 ± 0.43 a	98.9 ± 14.2 bc	136.7 ± 19.8 a	151.5 ± 55.5 abc	1481.3 ± 804.3 ab
'Campbell-28'	28.95 ± 0.22 ab	63.7 ± 13.9 d	90.5 ± 21.9 de	121.4 ± 12.8 cd	1414.2 ± 762.0 ab
'Goldene Königin'	26.71 ± 0.05 c	130.0 ± 6.7 a	138.2 ± 24.3 a	-	1270.2 ± 779.5 c
'Marmande'	26.19 ± 0.09 c	91.7 ± 11.2 bc	79.2 ± 15.2 e	125.8 ± 23.8 cd	1234.3 ± 618.2 c
'Suso'	25.73 ± 0.61 cd	84.3 ± 7.0 c	131.3 ± 14.5 a	72.6 ± 3.6 de	705.0 ± 28.2 c
'Yellow Pearshaped'	24.65 ± 0.11 d	63.1 ± 1.9 d	115.4 ± 2.5 c	-	643.6 ± 14.7 c
'CIMA'	26.03 ± 0.41 bc	102.6 ± 5.3 b	120.8 ± 1.3 b	164.7 ± 0.7 b	2005.7 ± 24.2 b
'Vyta'	29.97 ± 0.62 a	106.8 ± 2.3 b	109.0 ± 1.7 d	193.3 ± 1.1 a	2268.0 ± 72.1 a

FRAP—antioxidant capacity, LAA—L-ascorbic acid, GAE—gallic acid equivalents, DM—dry matter. Different letters indicate significant differences by Duncan test at $p \leq 0.05$ within the given processing method.

Table 2. Intake of selected bioactive compounds and contribution to antioxidant capacity by serving size as a function of tomato cultivar and processing level. Data are expressed on the basis of serving size: 200 g for fresh tomatoes, 60 mL for puree and 15 mL for ketchup.

Cultivar	**FRAP [mmol Fe^{2+}]**	**LAA [mg]**	**Lycopene [mg]**	**Total Phenolics [mg GAE]**
		Fresh fruits (200 g)		
'Rilia'	5.1 ± 1.62 d	38.5 ± 4.46 b	22.3 ± 7.06 c	46.9 ± 36.24 cd
'Roma'	5.4 ± 2.17 d	35.9 ± 2.16 c	20.7 ± 8.39 cd	42.3 ± 34.65 d
'Campbell-28'	4.6 ± 1.27 de	33.1 ± 7.37 c	23.5 ± 9.17 c	43.5 ± 30.53 d
'Goldene Königin'	6.7 ± 1.18 c	45.2 ± 4.76 ab	-	56.3 ± 36.05 c
'Marmande'	4.4 ± 1.27 e	27.9 ± 9.61 d	19.2 ± 3.43 cd	42.5 ± 30.61 d
'Suso'	5.2 ± 0.55 d	31.2 ± 2.14 cd	17.9 ± 0.66 d	21.4 ± 2.15 e
'Yellow Pearshaped'	5.0 ± 0.25 d	37.0 ± 3.13 bc	-	20.4 ± 1.66 e
'CIMA'	8.7 ± 0.08 b	40.9 ± 1.25 b	29.0 ± 0.92 b	96.6 ± 1.88 b
'Vyta'	9.6 ± 0.05 a	51.1 ± 2.10 a	40.5 ± 0.75 a	113.5 ± 2.01 a
		Tomato puree		
'Rilia'	3.8 ± 1.06 b	9.9 ± 1.20 a	18.2 ± 3.47 ab	45.5 ± 23.09 ab
'Roma'	3.6 ± 1.42 b	9.0 ± 2.20 ab	14.8 ± 6.98 c	45.9 ± 22.72 ab
'Campbell-28'	2.9 ± 0.53 cd	8.8 ± 0.95 ab	18.9 ± 4.26 ab	38.1 ± 18.51 b
'Goldene Königin'	3.0 ± 0.48 d	10.4 ± 0.79 a	-	40.9 ± 17.59 b
'Marmande'	2.5 ± 1.23 c	6.9 ± 0.99 c	14.3 ± 1.22 c	55.1 ± 26.56 ab
'Suso'	2.6 ± 0.06 cd	7.5 ± 0.94 ab	17.5 ± 0.59 b	27.1 ± 2.84 c
'Yellow Pearshaped'	2.6 ± 0.07 cd	10.1 ± 0.25 a	-	24.6 ± 0.32 c
'CIMA'	4.1 ± 0.06 b	10.7 ± 0.21 a	21.8 ± 0.42 a	63.7 ± 1.75 b
'Vyta'	4.7 ± 0.04 a	8.1 ± 0.29 b	22.2 ± 0.16 a	70.1 ± 2.12 a
		Ketchup		
'Rilia'	1.4 ± 0.20 bc	2.0 ± 0.41 a	1.8 ± 0.68 cd	20.8 ± 11.42 ab
'Roma'	1.5 ± 0.21 bc	2.1 ± 0.30 a	2.3 ± 0.83 abc	22.2 ± 12.06 ab
'Campbell-28'	1.0 ± 0.21 d	1.4 ± 0.33 de	1.8 ± 0.19 cd	21.2 ± 11.43 ab
'Goldene Königin'	2.0 ± 0.10 a	2.1 ± 0.36 a	-	19.1 ± 11.69 c
'Marmande'	1.4 ± 0.17 bc	1.2 ± 0.23 e	1.9 ± 0.36 cd	18.5 ± 9.27 c
'Suso'	1.3 ± 0.11 c	2.0 ± 0.22 a	1.1 ± 0.05 de	10.6 ± 0.42 c
'Yellow Pearshaped'	0.9 ± 0.03 d	1.7 ± 0.04 c	-	9.7 ± 0.22 c
'CIMA'	1.5 ± 0.08 b	1.8 ± 0.02 b	2.5 ± 0.01 b	30.1 ± 0.36 b
'Vyta'	1.6 ± 0.03 b	1.6 ± 0.03 d	2.9 ± 0.02 a	34.0 ± 1.08 a

FRAP—antioxidant capacity, LAA—L-ascorbic acid, GAE—gallic acid equivalents. Different letters indicate significant differences by Duncan test at $p \leq 0.05$ within the given processing method.

3.2. Ascorbic Acid

As expected, the content of ascorbic acid was highest in the fresh samples (Table 1) in comparison to the heat-treated samples (puree and ketchup), because a reduction during processing occurred (Table 1, Figure 2). The results varied among tomato cultivars and ranged from 139.4 to 255.7 mg kg^{-1} FM (fresh), 115.7 to 178.5 mg kg^{-1} FM (puree) and from 79.2 to 138.2 mg kg^{-1} FM (ketchup). A previous study reported wide ascorbic acid ranges in some vegetables, including tomato, with values between 10.9 and 229.8 mg kg^{-1} FM for the latter [2,8,21]. In the present study, cv. Vy (red), followed by cv. GK (yellow) showed the highest content of ascorbic acid in fresh samples, while in puree and ketchup cvs Ro, Rilia (Ri) and GK exhibited larger amounts of ascorbic acid. The cultivar Marmande (Ma) was characterized by the lowest content for all processed and fresh samples when compared with the other cultivars.

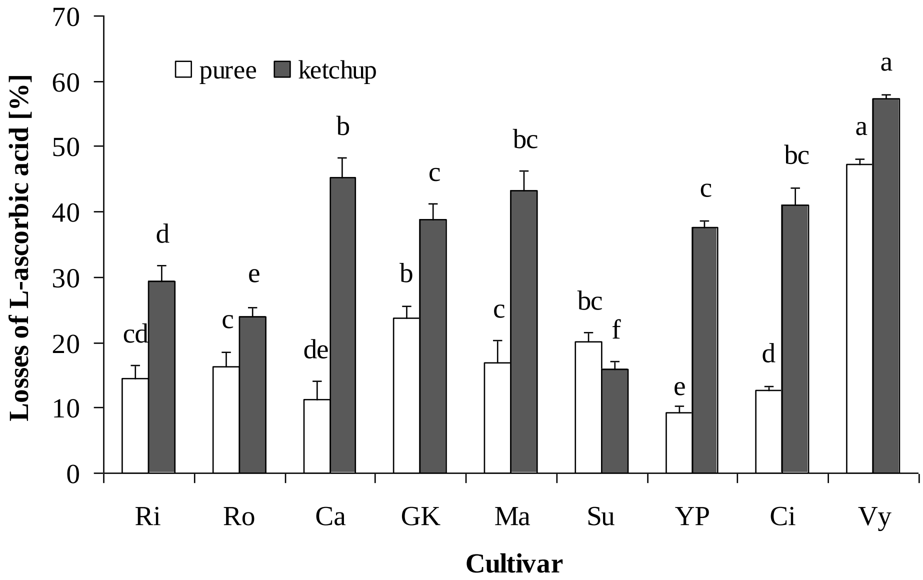

Figure 2. Losses of L-ascorbic acid in tomato cultivars after processing to puree and ketchup relative to fresh tomato fruits (Ri—'Rilia', Ro—'Roma', Ca—'Campbell-28', GK—'Goldene Königin', Ma—'Marmande', Su—'Suso' F1, YP—'Yellow Pearshaped', Ci—'CIMA', Vy—'Vyta'). The losses were calculated based on data presented in Table 1. The loss of ascorbic acid content of the fresh tomato fruits is 0 by definition. Different letters indicate significant differences within each processing method for cultivars by Duncan test at $p \leq 0.05$, $n = 16$.

The total percentage of ascorbic acid losses varied between 9.2% and 57.4% during the concentration from fresh samples to puree and ketchup, and the highest values were observed in cv. Vy for both processing methods, followed by cvs Campbell-28 (Ca), Ma, Ci and GK (Figure 2). The losses of ascorbic acid were observed in several studies [24,35,36]; however, they depended on the kind and processing level. Cultivars Su, Ro, and Ri were more stable to heating with regard to their ascorbic acid contents. Lower losses in these cvs are probably correlated with higher amounts of total organic acids irrespective of processing method, in the case of 'Su' with citric acid and in cvs Ro and Ri with malic acid (data not shown). The oxidation process of LAA to DHAA, followed by hydrolysis to 2,3-diketogulonic acid and further polymerization to other nutritionally inactive products during thermal treatments (blanching, cooking, pasteurization, dehydration or sterilization), can be slowed down by the presence of a high content of organic acids and low pH values of the medium, which facilitate the formation of complexes of LAA with metal ions and, moreover, inhibit the activity of oxidases.

3.3. Lycopene

The lycopene content of tomato products depends on the cultivar and processing method (Table 1). In fresh samples, it ranged from 89.7 mg kg^{-1} ('Su') to 202.5 mg kg^{-1} FM ('Vy'), while in puree samples lycopene contents varied from 238.4 mg kg^{-1} ('Ma') to 370.0 mg kg^{-1} ('Vy'), and in ketchup samples from 72.6 mg kg^{-1} ('Su') to 193.3 mg kg^{-1} FM ('Vy'). As expected, lycopene could not be detected in the yellow cultivars ('GK' and 'YP'). Although cv. 'Vy' had the highest content of lycopene for all processing methods, its relative change during processing was lower than in other cultivars (+82.7% for puree; −4,5% for ketchup) (Figure 3). The experiments showed an increase in lycopene levels in all red cvs as a result of processing to puree (concentration process). However, there are significant differences between cultivars. The largest increment in relation to the fresh samples was found for cvs Su (225.5%), Ca (186.0%) and Ri (182.8%). In the case of ketchup samples, in some cultivars (Ri, Ro, Ca, Ma and Ci) lycopene content rose, while in others (Su and Vy) it was diminished. The cultivar Ro showed the highest relative increase (48.6%), followed by 'Ma'. Lycopene was determined by several researchers [2,8,19,24,31,37] and values range from 43 to 111.1 mg kg^{-1} FM in different tomato cultivars. According to these authors, the variation is attributed to factors, such as plant nutrition, environment and genotype, which together can markedly affect the biosynthesis of carotenoids. Abushita et al. [16] reported variation in lycopene content ranging from 26 to 63 mg kg^{-1} FM in Hungarian cultivars. Lycopene content increased in the puree samples of all the cultivars due to the effect of heating and homogenization, which resulted in the concentration of dry matter and, thus, of lycopene. Lycopene shows a strong antioxidant capacity both in vitro and in vivo. It is the highest among all dietary antioxidants, and it is fairly stable during storage and cooking. In addition, heat processing, required for the preparation of tomato products, is recommended, because it increases the bioavailability of lycopene for the human body [37]. Food processing may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and the tissue matrix, thus making lycopene more accessible and enhancing cis-isomerization [38]. Ketchup samples had a significantly lower content of lycopene than puree, with significant differences among cultivars, probably because of the variation in lycopene stability, depending on several factors [35,39]. According to the literature [22,38,39], the main causes of lycopene degradation during tomato processing are isomerization and oxidation. Isomerization converts all-trans isomers to cis-isomers due to additional energy input and results in an unstable, energy-rich state. The cis-isomers increase with temperature and light irradiation only at the beginning of the treatment, and oxidation of all-trans and cis-isomers is the main tendency in long processing methods. That might be one of the reasons for the reduction in lycopene in ketchup, as well as the addition of the other ingredients of vinegar, sugar, onion and garlic (effect of dilution and relative reduction).

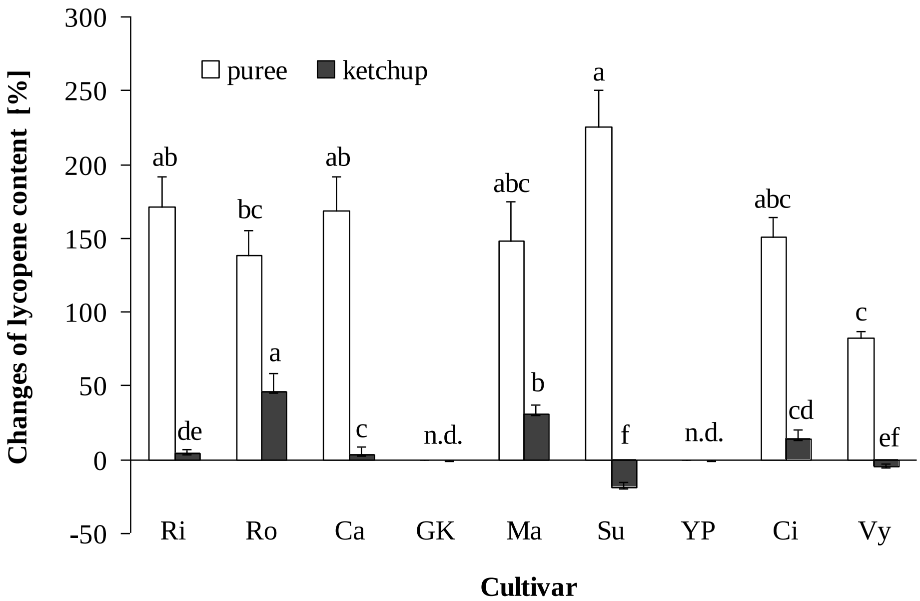

Figure 3. Relative variation in the lycopene content of tomato cultivars depending on processing method: puree and ketchup (Ri—'Rilia', Ro—'Roma', Ca—'Campbell-28', GK—'Goldene Königin', Ma—'Marmande', Su—'Suso' F1, YP—'Yellow Pearshaped', Ci—'CIMA', Vy—'Vyta'). The changes were calculated based on data presented in Table 1. The relative variation in the lycopene content of the fresh tomato fruit is 0 by definition. Different letters indicate significant differences within each processing method for cultivars by Duncan test at $p \leq 0.05$, $n = 16$.

3.4. Total Phenolic Compounds

Variation in total phenolic compounds for all cultivars depended on the processing method (Table 1, Figure 4). The contents ranged from 102.1 ('YP') to 567.0 mg kg^{-1} FM ('Vy') for the fresh tomatoes, from 409.6 ('YP') to 1168.3 mg kg^{-1} FM ('Vy') for puree, and from 643.6 ('YP') to 2268.0 mg kg^{-1} FM ('Vy') for ketchup. Minoggio et al. [40] obtained values of total polyphenols ranging from 258.8 to 443 mg kg^{-1} FM in fresh samples from different tomato lines and cultivars. Cultivar Vy, followed by cv. Ci, both Cuban varieties, were characterized by the highest polyphenol contents in fresh and processed samples, while cvs YP and Su showed the smallest amounts. In the present investigations, a statistically highly significant positive influence of processing method on the contents of total phenolic compounds was found (Table 1, Figure 4), which is in line with Chanforan et al. [41]. The amounts of total phenolic compounds increased with the intensity of the processing method (cooking time). The highest values were detected in ketchup in all cvs as a result of the concentration process, homogenization (better extractability), and because of the addition of ingredients with a high content of phenolic substances (e.g., garlic and onion puree). The increase in phenolic compounds varied between 105.9% ('Vy') and 467.6% ('Ma') for puree and from 299.6% ('Vy') to 940.5% ('Ro') for ketchup. Cultivar Ro was characterized by the largest increment (467.6%), with statistically significant differences compared to cvs Ci (119.6%) and Vy (105.8%). For ketchup as well, cv. Ro showed the most distinct increase in total phenolic compounds (940.6%), with significant differences to cvs GK (349.4%), Ci (314.8%) and Vy (299.6%). A previous study by Shen et al. [42] revealed that the content of phenolics in tomato rose by 34% for small tomato fruits and by 23% for large tomato fruits after blanching and heating at 100 °C for 30 min. It depends on the raw material, kind of processing and its duration. However, the results can also be contrary, as presented by Wu et al. [35].

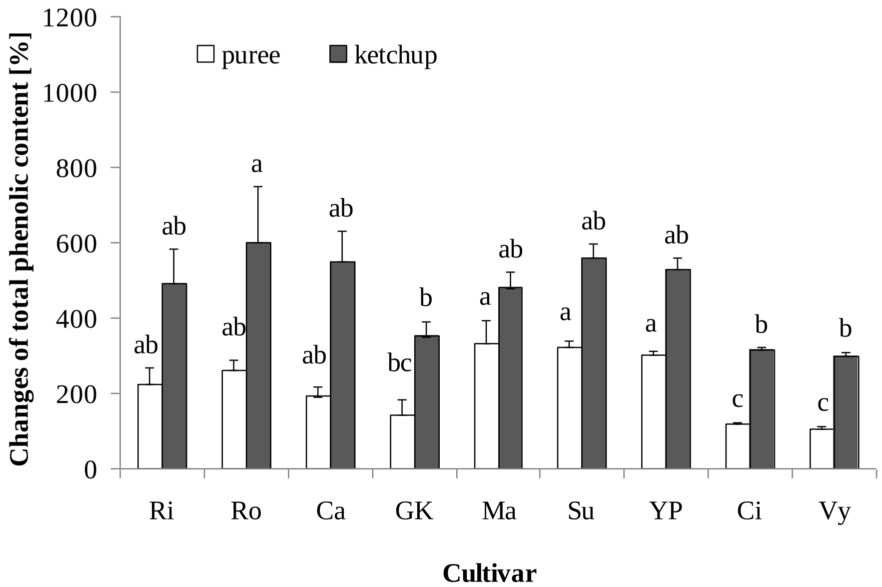

Figure 4. Relative changes in the content of total phenolic compounds of tomato cultivars depending on processing method: puree and ketchup (Ri—'Rilia', Ro—'Roma', Ca—'Campbell-28', GK—'Goldene Königin', Ma—'Marmande', Su—'Suso' F1, YP—'Yellow Pearshaped', Ci—'CIMA', Vy—'Vyta'). The changes were calculated based on data presented in Table 1. The relative change in the lycopene content of the fresh tomato fruit is 0 by definition. Different letters indicate significant differences within each processing method for cultivars by Duncan test at $p \leq 0.05$, $n = 16$.

3.5. Total Antioxidant Capacity

The evaluation of total antioxidant capacity represents a measure of the capacity of food extracts to delay oxidation processes in a controlled system, allowing the evaluation of potential synergistic and/or antagonistic effects of bioactive compounds when taking together the antioxidant capacity of such extracts [37]. The results of the FRAP assay conducted on fresh tomato, puree and ketchup revealed values ranging from 21.9 to 47.9 mmol Fe^{2+} kg^{-1} FM (fresh), 39.9 to 74.2 mmol Fe^{2+} kg^{-1} FM (puree) and from 61.3 to 106.8 mmol Fe^{2+} kg^{-1} FM (ketchup). The highest values for the fresh and processed samples were observed in cv. Vy, followed by 'Ci'. Similar results were published by

Binoy et al. [24], who found FRAP to vary between 6.4 and 23 mmol Fe^{2+} kg^{-1} FW in frozen samples of 12 tomato genotypes, reporting significant differences among them. The antioxidant capacity increased in all cultivars as a result of thermal treatment. The values were significantly higher in ketchup, followed by puree, than in fresh samples (Table 1, Figure 5). Dewanto et al. [23] as well reported an increase in the antioxidant capacity during heating due to an increase in the bioavailability of the main antioxidant substances (flavonoids, carotenoids and lycopene). In the presented investigation, a further increase in antioxidant capacity in ketchup resulted from the addition of ingredients during production showing antioxidant capacity.

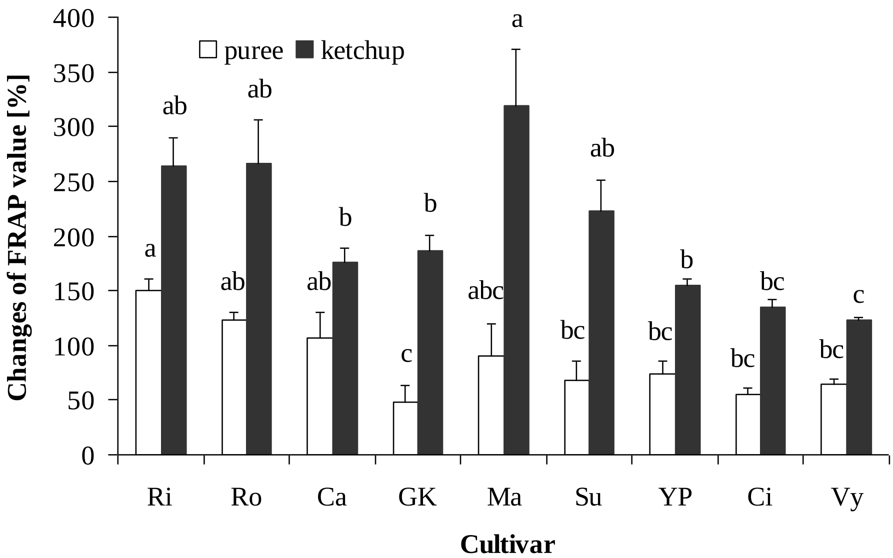

Figure 5. Relative variation in total antioxidant capacity measured as FRAP value of tomato cultivars as affected by processing to puree and ketchup (Ri—'Rilia', Ro—'Roma', Ca—'Campbell-28', GK—'Goldene Königin', Ma—'Marmande', Su—'Suso' F1, YP—'Yellow Pearshaped', Ci—'CIMA', Vy—'Vyta'). The changes were calculated based on data presented in Table 1. The relative variation in antioxidant capacity of fresh tomato fruits is 0 by definition. Different letters indicate significant differences within each processing method for cultivars by Duncan test at $p \leq 0.05$, $n = 16$.

The variation in total antioxidant capacity depended on the processing method [Figure 5] and varied widely between 45.3% and 148.1% for puree, and between 122.8% and 327.3% for ketchup. Among the puree samples, cv. Ri showed a significantly higher increment than cvs Vy, Ci, Su and GK. The latter cultivar was characterized by the smallest increase in antioxidant capacity, mainly due to the lack of lycopene and low stability during processing as a result of lower amounts of organic acids in the raw material (data not shown). In case of ketchup, the antioxidant capacity of cv. Ma increased more than that of cvs Ca, GK, YP, Ci and Vy. The variations might be due to different pre- as well as post-harvest factors such as the cultivars' responses to climate, temperature, hours of sunshine, soil type and composition, plant nutrition, carbon dioxide concentration in the atmosphere and application of naturally occurring compounds as well as the type and duration of processing.

3.6. Relationship between Antioxidant Capacity and Selected Bioactive Compounds

The correlation coefficients between total antioxidant capacity and bioactive compounds were higher for total phenolics (r = 0.90, $p \leq 0.01$) and lycopene (r = 0.90, $p \leq 0.01$) than for ascorbic acid (r = 0.76, $p \leq 0.01$). For comparison, Lenucci et al. [29] reported that the total antioxidant hydrophilic capacity was correlated with the levels of all of the major antioxidants (ascorbic acid, total flavonoids, and hydrophilic phenolics) in selected tomato cultivars. Other literature [27,43] indicated strong correlations between antioxidant capacity (FRAP) and total phenols, but did not confirm a correlation between ascorbic acid and antioxidant capacity. In the case of processed samples, a significant correlation between antioxidant capacity and ascorbic acid could not be found, while for lycopene and total phenolics the correlations were significant at the 1% level, although the correlation coefficients were smaller when compared with fresh samples (r for lycopene: 0.50 for puree, 0.49 for ketchup; r for total phenolics: 0.70 for puree, 0.43 for ketchup). Weak positive correlations were also found between lycopene and total phenolic content of fresh samples and between lycopene and the antioxidant capacity of processed products. According to the literature [43,44], the antioxidant capacity of onion and garlic measured by FRAP ranges between 6.3 and 8.5 mmol kg^{-1} for onion and from 1.9 to 2.5 mmol kg^{-1} for garlic. Considering that ketchup was prepared with 5% onion and 1% garlic, based on 100 g of ketchup, respectively, the contribution of these spices to the total antioxidant capacity of this product should be approximately 0.32 mmol Fe^{2+} kg^{-1} and 0.019 mmol Fe^{2+} kg^{-1} of the end product, respectively. The correlations obtained in this study suggest that total phenolics and lycopene contributed to a great extent to the antioxidant capacity of fresh tomato, while ascorbic acid to a lesser extent.

Both the content of bioactive compounds and the antioxidant capacity depended on cultivar and processing. Heating enhanced the content of lycopene (except of cvs Su and Vy in ketchup) and total phenolics, resulting in an increase in total antioxidant capacity, despite the reduction in ascorbic acid as a consequence of heating. Cultivar Vy, followed by 'Ci', was characterized by high contents of all analyzed parameters in the fresh and processed samples, although the percentage of increment after processing was lower. In cv. 'Ro', the content of antioxidants and antioxidant capacity rose for the used processing methods.

Comparing the origin of cvs, the Cuban cvs were characterized by higher antioxidant capacity measured as FRAP, especially the cvs Vy (106.8 mmol Fe^{2+} kg^{-1} FM) and Ci (102.6 mmol Fe^{2+} kg^{-1} FM). However, in the case of ketchup, the highest antioxidant capacity was found in the yellow cv. GK (130.0 mmol Fe^{2+} kg^{-1} FM), caused mainly by higher amounts of ascorbic acid and polyphenolic substances in addition to carotenoids instead of lycopene.

3.7. Contribution of Tomato Products to the Antioxidant Pool in Human Diet

More interesting is the contribution of the end product to the pool of antioxidants in the human dietary intake (Table 2) due to the different serving size of tomato products: fresh (200 g), puree (60 mL) and ketchup (15 mL) [45]. In consequence, with respect to the antioxidant capacity, the most important source of antioxidants was fresh tomatoes followed closely by tomato puree, irrespective of cultivar. In the case of antioxidant capacity measured as FRAP and other antioxidant compounds, the fresh tomatoes were characterized by the highest amounts of the investigated parameters, based on the serving size and because of the lack of thermal processing and the consequent lower degree of decomposition of temperature-sensitive compounds. In the case of ascorbic acid, the fresh tomatoes were the best source (LAA, Table 2). Losses due to the thermal processing were higher than the effect of concentration. In consequence, ketchup delivers almost no ascorbic acid by intake in line with serving size.

A lower serving portion of tomato puree was compensated for by a higher content of lycopene and total phenolic compounds; hence, both sources deliver a comparable amount of antioxidants. The smallest amounts of antioxidative compounds with respect to serving size are delivered by ketchup, despite the addition of further ingredients with high antioxidative capacity, e.g., garlic and onion puree. The investigations did not reveal any considerable differences between the contribution of lycopene and total phenolic compounds by intake of typical serving sizes of fresh tomato and puree. In order to consume the needed and recommended lycopene daily intake of about 20–30 mg d^{-1} [15] for protection against cancer and CVD, one to two portions of fresh tomato or tomato puree are required.

4. Conclusions

The results of this study revealed that the investigated tomato cultivars and their products (puree and ketchup) contain antioxidant compounds that contribute to their total antioxidant capacity, which is important for human nutrition due to their health benefits. Both the content of bioactive compounds and the antioxidant capacity depended on cultivar and processing. The heating process (90–100 °C/15 min) in puree production concentrated the contents of lycopene and total phenolics, resulting in an increase in total antioxidant capacity, despite the reduction in ascorbic acid. The correlation coefficients confirmed the major contribution of lycopene and total phenolics to the antioxidant capacity of fresh fruits, while ascorbic acid contributed less. The Cuban cvs Vy and Ci are most suitable for processing due to the highest amounts of investigated bioactive compounds in fresh matter as well as their very good retention during processing.

For future studies, using new breeds with high amounts of bioactive compounds and alternative processing methods, especially with less energy input, should be addressed. Furthermore, the blending of different cultivars with complementary properties for a stable and high content of the health-promoting compounds as a targeted quality of a final product, also in the context of sensory quality and consumer acceptance, should be studied.

Author Contributions: Conceptualization, E.P., A.J.K. and A.G.R.; methodology, A.J.K.; validation, A.G.R., A.J.K. and E.P.; formal analysis, A.G.R. and A.J.K.; investigation, A.G.R. and A.J.K.; resources, E.P. and A.J.K.; data curation, A.G.R., A.J.K. and E.P.; writing—original drafts preparation, A.G.R. and A.J.K.; writing—review and editing, A.G.R., A.J.K. and E.P.; visualization, A.G.R. and A.J.K.; supervision, E.P. and A.J.K.; funding acquisition A.G.R. and E.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the German Academic Exchange Service (DAAD) fellowship number A/03/15976.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Lidcay Herrera Isla (Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, 50100 Santa Clara, Cuba) for his contribution to research initiation and its conceptualization, and dedicate this paper to him. Furthermore, the authors thank the German Academic Exchange Service (DAAD) for supporting the research in the form of a PhD fellowship for Annia Gonzalez Rivero.

Conflicts of Interest: The authors declare no conflict of interest.

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