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Lipophilic and hydrophilic quantitative analysis of antioxidant activity in tomatoes and tomato products

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Lipophilic and Hydrophilic Quantitative Analysis of Antioxidant Activity in Tomatoes

and Tomato Products

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<u>ABSTRACT</u>

The amount of antioxidants present in food varies depending on environmental conditions in which produce was grown and how products were processed prior to consumption. It would, therefore, be useful to quantify antioxidant activities in these foods. This study focused on quantitative analysis of antioxidant activities in commercially produced whole tomatoes and processed tomato products. For commercially processed tomatoes, diced tomatoes had total antioxidant activities (TAA) ranging from 1.243 to 2.243 μmol TE/g fresh weight (fw), juice 1.573 to 6.86 μmol TE/g fw, paste 6.3 to 13.248 μmol TE/g fw, sauce 1.62 to 3.168 μmol TE/g fw, and soup 1.073 to 3.773 μmol TE/g fw. In commercial whole tomatoes, cherry tomatoes had TAA ranging from 2.303 to 3.66 μmol TE/g fw, grape tomatoes 2.443 to 2.825 μmol TE/g fw, roma tomatoes 0.535 to 3.033 μmol TE/g fw, and slicer tomatoes 1.448 to 2.788 μmol TE/g fw. Variations and significant differences were observed in different samples of the same type of tomatoes, between different types of tomatoes, in different batches of the same brand and kind of processed tomatoes, and between different types of processed tomatoes. These variations could be attributed to the different locales tomatoes were grown, type of tomatoes used for processed tomato products, or additives such as herbs and spices used for flavoring. Additionally, the effect of light intensity on antioxidant accumulation in tomatoes was investigated by experimentally growing plants in different light intensities. Data indicated statistical differences between tomatoes grown under the same as well as different light intensities, on a fresh weight basis. Tomatoes grown in 100% light had TAA ranging from 1.898 to 3.565 μmol TE/g fw (7.73 to 13.405 μmol TE/g dw), 2.375 to 2.523 μmol TE/g fw (8.558 to 13.223 μmol TE/g dw) in 50% light,

and 1.623 to 1.958 μmol TE/g fw (8.068 to 13.073 μmol TE/g dw) in 25% light. While small differences in data proved to be statistically significant, some of these differences may be too small to be of biological consequence. Data from this study, along with currently available data on antioxidants in foods, can provide useful information to consumers interested in purchasing products that are most beneficial to their health and to dietitians when making dietary recommendations to patients.

INTRODUCTION

Plant based foods have been found to have the greatest antioxidant activities (mean 11.57 mmol/100 g) compared to animal based foods (mean 0.18 mmol/100 g) and mixed food products (mean 0.91 mmol/100 g) (Carlsen et al 2010). Within plant foods, herbal or traditional plant medicines, herbs, and spices are the most antioxidant rich products. Fruits, vegetables, nuts, and grains are also rich sources of antioxidants. Antioxidant quantity among the same foods and between different foods can vary due to environmental factors such as growing conditions, genotypic differences, and ripeness when picked. For example, Scalzo et al. (2005) found that genotypically different strawberries or apricots from different rootstocks display different degree of antioxidant activities. Thornless blackberries, red and black raspberries, and strawberries were found to have increased anthocyanin content if picked later in fruit development (Wang and Lin 2000).

The effect of processing of biological materials into different forms for human consumption may affect the antioxidant content of the materials. Gil-Izquierdo et al. (2002) found that total antioxidant capacity of orange juice pulp was reduced by 47% due to pasteurization, concentration, and freezing. However, Murphy et al. (2009) demonstrated that cooking increases hydrophilic antioxidant capacity of wild blueberries. Moreover, sprouting, followed by oil-frying of Jack bean seeds leads to an increase in total free phenolic content, while soaking, followed by cooking or open-pan roasting diminishes the free-radical scavenging capacity of extracts (Vadivel et al. 2012).

Solanum lycopersicum

Solanum lycopersicum L. (*Lycopersicon esculentum* Mill.), commonly known as tomato, is considered a good source of antioxidants and is consumed in many different forms, such as paste, sauce, soup, and fresh fruits. Tomatoes are known for many nutritive components such as carotenoids such as β-carotene and lycopene, ascorbic acid (vitamin C), vitamin E, folate, phenolic compounds (flavonoids), potassium, protein and dietary fibers. Of these nutrients, lycopene, ascorbic acid, and phenolic compounds are the antioxidants responsible for most of the antioxidant activity observed in tomatoes (Sahlin et al. 2004). Since 2000, tomato production in the United States alone has increased from 12.6 to 13.7 million tons in 2008 (FAO STAT 2010). A major proportion of these tomatoes are processed into the tomato products mentioned above. Due to its popular consumption, we are interested in studying the variations that may be present in these different tomato products.

Environmental Effects on Antioxidant Activities in Solanum lycopersicum

Processing can greatly affect the amount of antioxidant present in the final products. Other factors affecting antioxidant activities include genotypic differences among tomato varieties (George et al. 2004), ripeness of tomatoes when they were picked (Arias et al. 2000, Cano et al. 2003, Ilie et al. 2009, Horchani et al. 2010), and environmental conditions in which tomato plants were grown (Arias et al. 2000, Pernice et al. 2010, Horchani et al. 2010). In terms of genotypic differences between tomato varieties, it was observed that different genotypes of *L. esculentum* exhibited a wide range of antioxidant activities. This genotypic effect on antioxidant activity was examined in a study where 12 different genotypes of tomatoes were planted in the same

controlled environmental conditions. Upon analyzing samples from these 12 genotypes, it was shown that the lycopene content ranged from 4.83 to 14.1 mg per 100 g fresh weight peel (skin removed from tomato fruit) and 2.04 to 6.94 mg per 100 g fresh weight pulp (fruit excluding the skin and seed). Ascorbic acid ranged from 8.55 to 56 mg in peel and 8.40 to 32.4 mg in pulp per 100 g fresh weight. Phenolics ranged from 10.4 to 40 mg in peel and 9.20 to 27 mg in pulp per 100 g fresh weight (George et al. 2004). Although these tomatoes were grown under the same conditions, the results indicated differences in antioxidant activities, portraying a significant role of genetics in antioxidant accumulation.

Not only does genetics play an important role in antioxidant quantity but the ripening stage at which tomato fruits are picked also influences antioxidant quantities present (Cano et al. 2003). Fruit picked at the latest stage of ripeness with the deepest red color exhibited the highest antioxidant activities, mainly due to an increase in the lipophilic antioxidant, lycopene, while other antioxidants such as ascorbic acid and phenols either remained fairly constant or increased only slightly. An increase in lycopene activity is expected as the fruit ripens because lycopene is responsible for the red color exhibited during ripening (Ilie et al. 2009, Horchani et al. 2010). How tomatoes ripen, whether on the vine or detached from the vine, also plays a vital role in antioxidant accumulation. A comparison of on-vine and off-vine ripening indicated 33% lower antioxidant activity in tomatoes that were allowed to ripen off-vine (Arias et al. 2000). Oftentimes commercial tomatoes are picked green before they are delivered to groceries stores, decreasing the maximum antioxidant content normally observed in on-vine ripened tomatoes.

Aside from how the tomatoes are ripened, environmental conditions in which plants are grown also determine the antioxidant content present in tomatoes. One of these conditions is root hypoxia, where the soil in which plants are grown is flooded with water at first anthesis. Tomato samples from plants submitted to prolonged root hypoxia were shown to have limited accumulations of carotenoids and ascorbate. Specifically, lycopene and β-carotene contents were significantly reduced in fruits subjected to root hypoxia compared to the control. This reduction is most evident at the late ripening stages when growth conditions are important for healthy fruit development (Horchani et al. 2010), indicating the importance of proper irrigation in fruit quality, in terms of antioxidant content. The effect of irrigation on antioxidant activities have previously been examined by Pernice et al. (2010) where three different micro-irrigation treatments (normal, reduced, and none) were applied to plants at fruit-setting to analyze their effects on quality and productivity of tomato fruit. Their results indicated an increase in carotenoid contents, on a fresh weight basis, when the plants were not irrigated.

Effect of Processing on Antioxidant Activities in Solanum lycopersicum

In addition to environmental effects, processing methods have also been found to control how much of the antioxidant are in the final products. Although tomatoes are highly consumed in the United States, a fairly high percentage of consumed tomato comes in the form of a paste, sauce, or soup rather than fresh tomatoes. Therefore, many studies have focused on quantifying antioxidant activity in these products to determine how processing methods affect antioxidant contents of processed products compared to fresh fruits. One way tomatoes are processed is thermal treatment. During thermal treatment, brown melanoidins or MRPs (Maillard reaction products) are formed as a

result of sugars condensing with amino acids, peptides or proteins. These MRPs have been shown to exhibit antioxidant activity as well as minimizing the loss of natural antioxidants during processing (Nicoli et al. 1997). Therefore, the overall antioxidant properties in these samples seem to be enhanced in thermal treatment, which may result in increased bioavailability of lycopene content due to its release from the tissue matrix (Rao et al. 1998). Contrasting with thermal treatment, dehydration results in a loss of lycopene activity. When tomato samples are dehydrated, the all-trans lycopene isomers are converted to cis-isomers, leading to a loss in lycopene bioavailability and an increase in its susceptibility to oxidation (Shi et al. 1999). It was suggested that vacuum-drying or air-drying leads to isomerization and oxidation, which are the main causes of lycopene biodegradation in these dehydration processes.

Storing, marinating and cooking of fresh tomatoes prior to serving are other forms of food processing methods that, as expected, also result in changes in the total antioxidant activities in tomatoes. Research by Lana et al. (2006) indicates that storing tomato slices instead of whole tomatoes decreases the hydrophilic antioxidant values of the tomatoes while there are no observable changes in the lipophilic antioxidant contents. Marinating tomato slices in oil and/or vinegar prior to eating has also been found to affect nutritional quality of tomatoes. The greatest loss of antioxidant activity was observed when tomato slices were soaked in both vinegar and oil, followed by in oil, and then in vinegar alone. Soaking slices in any of these ingredients significantly decreased the total antioxidant activity compared to raw slices (Sahlin et al. 2004). Boiling, baking and frying, however, indicated no significant loss of antioxidant activity compared to raw tomatoes, although frying tomatoes was found to result in the highest loss of nutrients

and total antioxidant activity. Sahlin et al. (2004) suggested that high loss could be due to the higher temperatures used in frying.

Significance of Antioxidants on Human Health

While beyond the scope of the investigation, antioxidants have been investigated in relation to human health. Antioxidants have been widely studied and recognized as potential agents against oxidative stress, caused by over-production of reactive oxygen species (ROS), which can undergo oxidation to produce more free radicals. Free radicals, such as superoxide (O_2^-), nitric oxide (NO), and hydrogen peroxide (H_2O_2), are important in biological processes necessary for life. However, these same molecules can participate in side chain reactions that are damaging to cells (Ames et al. 1993). Sources of oxidants are generally endogenous if they are produced by the body from natural life processes and exogenous if they are introduced into the body from the external environment. Some major sources of endogenous oxidants produced in the human body include mitochondrial activities, cells of the immune system, and peroxisomes. Mitochondria reduce oxygen to produce water and in the process also produce by-products such as O_2-, H_2O_2, and $^{\bullet}OH$. Phagocytic cells of the immune system can generate radicals while destroying infected cells. Peroxide can escape from degradation by catalase in peroxisomes. Aside from endogenous oxidants, exogenous oxidants can also be introduced from environmental chemicals, toxins, and pollutants such as cigarette smoke and certain acids in plant food. Oxides of nitrogen in cigarette smoke can deplete the endogenous antioxidant level. Chlorogenic and caffeic acid in plant food can generate oxidants via redox cycling (Wu et al. 2004, Ames et al. 1993).

As a defense against excessive oxidation, our body naturally produces and secretes (endogenous) antioxidants to protect our biological system from cell damage. Some of these endogenous antioxidants include melatonin, glutathione peroxidase, and superoxide dismutase (Bandyopadhyay and Chattopadhyay 2006). In addition, consumption of food such as fruits, vegetables, and grains rich in antioxidants such as vitamin E, selenium, ascorbic acid, quercetin, and phenolic compounds can augment and strengthen our fight against excessive oxidation.

Study on antioxidants might have a broader relevance because of the potential effect of antioxidants on human health and diseases. Various degenerative diseases such as cancer, cardiovascular disease, Alzheimer's disease, and Parkinson's disease have been associated with damages due to excessive oxidations. Consumption of fruit and vegetables, foods rich in antioxidants has been correlated with a decrease in biomarkers of oxidative stress (Khan et al 2008). An epidemiologic study on consumptions of fruit and vegetables, at least three times a day, demonstrated an inverse relationship between consumption and stroke incidence, stroke mortality, ischemic heart disease mortality, and cardiovascular disease mortality (Bazzano et al. 2002). A three year investigation by Aviram et al. (2004) found that consumption of pomegranate juice, rich in polyphenols, by carotid artery stenosis patients reduces blood pressure and LDL oxidation after one year. Consumption of at least 24μg of quercetin, highly concentrated in apples, grapes, soyeans, and broccoli, a day reduces the risk for prostate cancer by 27% (McCann et al. 2005). These studies provide promising results on the protective properties of antioxidants on human health. Further researches on antioxidants should be encouraged to extend our knowledge in this field.

Present Study

The purpose of the present study was to provide a comprehensive analysis of antioxidant activities in tomatoes and investigate the biological variability in tomatoes due to environmental effects and commercial processing methodologies. Commercially produced whole tomatoes and processed tomato products were used in the analysis. In addition, tomato plants were experimentally grown under different light intensities to study its effect on antioxidant accumulation in tomato fruits. There were three objectives upon which the study was conducted. First, antioxidant quantity across different tomato varieties including roma, cherry, grape, and slicer were analyzed and compared. Whole tomatoes were analyzed for both their lipophilic and hydrophilic antioxidant activities. Second, the effects processing has on the total concentration of antioxidants in tomato products was determined by analyzing commercially produced tomato paste, sauce, puree, and soup. Previous studies on environmental growth conditions have primarily focused on irrigation systems but few on light intensity (Hamner 1944, McCollum 1954). There is insufficient information regarding the effect of sunlight on antioxidant accumulation in tomato fruits. This led to the third objective where the effect of light on antioxidant content in tomatoes was determined. All tomato plants were grown under the same conditions until first anthesis, when sunlight intensity was artificially modified.

Many studies have been conducted on tomato antioxidant activities but not all were analyzed using the same methodology, hence antioxidant quantities were not expressed in the same units. Therefore, it is difficult to perform cross comparison between different tomato products using data from previous studies. Data from this

research will supply useful comprehensive comparative values for future studies that aim to focus on the same methods.

Current Methodologies

Many methods have been introduced and employed attempting to quantify the total antioxidant capacity of antioxidant-containing samples. Because antioxidants can be augmented from exogenous sources, quantifying the antioxidant activities in food can provide useful data to consumers in terms of food choices. Some of the more extensively used methods have been Trolox Equivalence Antioxidant Capacity (TEAC), Ferric Reducing Ability of Plasma (FRAP), and Oxygen Radical Absorbance Capacity (ORAC). FRAP and TEAC are based on single electron transfer between oxidant and free radical, whereas ORAC is based on hydrogen atom transfer (Ou et al. 2002). The FRAP assay measures increased absorbance of ferrous ions following the reduction of ferric ions by antioxidants whereas ORAC measures the inhibition of peroxyl radicals by antioxidants. The TEAC assay is based on the drop in absorbance of radical cation of ABTS as it is reduced by antioxidants. ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), in the presence of H_2O_2, is oxidized to the cation $ABTS^{\bullet+}$, which is photometrically measured by monitoring the absorbance at 735 nm. The absorbance can be continuously recorded, after loading of antioxidant sample (Alamed et al. 2009), until an end point is reached.

For this research, total antioxidant activity in different types of tomato products was quantified by employing the TEAC method as described by Arnao et al. (2001). Tomatoes have been found to be high in hydrophilic antioxidants such as ascorbic acid, dehydroascorbic acid, and aqueous phenols, as well as lipophilic antioxidants such as β-

carotene, lycopene and organic phenols (Cano et al. 2003). Therefore, the TEAC method is most suitable for analysis of antioxidant activities because it measures both hydrophilic and lipophilic antioxidant activity (HAA and LAA, respectively). Additionally, the time required for the sample to be analyzed is shorter (6 minutes) which allows the results to be more quickly obtained.

FRAP and ORAC are not well suited for this study due to the following reasons. The FRAP method does not measure antioxidants containing an SH-group (thiol) (Prior et al. 2005). Therefore not all antioxidants in the sample would be quantified, leading to skewed results (Gliszczynska-Swiglo 2006). Similar to TEAC, the ORAC assay has also been adapted to measure HAA. However, this assay is temperature-sensitive therefore requires close monitoring of the temperature of reactants. Greater time and more control are required for ORAC analysis, and equipment for ORAC analysis is expensive and not readily available, thus making TEAC a more suitable method for analyzing antioxidant activities in tomato samples. Not only is TEAC fast and easy to apply in measuring antioxidant activity, the method also avoids undesirable side reactions and can be used with foods having a wide range of pH (Cano et al. 2003).

MATERIALS & METHODS

Preparation of Reagents

The HAA reagent consisted of 2 mM ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), 0.25 µM horseradish peroxidase (HRP), and 45 µM H_2O_2 in 50 mM Na-phosphate buffer. ABTS, in its reduced state, is colorless. Addition of H_2O_2 leads to transfer of an electron from ABTS to H_2O_2 by peroxidase, changing the color of solution to dark blue-green. Loss of electrons generates ABTS radicals (ABTS•) in an oxidized state. Antioxidants, when loaded, would scavenge the ABTS•, reducing ABTS• back to its colorless form, ABTS. The color change is detected by a spectrophotometer and is recorded as a drop in absorbance of the solution at 730 nm.

The LAA reagent consisted of 1 mM ABTS, 5 µM HRP, and 45 µM H_2O_2 in acidified ethanol (0.7% phosphoric acid). Solution was capped, wrapped in aluminum foil, and allowed to develop for at least one week prior to usage.

Trolox (6-hydroxy-2, 5, 7, 8, -tetramethylchroman-2-carboxylic acid), a fat-soluble vitamin E analog, was used as the standard to convert all data into Trolox equivalent (TE) units (Figure A). Trolox acts as an antioxidant, donating an electron to ABTS radicals, turning the solution from green to colorless. Two different Trolox standards, freshly prepared, were made; one for HAA and another for LAA analysis. For HAA Trolox standard, a 1mM Trolox solution was prepared in 50 mM Na-phosphate buffer (pH 7.5). For LAA Trolox standard, a 1mM Trolox solution was prepared in absolute ethanol.

Biological Samples and Extractions

Commercial canned tomatoes (paste, puree, sauce, juice, and soup) from different brands with tomatoes as the major ingredient and whole tomatoes (cherry, grape, roma, and slicer) were purchased mostly from local grocery stores in Harrisonburg, VA 22801 and the remainder from a local farmer's market. All samples were analyzed the same day they were purchased. Reference herein to any specific commercial product, process, or service by trade names, trademark, manufacturer or otherwise does not constitute or imply its endorsement, recommendation, or favoring by James Madison University or the Commonwealth of Virginia. Seeds (Heirloom Beefsteak cultivar) for the light experiment were purchased from El Dorado Heirloom Seeds, Lipscomb Enterprises (KS, 67042). After seeds germinated, seedlings were transplanted into individual pots containing uniform pro-mix soil and placed in full light until first anthesis when they are randomly placed into one of the three different light treatments (100%, 50%, or 25% light). In the shaded treatments (50 and 25%), shading cloths were wrapped around the treatment lot to reduce the light to the desire intensity. Each treatment consisted of one light sensor (HOBO PAR Smart Sensors) that measures the light intensity during the entire duration of the experiment. Plants were watered once a day every day for the first week and every other day thereafter. When anthesis was first detected, flowers were tagged to keep track of fruiting (days post anthesis). Fruits from flowers set on the same day were picked from each shade as they ripened (ca. 35 days) and were analyzed on the same day for antioxidant activities.

For whole, at least three tomatoes combined, or chunky tomato samples, samples (if whole, placental tissue was removed along with seeds) were diced into smaller pieces.

For every 2 g of sample, 1 mL of Na-phosphate buffer (for HAA extraction) or 2 mL of ethyl acetate (for LAA extraction) was added. Samples were ground using a mortar and pestle while covered and were allowed to extract for 30 minutes. Extract was filtered into micro-centrifuge tubes, using a 5mL disposable syringe with a 0.45 μm (pore size) Luer-Lok syringe filter. Extracts were immediately analyzed for antioxidant activity.

For liquid or semi-liquid samples, for every milliliter of sample, 1 mL of Na-phosphate buffer and 1 mL of ethyl acetate were added. Mixture was emulsified and allowed to extract for 30 minutes. To separate HA and LA from the mixture, the mixture was pipetted into micro-centrifuge tubes and subjected to centrifugation for 2 minutes at 7500 rpm. After centrifugation, the mixture separated into three distinct layers; the top layer is the organic layer (LA), middle layer HA, and the bottom solid materials. Top organic LA layers were pipetted into a new micro-centrifuge tube and any remaining organic content from LA layer was discarded. Then, the rest of the content in the tubes was poured into a 5mL disposable syringe with a 0.45 μm (pore size) Luer-Lok syringe filter to filter HA into a new micro-centrifuge tube to remove solid materials that were on the bottom. Extracts were immediately analyzed.

Spectrophotometric Measurement of Antioxidant Activity

I measured antioxidant activities of sample extracts by using both HAA and LAA assays. Trolox standards were run first to create a dose-response curve (Figure 1) that was used as a basis for calculation of Trolox equivalence (TE) per gram of tissue. Trolox was used as the standard for measuring the reducing power of sample extracts because it is very stable in solution and has well-characterized reducing potential (Huang et al. 1996).
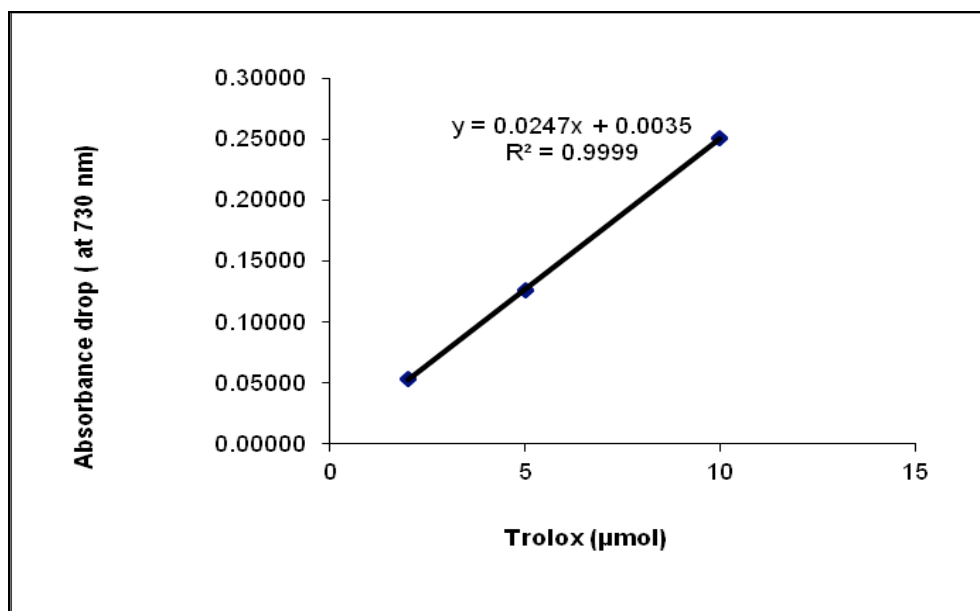
Figure 1. Dose-response Trolox standard showing linearity in absorbance of Trolox at 730 nm.

Once the Trolox curve was generated, measurement of antioxidant activities were made by loading cuvettes with 1mL HAA or LAA reagent, sealing them with latex septum and purging headspace with dry nitrogen gas to remove oxygen. Approximately 2 to 10 μL of Trolox standard or sample extract (four loadings or measurements per sample extract) was loaded into a cuvette and the redox reaction was spectrophotometrically measured and recorded as drop in absorbance at 730 nm, the wavelength at which ABTS has an absorption peak in the oxidized form and at which there is minimal interference from other biological molecules (Arnao 2000). The measured drops in absorbance were converted to TE using a standard curve of known Trolox concentrations and measured drops in absorbance.

Statistical Analysis

Statistical analysis was performed on data using one-way ANOVA, followed by a comparison of the means using a Dunnett T3 test ($p < 0.05$) to determine if means of samples were significantly different.

Moisture Content

The dry weight of each sample was measured to determine their moisture content by incubating samples in a drying oven for 5 days at 33°C. Moisture content was calculated using the equation:

$$\frac{(wet\ weight) - (dry\ weight)}{(wet\ weight)} \times 100$$

Fresh weight measurements were then adjusted for moisture content to determine antioxidant concentration on a dry weight basis. Dry weight measurements helped rule out the variability in moisture content between samples.

RESULTS

Commercially Produced Canned Tomatoes

Analysis of commercially processed tomato products (diced, paste, puree, sauce, soup) indicated high variations and wide ranges in antioxidant activities. HAA in diced tomato ranged from 0.762 to 1.533 µmol TE per gram fresh (fw) and 8.577 to 22.325 µmol TE per gram dry weight (dw). LAA ranged from 0.481 to 1.213 µmol TE per gram fw (5.414 to 11.863 µmol TE per gram dw) (Table 1). All four brands of diced tomato are significantly different ($p<0.05$) in their total antioxidant activity (TAA) on both fresh and dry weight basis (Table 2). Campbell's juices and Clamato cocktail showed an HAA range of 1.216 to 5.975 and LAA range of 0.357 to 0.85 µmol TE per gram sample, on a fresh weight basis (Table 3). TAA (fresh weight) analysis of these drinks, with values ranging from 1.573 to 6.861 µmol TE/g fw, indicated significant differences between all three samples (Table 4). Tomato pastes had an average HAA range of 2.846 to 9.916 µmol TE/g fw (11.840 to 121.338 µmol TE/g dw); LAA ranged from 2.763 to 5.460 µmol TE/g fw (13.374 to 48.464 µmol TE/g dw) (Table 5). TAA analysis indicated significant differences between brands and batches of the same brands (Table 6). TAA in pastes ranged from 6.301 to 13.245 and 26.903 to 162.068 µmol TE/g fresh and dw, respectively. Tomato sauce exhibited much lower antioxidant activities compared to other processed tomato products. Significant differences were also observed between different brands of tomato sauce. HAA values for sauce ranged from 0.895 to 1.580 µmol TE/g fw (8.690 to 19.761 µmol TE/g dw); LAA ranged from 0.898 to 1.876 µmol TE/g fw (8.491 to 19.761 µmol TE/g dw) (Table 7); TAA ranged from 1.933 to 3.168 µmol TE/g fw (18.288 to 33.624 µmol TE/g dw) (Table 8). Tomato soup had HAA ranging

from 0.684 to 2.779 µmol TE/g fw (5.374 to 13.717 µmol TE/g dw), LAA 0.389 to 1.166

µmol TE/g fw (3.055 to 7.561 µmol TE/g dw) (Table 9), and TAA ranging from 1.073 to

3.769 mmol TE/g fw (8.428 to 18.602 mmol TE/g dw) (Table 10). There were significant

differences between samples of soup. HAA analysis of all different types of processed

tomato indicated no significant difference among most samples on fw basis except

tomato paste (Table 11). TAA analysis indicated significant differences between paste

and sauce, puree, and soup, while no significant differences were observed between

sauce, puree, and soup (Table 12). Additionally, TAA in diced tomatoes were

significantly lower than paste, sauce, puree, and soup.

Table 1. Mean HAA and LAA (µmol TE/g sample) in different brands of canned diced
tomatoes. Subscripts represent fresh (f) and dry (d) weight. Different letters following the
means indicate significant differences at 95% confidence, within the same analysis
(HAAf, LAAf, HAAd, or LAAd).

	Brand of Diced Tomato	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	Hunts	1.533a	0.005	1.518	1.548
	Muir Glen	1.028b	0.012	0.991	1.065
	Walmart	0.943c	0.014	0.898	0.988
	Delmonte	0.762d	0.003	0.752	0.772
LAA_f	Muir Glen	1.213a	0.007	1.189	1.236
	Walmart	0.639b	0.005	0.623	0.654
	Hunts	0.509c	0.006	0.489	0.528
	Delmonte	0.481c	0.006	0.463	0.500
HAA_d	Hunts	22.325	0.071	22.098	22.551
	Walmart	14.527	0.219	13.831	15.222
	Muir Glen	10.057	0.114	9.694	10.419
	Delmonte	8.577d	0.038	8.457	8.697
LAA_d	Muir Glen	11.863	0.071	11.637	12.089
	Walmart	9.830b	0.078	9.583	10.077
	Hunts	7.402c	0.090	7.116	7.688
	Delmonte	5.414d	0.065	5.206	5.621

Table 2. Mean TAA (µmol TE/g sample) in different brands of diced tomato. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	Brand of Diced Tomato	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Muir Glen	2.240a	0.014	2.213	2.267
	Hunts	2.042b	0.008	2.027	2.057
	Walmart	1.582c	0.015	1.552	1.611
	Delmonte	1.243d	0.007	1.230	1.256
Dry weight	Hunts	29.727a	0.115	29.502	29.952
	Walmart	24.356b	0.232	23.902	24.811
	Muir Glen	21.920c	0.134	21.656	22.183
	Delmonte	13.991d	0.075	13.843	14.138

Table 3. Mean HAA and LAA (µmol TE/g sample) in tomato juice and cocktail from Campbell and Clamato. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf). *Low sodium.

	Brand of Tomato Juice	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA$_f$	Campbell*	5.975a	0.024	5.899	6.050
	Campbell	4.920b	0.100	4.600	5.240
	Clamato	1.216c	0.010	1.185	1.248
LAA$_f$	Campbell*	0.887a	0.019	0.825	0.948
	Campbell	0.741b	0.017	0.688	0.794
	Clamato	0.357c	0.018	0.300	0.413

Table 4. Mean TAA (µmol TE/g sample) in different brands of tomato juice. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight). *Low sodium.

	Brand of Tomato Juice	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Campbell*	6.861a	0.031	6.801	6.921
	Campbell	5.661b	0.102	5.461	5.860
	Clamato	1.573c	0.020	1.533	1.613

Table 5. Mean HAA and LAA (μmol TE/g sample) in different brands of canned tomato paste. Samples of the same brand represent different batches purchased on different dates or from different stores. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

	Brand of Tomato Paste	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	Contadina2	9.916a	0.136	9.484	10.349
	Hunts1	8.425b	0.121	8.040	8.810
	Muir Glen1	8.346b	0.086	8.072	8.619
	Walmart	5.081c	0.056	4.842	5.320
	Muir Glen2	3.706cde	0.238	2.950	4.462
	Hunts2	3.639d	0.041	3.508	3.769
	Contadina1	3.566d	0.011	3.530	3.601
	Contadina3	2.846e	0.016	2.796	2.895
LAA_f	Walmart	5.460a	0.050	5.300	5.620
	Hunts2	4.629b	0.072	4.398	4.859
	Muir Glen2	4.490b	0.034	4.382	4.598
	Muir Glen1	4.191c	0.039	4.068	4.314
	Hunts1	4.110c	0.045	3.967	4.253
	Contadina3	3.455d	0.033	3.351	3.559
	Contadina2	3.328d	0.038	3.206	3.451
	Contadina1	2.763e	0.022	2.693	2.833
HAA_d	Contadina2	121.338a	1.663	116.044	126.632
	Muir Glen1	96.510b	0.994	93.347	99.673
	Contadina1	43.608c	0.136	43.173	44.042
	Contadina3	34.821d	0.190	34.215	35.427
	Hunts1	27.418e	0.394	26.165	28.670
	Walmart	17.133f	0.187	16.329	17.937
	Muir Glen2	14.068fg	0.902	11.199	16.938
	Hunts2	11.840g	0.133	11.417	12.264
LAA_d	Muir Glen1	48.464a	0.448	47.040	49.888
	Contadina3	42.276b	0.397	41.011	43.540
	Contadina2	40.730b	0.470	39.235	42.226
	Contadina1	33.790c	0.270	32.931	34.649
	Walmart	18.410d	0.169	17.870	18.949
	Muir Glen2	17.044e	0.129	16.634	17.453
	Hunts2	15.062f	0.236	14.312	15.813
	Hunts1	13.374g	0.146	12.909	13.839

Table 6. Mean TAA (µmol TE/g sample) in different brands of tomato paste. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	Brand of Tomato Paste	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Contadina2	13.245a	0.141	12.968	13.522
	Muir Glen1	12.537b	0.094	12.352	12.721
	Hunts1	12.535b	0.129	12.282	12.788
	Walmart	10.541c	0.075	10.394	10.687
	Hunts2	8.267d	0.083	8.104	8.430
	Muir Glen2	8.196d	0.240	7.725	8.666
	Contadina1	6.328e	0.025	6.280	6.377
	Contadina3	6.301e	0.036	6.230	6.371
Dry weight	Contadina2	162.068a	1.729	158.680	165.456
	Muir Glen1	144.974b	1.090	142.837	147.110
	Contadina1	77.398c	0.302	76.805	77.991
	Contadina3	77.097c	0.441	76.233	77.960
	Hunts1	40.792d	0.420	39.969	41.615
	Walmart	35.542e	0.252	35.048	36.036
	Muir Glen2	31.112f	0.911	29.327	32.897
	Hunts2	26.903g	0.271	26.372	27.433

Table 7. Mean HAA and LAA (µmol TE/g sample) in different brands of tomato sauce. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

	Brand of Tomato Sauce	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA$_f$	DW	1.580a	0.010	1.549	1.612
	Kroger	1.321b	0.009	1.292	1.350
	Paradiso	1.299b	0.009	1.269	1.329
	Walmart	1.292b	0.011	1.257	1.328
	Hunts	1.179c	0.006	1.158	1.199
	Stokelys	1.163c	0.008	1.136	1.189
	DelFeurte	1.089d	0.011	1.053	1.125
	Bestwest	1.035d	0.007	1.012	1.059
	Delmonte	0.895e	0.008	0.871	0.919
LAA$_f$	Walmart	1.876a	0.011	1.840	1.912
	Paradiso	1.468b	0.014	1.424	1.512
	Delmonte	1.448b	0.000	1.448	1.448
	Stokelys	1.335bcd	0.045	1.193	1.476
	DW	1.299bcd	0.047	1.148	1.449
	DelFeurte	1.151d	0.014	1.106	1.196
	Hunts	1.033c	0.002	1.028	1.037
	Kroger	0.927e	0.014	0.884	0.970
	Bestwest	0.898e	0.008	0.871	0.924
HAA$_d$	Kroger	19.761a	0.136	19.327	20.194
	DW	15.976b	0.103	15.648	16.304
	Walmart	13.611c	0.116	13.241	13.980
	Hunts	12.550d	0.067	12.336	12.764
	Stokelys	10.930e	0.078	10.683	11.177
	Paradiso	10.555e	0.078	10.308	10.802
	Bestwest	9.797f	0.069	9.577	10.018
	DelFeurte	9.448f	0.100	9.130	9.767
	Delmonte	8.690g	0.072	8.460	8.920
LAA$_d$	Walmart	19.761a	0.121	19.378	20.145
	Delmonte	14.055b	0.004	14.010	14.099
	Kroger	13.863b	0.201	13.224	14.502
	DW	13.129bcde	0.479	11.605	14.652
	Stokelys	12.544bcde	0.419	11.211	13.877
	Paradiso	11.926d	0.114	11.564	12.288
	Hunts	10.992c	0.017	10.938	11.045
	DelFeurte	9.984e	0.125	9.587	10.382
	Bestwest	8.491f	0.079	8.241	8.741

Table 8. Mean TAA (µmol TE/g sample) in different brands of tomato sauce. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	Brand of Tomato Sauce	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Walmart	3.168a	0.016	3.137	3.199
	DW	2.879b	0.048	2.784	2.974
	Paradiso	2.767b	0.017	2.734	2.800
	Stokelys	2.497c	0.045	2.408	2.586
	Delmonte	2.343d	0.008	2.329	2.358
	Kroger	2.248e	0.016	2.216	2.280
	DelFeurte	2.240de	0.018	2.204	2.276
	Hunts	2.211e	0.007	2.198	2.224
	Bestwest	1.933f	0.011	1.911	1.955
Dry weight	Kroger	33.624a	0.243	33.148	34.099
	Walmart	33.372a	0.167	33.044	33.700
	DW	29.105b	0.490	28.145	30.064
	Hunts	23.542c	0.069	23.406	23.678
	Stokelys	23.474cd	0.426	22.639	24.309
	Delmonte	22.745d	0.072	22.603	22.886
	Paradiso	22.481d	0.138	22.211	22.751
	DelFeurte	19.433e	0.160	19.119	19.746
	Bestwest	18.288f	0.105	18.083	18.494

Table 9. Mean HAA and LAA (µmol TE/g sample) in different brands of canned tomato soup. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd). *With basil.

	Brand of Tomato Soup	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	Walmart	2.779a	0.033	2.672	2.885
	Campbell	1.276b	0.006	1.257	1.295
	Progresso*	1.155c	0.019	1.096	1.214
	Campbell*	0.684d	0.013	0.642	0.726
LAA_f	Progresso*	1.166a	0.017	1.112	1.219
	Walmart	0.990b	0.027	0.905	1.074
	Campbell	0.763c	0.011	0.729	0.797
	Campbell*	0.389d	0.034	0.282	0.496
HAA_d	Walmart	13.717a	0.165	13.190	14.243
	Progresso*	7.493b	0.120	7.112	7.875
	Campbell	5.889c	0.028	5.800	5.979
	Campbell*	5.374d	0.102	5.049	5.698
LAA_d	Progresso*	7.561a	0.108	7.217	7.906
	Walmart	4.885b	0.132	4.466	5.305
	Campbell	3.522c	0.049	3.366	3.677
	Campbell*	3.055c	0.265	2.213	3.897

Table 10. Mean TAA (µmol TE/g sample) in different brands of tomato soup. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight). *With basil.

	Brand of Tomato Soup	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Walmart	3.769a	0.043	3.685	3.852
	Progresso*	2.321b	0.025	2.272	2.369
	Campbell	2.039c	0.012	2.015	2.063
	Campbell*	1.073d	0.036	1.002	1.143
Dry weight	Walmart	18.602a	0.211	18.188	19.017
	Progresso*	15.055b	0.161	14.738	15.371
	Campbell	9.411c	0.056	9.301	9.522
	Campbell*	8.428c	0.284	7.873	8.984

Table 11. Mean HAA and LAA (µmol TE/g sample) in different types of commercially processed tomatoes. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

	Type of Processed Tomato	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	Paste	5.710a	0.481	4.728	6.692
	Sauce	1.763b	0.265	1.224	2.303
	Soup	1.473b	0.203	1.041	1.906
	Puree	1.377b	0.123	1.085	1.668
	Diced	1.067b	0.074	0.909	1.224
LAA_f	Paste	4.053a	0.144	3.760	4.346
	Sauce	1.356b	0.064	1.226	1.486
	Puree	1.076c	0.053	0.951	1.200
	Soup	0.827de	0.076	0.665	0.988
	Diced	0.710e	0.076	0.547	0.873
HAA_d	Paste	46.768a	7.040	32.390	61.146
	Sauce	18.342b	2.830	12.590	24.093
	Puree	15.870b	1.424	12.504	19.237
	Diced	13.871b	1.382	10.925	16.818
	Soup	8.118c	0.860	6.285	9.952
LAA_d	Paste	28.644a	2.385	23.780	33.508
	Sauce	13.807b	0.686	12.413	15.202
	Puree	12.399b	0.605	10.968	13.830
	Diced	8.627c	0.630	7.284	9.970
	Soup	4.756d	0.458	3.779	5.733

Table 12. Mean TAA (µmol TE/g sample) in different commercially processed tomato products. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	Type of Processed Tomatoes	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Paste	9.763a	0.502	8.780	10.747
	Sauce	3.119b	0.273	2.584	3.655
	Puree	2.452b	0.134	2.189	2.715
	Soup	2.300b	0.217	1.875	2.725
	Diced	1.777c	0.106	1.568	1.985
Dry weight	Paste	75.412a	7.433	60.842	89.981
	Sauce	32.149b	2.912	26.441	37.856
	Puree	28.270b	1.547	25.237	31.301
	Diced	22.498b	1.519	19.521	25.476
	Soup	12.874c	0.975	10.964	14.784

Commercially Produced Whole Tomatoes

Four types of commonly consumed whole tomatoes (cherry, grape, roma, and slicer) were analyzed for their HAA and LAA. Cherry tomatoes had an average HAA range of 1.706 to 3.187 µmol TE/g fw (24.446 to 42.208 µmol TE/g dry weight), LAA 0.277 to 0.905 µmol TE/g fw (3.589 to 14.440 µmol TE/g dry weight) (Figure 2), and TAA ranging from 1.983 to 3.524 µmol TE/g fw (30.307 to 56.647 µmol TE/g dry weight) (Table 13). Significant differences were observed in all analyses (HAA, LAA, and TAA). Analysis of grape tomatoes indicated significant differences between samples on a fresh and dry weight basis for both HAA (1.889 to 2.634 µmol TE/g fw and 25.032 to 40.466 µmol TE/g dw) and LAA (0.185 to 0.58 µmol TE/g fw and 2.685 to 9.453 µmol TE/g dw) (Figure 3). Analysis of TAA also indicated significant differences between different batches of cherry tomatoes (Table 14). Similarly, analysis of roma tomatoes indicated significant differences between different batches across HAA (0.412 to 2.716 and 8.614 to 50.876 µmol TE/g fw and dw, respectively), LAA (0.137 to 0.371 and 4.127 to 6.689 µmol TE/g fw and dw, respectively) (Figure 4) and TAA (0.549 to

3.038 and 12.741 to 56.907 μmol TE/g fw and dw, respectively) (Table 15). Slicer

tomatoes indicated more homogenous results, especially on LAA (Figure 5). TAA

analysis indicated significant differences between different types of slicer tomatoes but

on a lower scale compared to cherry, grape, and roma (Table 16). Analysis of all four

types of tomatoes (roma, slicer, grape, and cherry) indicated that cherry and grape

tomatoes are significantly different from roma and slicer tomatoes, on a fresh weight

basis (Figure 6). On dry weight basis, LAA of all samples were similar. Analysis of TAA

indicated small differences among the different types of whole tomatoes on a fresh

weight basis, similar to that of HAA fw and LAA fw (Table 17).
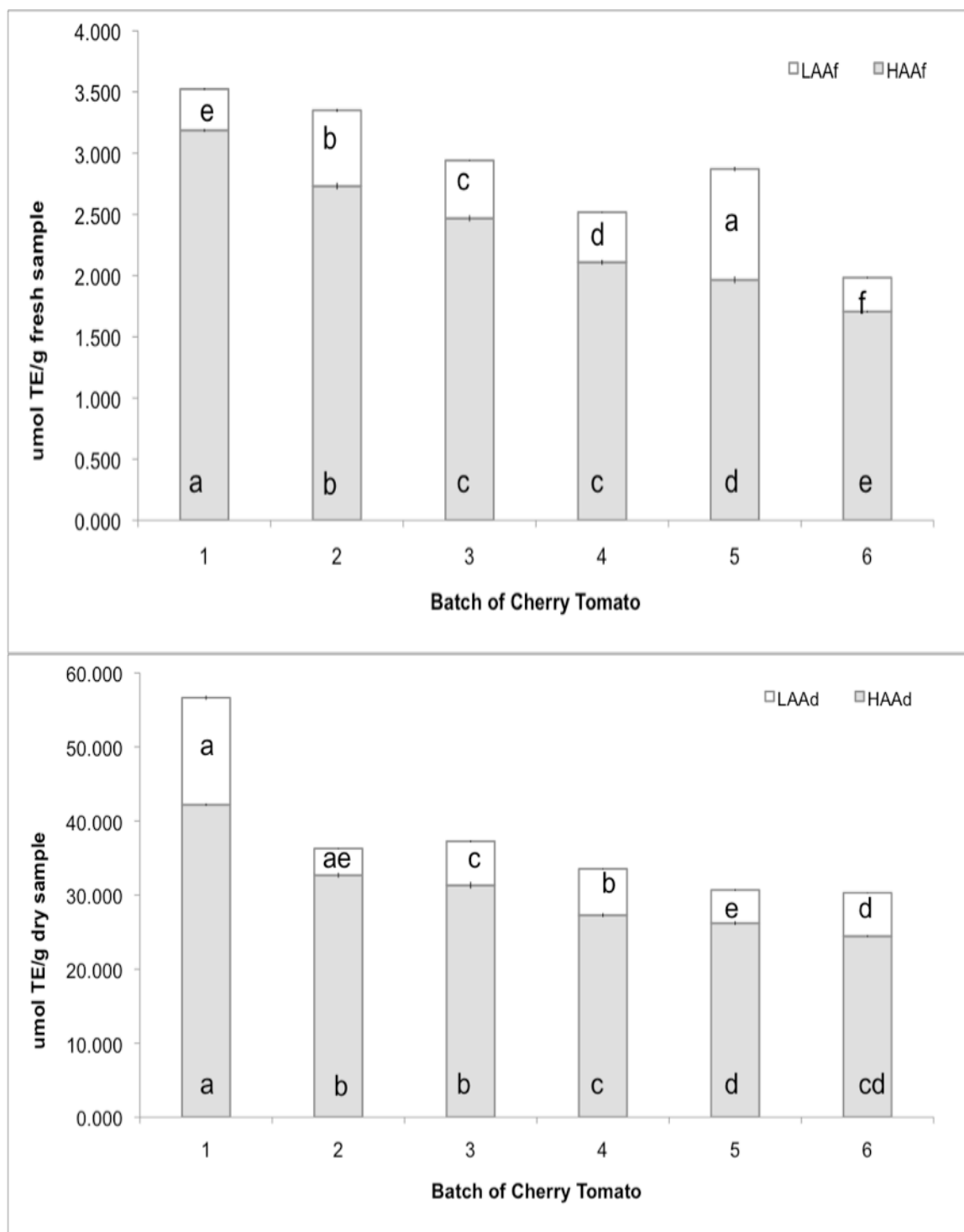
Figure 2. Mean HAA and LAA (μmol TE/g sample) in different batches of commercially produced cherry tomatoes. Letters "f" and "d" following "HAA" and "LAA" represent fresh (top panel) and dry (bottom panel) weight, respectively. Different letters indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

Table 13. Mean TAA (µmol TE/g sample) in different batches of cherry tomatoes.
Different letters following the means indicate significant differences at 95% confidence,
within the same analysis (fresh or dry weight).

	Batch of Cherry Tomatoes	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	1	3.524a	0.015	3.494	3.555
	3	3.3450b	0.030	3.290	3.409
	2	2.941c	0.028	2.887	2.995
	5	2.870c	0.035	2.802	2.939
	4	2.518d	0.021	2.477	2.578
	6	1.983e	0.011	1.963	2.004
Dry weight	1	56.647a	0.357	55.947	57.347
	4	37.267b	0.476	36.337	38.203
	5	36.282b	0.369	35.558	37.006
	2	33.554c	0.270	33.024	34.084
	6	30.689d	0.287	30.127	31.252
	3	30.307d	0.128	30.055	30.559

Table 14. Mean TAA (µmol TE/g sample) in different batches of grape tomatoes.
Different letters following the means indicate significant differences at 95% confidence,
within the same analysis (fresh or dry weight).

	Batch of Grape Tomatoes	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	2	2.848ab	0.038	2.773	2.923
	5	2.823a	0.012	2.798	2.847
	3	2.702b	0.028	2.647	2.756
	1	2.528c	0.010	2.508	2.547
	4	2.444c	0.026	2.394	2.494
Dry weight	5	46.009a	0.205	45.606	46.411
	2	43.758a	0.590	42.602	44.913
	3	39.136b	0.402	38.348	39.924
	1	36.604c	0.147	36.316	36.892
	4	32.386d	0.339	31.722	33.050
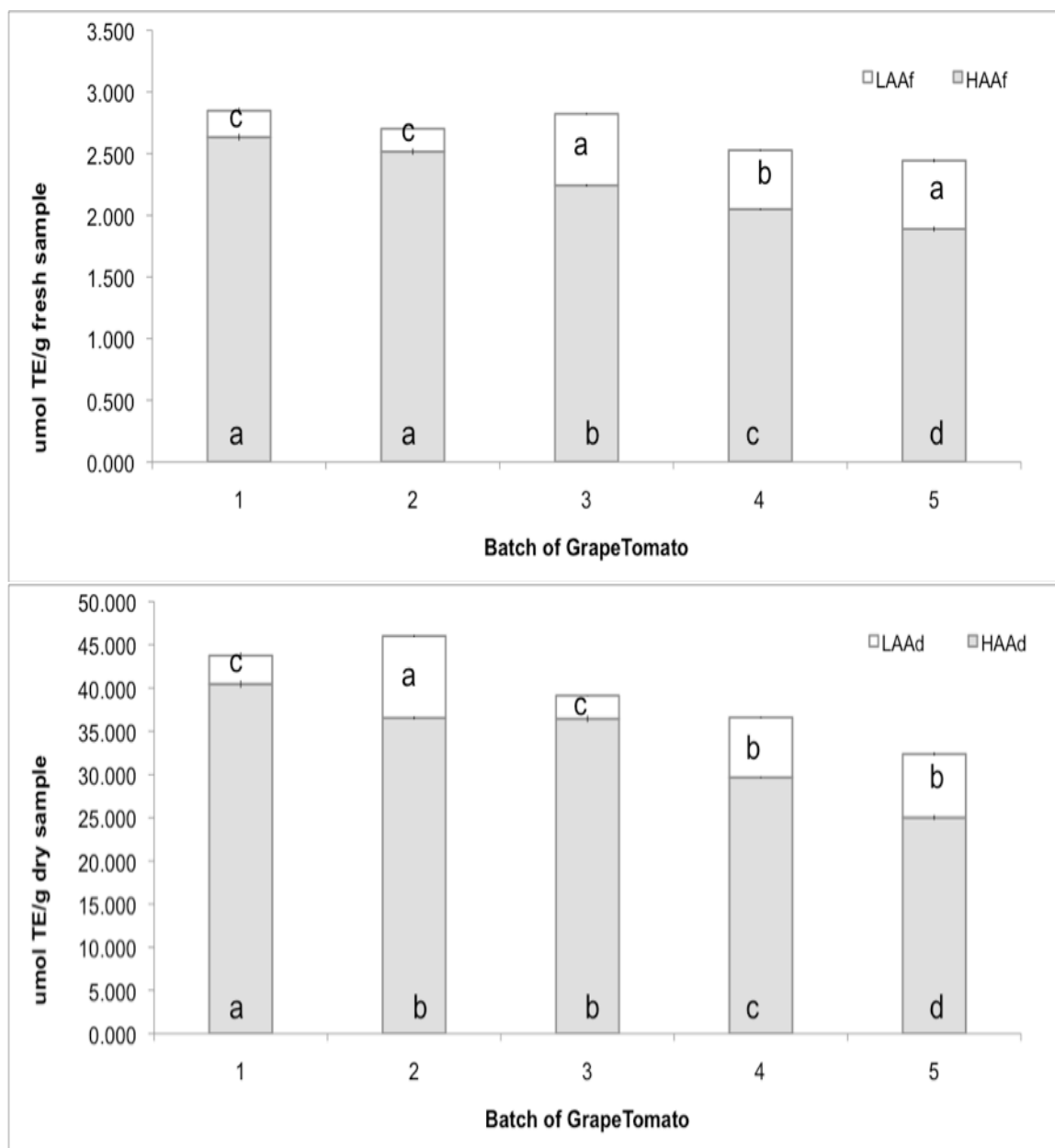
Figure 3. Mean HAA and LAA (µmol TE/g sample) in different batches of commercially produced grape tomatoes. Letters "f" and "d" following "HAA" and "LAA" represent fresh (top panel) and dry (bottom panel) weight, respectively. Different letters indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

Figure 4. Mean HAA and LAA (μmol TE/g sample) in different batches of commercially produced roma tomatoes. Letters "f" and "d" following "HAA" and "LAA" represent fresh (top panel) and dry (bottom panel) weight, respectively. Different letters indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

Table 15. Mean TAA (μmol TE/g sample) in different batches of roma tomatoes.
Different letters following the means indicate significant differences at 95% confidence,
within the same analysis (fresh or dry weight).

	Batch of Roma Tomatoes	Mean TAA	Standard Error	95% Confidence Interval for mean	
				Lower Bound	Upper Bound
Fresh weight	5	3.038a	0.032	2.974	3.101
	4	2.217b	0.011	2.195	2.238
	3	1.851c	0.020	1.811	1.890
	1	1.374d	0.019	1.338	1.410
	2	0.549e	0.020	0.509	0.589
Dry weight	5	56.907a	0.602	55.727	58.086
	3	53.471a	0.591	52.312	54.630
	4	39.963b	0.201	39.568	40.357
	1	28.753c	0.389	27.991	29.514
	2	12.741d	0.554	11.655	13.827

Table 16. Mean TAA (μmol TE/g sample) in different types of slicer tomatoes. Different
letters following the means indicate significant differences at 95% confidence, within the
same analysis (fresh or dry weight).

|	Type of Slicer Tomatoes	Mean TAA	Standard Error	95% Confidence Interval for mean	
				Lower Bound	Upper Bound
Fresh weight	red slicers	1.989a	0.101	1.791	2.187
	blossers	1.780a	0.016	1.748	1.811
	yellow slicers	1.730a	0.010	1.710	1.749
	early girls	1.520b	0.012	1.497	1.543
Dry weight	red slicers	43.816a	1.189	41.486	46.147
	blossers	29.588b	0.267	29.064	30.111
	yellow slicers	29.581b	0.173	29.241	29.920
	early girls	28.800b	0.222	28.365	29.235
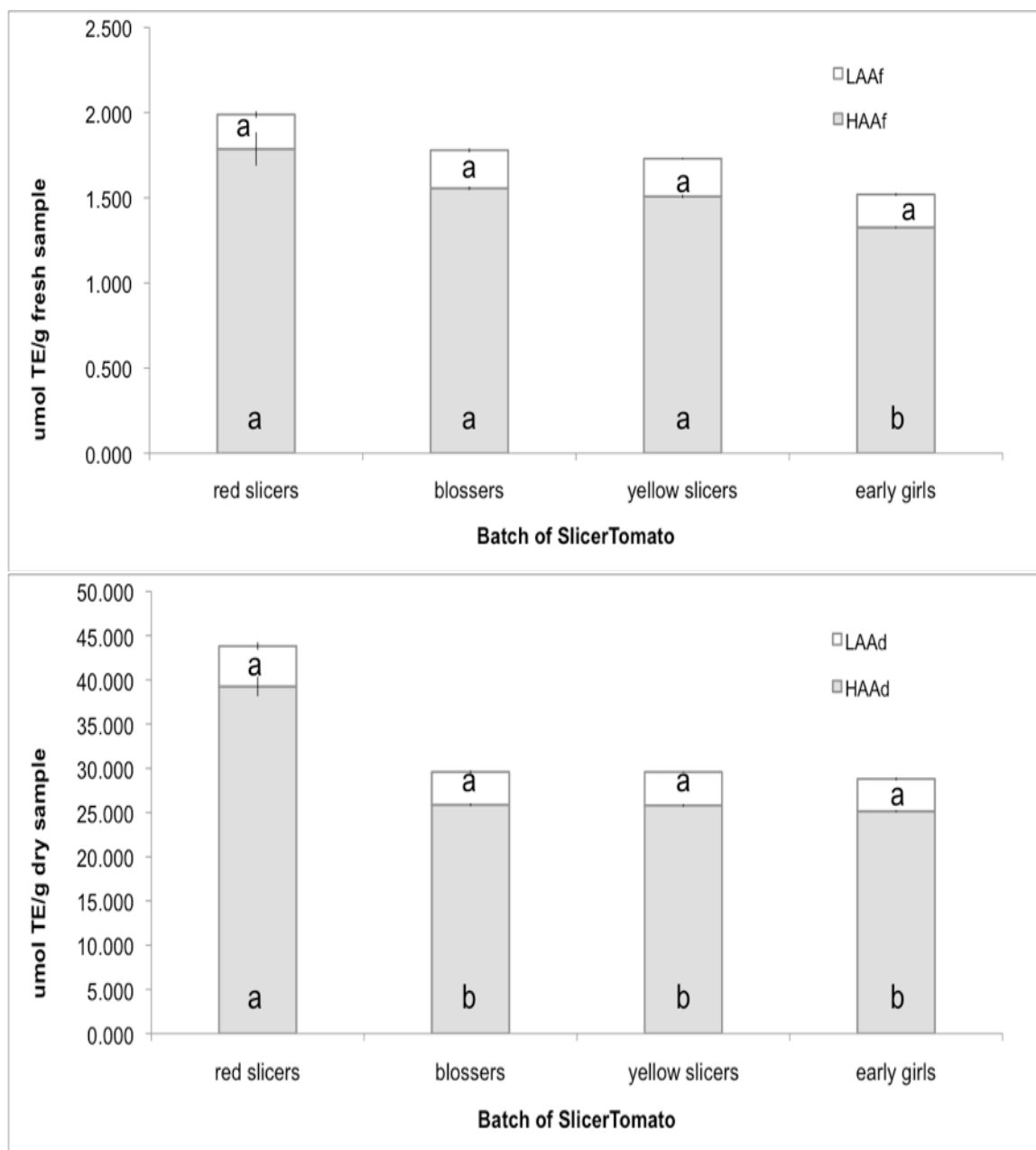
Figure 5. Mean HAA and LAA (μmol TE/g sample) in different batches of commercially produced slicer tomatoes. Letters "f" and "d" following "HAA" and "LAA" represent fresh (top panel) and dry (bottom panel) weight, respectively. Different letters indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).
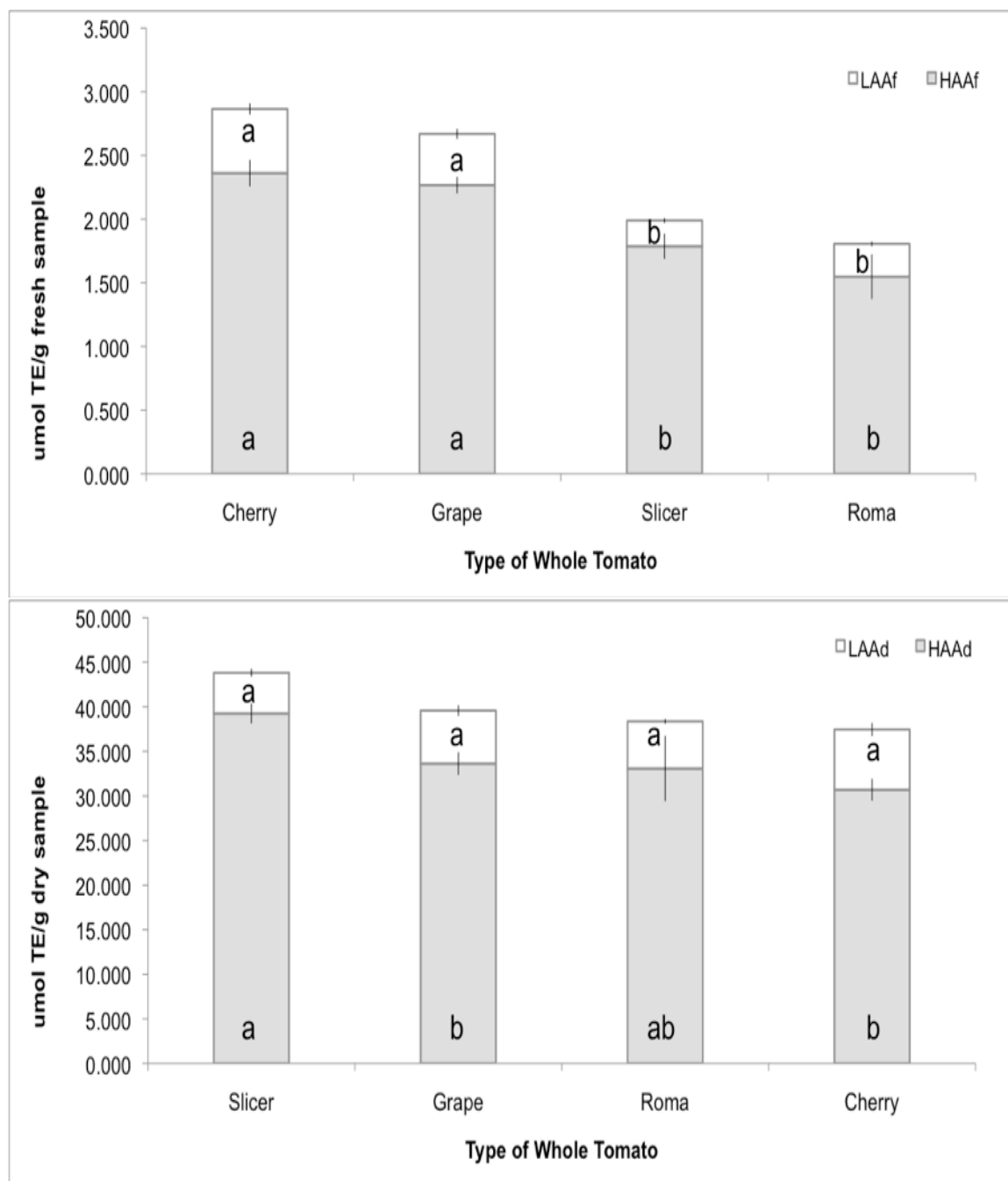
Figure 6. Mean HAA and LAA (µmol TE/g sample) in different types of tomatoes. Letters "f" and "d" following "HAA" and "LAA" represent fresh (top panel) and dry (bottom panel) weight, respectively. Different letters indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).
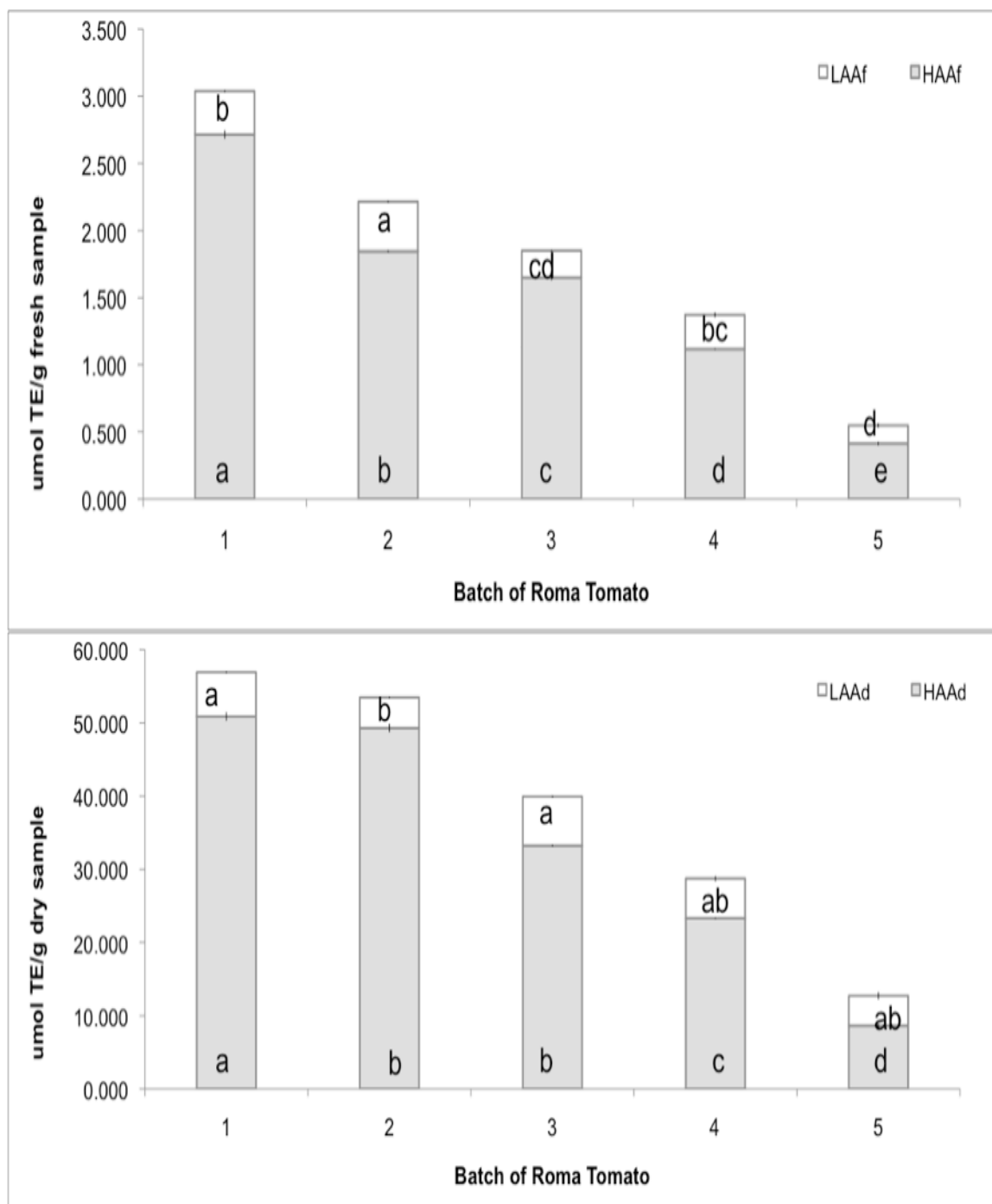
Table 17. Mean TAA (μmol TE/g sample) in different type of whole tomato. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	Type of Whole Tomato	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	Cherry	2.864a	0.113	2.643	3.085
	Grape	2.669a	0.075	2.521	2.816
	Slicer	1.989b	0.101	1.791	2.187
	Roma	1.805b	0.177	1.458	2.153
Dry weight	Slicer	43.816a	1.189	41.486	46.147
	Grape	39.578a	1.405	36.824	42.333
	Roma	38.367a	3.673	31.167	45.566
	Cherry	37.458a	1.439	34.639	40.278

Experimentally Grown Tomatoes in Different Light Treatments

Experimentally grown tomatoes were subjected to different light intensity to determine the effect of light on antioxidant accumulation in the fruit. HAA and LAA analysis of samples from 100% light intensity indicated significant differences between samples, especially more so for HAA (fresh and dry weight basis) (Table 18). When subjected to 50% light intensity, range of HAA was slightly lower (1.887 to 2.063 μmol TE/g fw) than 100% light (1.3 to 2.985 μmol TE/g fw) samples (Table 19, Table 20, Table 21). Tomatoes grown under only 25% light intensity showed even lower HAA ranges, 1.381 to 1.769 μmol TE/g fw (Table 22). TAA analysis indicated significant differences among samples grown in 25% light intensity (Table 23). Analysis of all three treatments indicated significant differences in HAA between all three treatments, on fresh weight basis (Figure 7). On dry weight basis, tomatoes grown in 100% light exhibited significantly higher HAA than in 25% light. TAA analysis indicated a trend similar to that of HAA analysis (Table 24).

Table 18. Mean HAA and LAA (μmol TE/g sample) in different batches of tomatoes grown experimentally in 100% light intensity. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

|	100% Light Tomato Sample	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	4	2.985a	0.019	2.925	3.044
	5	2.886a	0.028	2.797	2.975
	3	2.626b	0.009	2.599	2.653
	6	2.544bc	0.018	2.486	2.603
	2	2.464c	0.006	2.443	2.484
	1	1.300d	0.008	1.273	1.326
LAA_f	1	0.596a	0.011	0.561	0.631
	4	0.581a	0.011	0.545	0.616
	6	0.573a	0.030	0.477	0.669
	3	0.536a	0.011	0.501	0.570
	2	0.315b	0.011	0.280	0.349
	5	0.292b	0.011	0.257	0.327
HAA_d	4	38.710a	0.243	37.936	39.484
	5	33.568b	0.325	32.534	34.602
	6	32.829b	0.238	32.070	33.588
	2	27.301c	0.073	27.068	27.533
	3	26.800d	0.087	26.522	27.078
	1	15.902e	0.103	15.573	16.231
LAA_d	4	7.531a	0.143	7.075	7.988
	6	7.395ab	0.388	6.159	8.630
	1	7.294a	0.135	6.863	7.724
	3	5.468b	0.111	5.114	5.822
	2	3.485c	0.117	3.112	3.859
	5	3.391c	0.127	2.988	3.794

Table 19. Mean TAA (µmol TE/g sample) in different batches of tomatoes grown under 100% light intensity. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	100% Light Tomato Sample	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	4	3.565a	0.022	3.523	3.608
	5	3.178b	0.030	3.119	3.236
	3	3.162b	0.014	3.135	3.189
	6	3.117b	0.035	3.048	3.186
	2	2.778c	0.013	2.754	2.803
	1	1.895d	0.014	1.868	1.922
Dry weight	4	46.241a	0.282	45.688	46.794
	6	40.223b	0.456	39.330	41.116
	5	36.959c	0.349	36.275	37.643
	3	32.268d	0.141	31.991	32.545
	2	30.786e	0.138	30.515	31.057
	1	23.196f	0.170	22.862	23.529

Table 20. Mean HAA and LAA (µmol TE/g sample) in different batches of tomatoes grown experimentally in 50% light intensity. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

	50% Light Tomato Sample	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	3	2.063a	0.009	2.034	2.091
	2	1.999b	0.012	1.961	2.037
	1	1.887c	0.006	1.867	1.906
LAA_f	1	0.524a	0.008	0.497	0.550
	3	0.461b	0.005	0.445	0.476
	2	0.373c	0.011	0.337	0.408
HAA_d	2	31.906a	0.193	31.292	32.520
	3	25.154b	0.110	24.805	25.503
	1	20.092c	0.065	19.886	20.299
LAA_d	2	5.948a	0.177	5.386	6.509
	3	5.617a	0.062	5.420	5.814
	1	5.578a	0.090	5.293	5.863

Table 21. Mean TAA (µmol TE/g sample) in different batches of tomatoes grown in 50% light intensity. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	50% Light Tomato Sample	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	3	2.523a	0.010	2.503	2.543
	1	2.411b	0.010	2.390	2.431
	2	2.372b	0.016	2.340	2.404
Dry weight	2	37.854a	0.262	37.341	38.367
	3	30.771b	0.126	30.524	31.018
	1	25.670c	0.111	25.453	25.887

Table 22. Mean HAA and LAA (µmol TE/g sample) in different batches of tomatoes grown experimentally in 25% light intensity. Subscripts represent fresh (f) and dry (d) weight. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

	25% Light Tomato Sample	Mean	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper bound
HAA_f	2	1.769a	0.021	1.703	1.835
	4	1.439b	0.006	1.421	1.457
	3	1.400b	0.009	1.373	1.427
	1	1.381b	0.014	1.336	1.425
LAA_f	3	0.326a	0.007	0.304	0.348
	4	0.252b	0.007	0.229	0.274
	1	0.241b	0.008	0.216	0.266
	2	0.191b	0.014	0.146	0.236
HAA_d	2	25.772a	0.304	24.805	26.738
	1	25.549a	0.255	24.737	26.361
	3	24.740a	0.150	24.264	25.216
	4	16.019b	0.064	15.816	16.223
LAA_d	3	5.764a	0.119	5.386	6.142
	1	4.462b	0.145	4.002	4.922
	4	2.797c	0.079	2.544	3.050
	2	2.782c	0.210	2.114	3.451

Table 23. Mean TAA (µmol TE/g sample) in different batches of tomatoes grown in 25% light intensity. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh or dry weight).

	Batch	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	2	1.960a	0.025	1.911	2.009
	3	1.727b	0.011	1.705	1.748
	4	1.691b	0.009	1.673	1.709
	1	1.622c	0.016	1.590	1.653
Dry weight	3	30.504a	0.191	30.129	30.879
	1	30.011ab	0.293	29.436	30.586
	2	28.554b	0.369	27.830	29.278
	4	18.817c	0.102	18.617	19.016
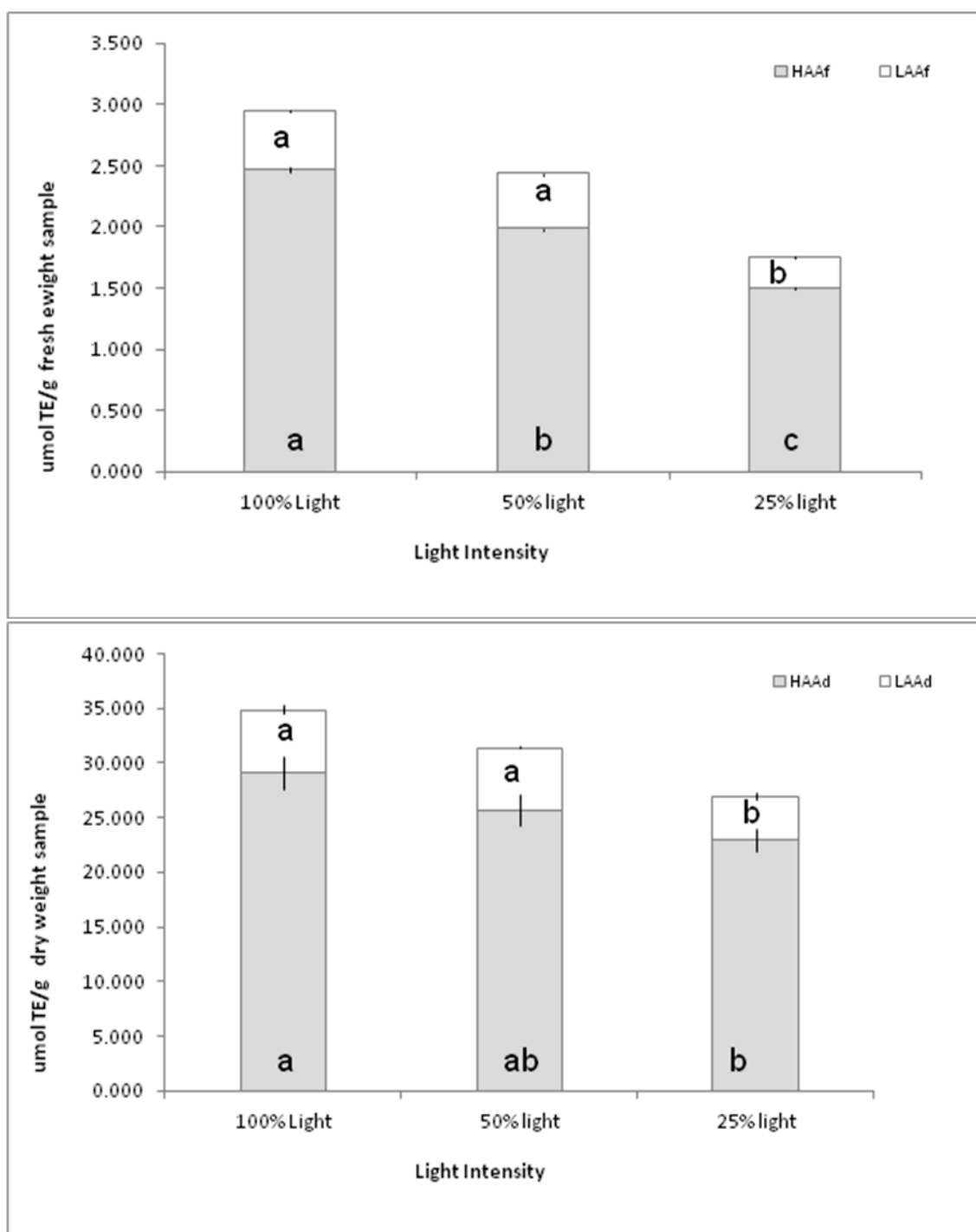
Figure 7. Mean HAA and LAA (µmol TE/g sample) in tomatoes grown experimentally under different light intensities (100%, 50%, and 25%). Letters "f" and "d" following "HAA" and "LAA" represent fresh (top panel) and dry (bottom panel) weight, respectively. Different letters indicate significant differences at 95% confidence, within the same analysis (HAAf, LAAf, HAAd, or LAAd).

Table 24. Mean TAA (µmol TE/g sample) in tomatoes grown under different light intensities. Different letters following the means indicate significant differences at 95% confidence, within the same analysis (fresh weight or dry weight).

	Light Intensity	Mean TAA	Standard Error	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Fresh weight	100% Light	2.949a	0.119	2.717	3.182
	50% light	2.435b	0.030	2.377	2.493
	25% light	1.750c	0.043	1.665	1.835
Dry weight	100% Light	34.945a	1.545	31.917	37.973
	50% light	31.432ab	1.463	28.564	34.299
	25% light	26.971b	1.103	24.810	29.133

<u>DISCUSSION</u>

Different brands and types of canned tomatoes were analyzed to determine the effect of processing on the antioxidant content of final products. Analysis of diced tomatoes of different brands showed significantly different antioxidant activities (Table 1, Table 2). Tomato juices (Table 3, Table 4) had almost three times more HAA than diced tomato, most likely due to the addition of vitamin C, a hydrophilic antioxidant, during the bottling process. The LAA of these juices, however, were similar to that of diced tomatoes. Tomato paste showed a very large range in antioxidant activities across all four brands (Table 5, Table 6), showing statistically significant differences not only between brands but also among different batches of the same brand, especially in HAA. Tomato sauces also exhibited statistically significant differences between different brands and among different batches of the same brands (Table 7, Table 8). Interestingly on a dry weight basis, sauces had a HAA range (8.690 to 19.731 μmol TE/g) very close to its LAA range (8.491 to 19.761 μmol TE/g). HAA and LAA in tomato soups (Table 9) were significantly different across different brands. TAA calculation indicated lower variation, still significantly different, among different brands of tomato soup (Table 10). Overall, variations in antioxidant activities existed between different brands and types of processed tomato products and more importantly between different batches of the same brands (Table 11, Table 12). On the fresh weight basis, HAA in tomato pastes were significantly different from and higher than other processed tomatoes, which were not significantly different from each other; LAA showed more variations among different processed tomatoes. On the dry weight basis, diced tomato, tomato sauce, and tomato

puree (group 1) are not significantly different; however group 1, tomato soup, and tomato paste are significantly different from each other (Table 12).

Similar results were observed in a study conducted by Rao et al. (1998), mainly on lycopene (a lipophilic antioxidant) content, indicating that paste had the most lycopene activity (343.1 ppm), followed by puree (196 ppm), sauce (156.7ppm), juice (116.9 ppm), soup (75.8 ppm), and lastly, cocktail (43.3 ppm). All variations observed in samples, with moisture content taken into account, could be due to many factors such as types, origin and growth conditions of tomatoes used, processing of products, and additives such as herb and spices added to enhance flavors. Tomato paste, typically containing no seeds or skin, is highly concentrated therefore is expected to have the highest antioxidant activity compared to other commercial tomato products. The mean TAA in tomato paste was 9.763 µmol TE/g fw (75.412 µmol TE/g dw) (Table 12), which was significantly different from other processed tomatoes. High LAA activity in tomato paste could be due to the heating process involved in making tomato paste. Thermal processing has been observed to increase bioavailability of lycopene content (Shi et al. 1999). Tomato puree and sauce are less concentrated than tomato puree and therefore are expected to have lower antioxidant activities than paste. Tomato sauce is usually processed using diluted tomato paste or puree therefore is expected to have lower antioxidant activities than either product. However, tomato sauce usually contains additives such as onion, garlic and spices to impart more flavors to the sauce. Herbs and spices are known to have antioxidant activities (Carlsen et al. 2010). Therefore flavoring with herbs and spices should increase antioxidant content of sauces, as observed in results where there were no significant differences between TAA in tomato puree (26.270 µmol

TE/g fw) and tomato sauce (32.179 µmol TE/g fw) (Table 12). Diced tomato and tomato soup are highly diluted, therefore, as observed, should have lower antioxidant activity (TAA 22.498 and 12.874 µmol TE/g dw in diced and soup, respectively) compared to tomato paste, puree and sauce. Lower antioxidant activity in diced tomatoes compared to tomato soup could be a result of leaching of antioxidant, especially LAA, from tomato tissue into the liquid portion. Analysis of the liquid portion in diced tomatoes indicated almost three times more LAA (2.129 µmol TE/g fw) than in solid content (0.710 µmol TE/g fw).

Analysis of commonly consumed whole commercial tomatoes (cherry, grape, roma, and slicer) indicated high variation between batches of the same types of tomatoes. Variation is broadest for roma tomatoes, with TAA ranging from 12.741 to 56.907 µmol TE/g dw (Figure 4, Table 15), and slicer tomatoes, with TAA ranging from 28.80 to 43.86 µmol TE/g dw (Figure 5, Table 16). Variations could be due to different degrees of ripeness (Arias et al. 2000, Cano et al. 2003, Ilie et al. 2009, Horchani et al. 2010), of tomatoes when analyzed. Statistical analysis of TAA indicated significant differences across all four different types of tomatoes (Table 17). Cherry and grape tomatoes contained significantly more TAA than slicer and roma tomatoes per gram fw while there were significant differences between all four types per gram dw. In a consumer's perspective, cherry and grape tomatoes should be preferred, to maximize the amount of antioxidant consumed per serving, followed by slicer, then roma.

Overall, data on commercially produced products showed high variations among similar and between different tomato products. These variations could be due to the way products were processed prior to canning, other ingredients added, or different

environmental conditions in which whole tomatoes were originally grown.

Environmental effects have been found to greatly affect antioxidant accumulation in many different plant foods. For example, Horchani et al. (2010) found that root hypoxia greatly reduced lyopene and β-carotene contents in fruits, portraying the importance of proper irrigation during fruit maturation. Arias et al. (2000) analyzed on-vine and off-vine ripened tomatoes and observed that on-vine ripened tomatoes exhibited higher antioxidant activities than off-vine ripened tomatoes. Therefore, it is important to thoroughly study and not overlook the impact the environment has on antioxidant accumulation.

As part of this research, the effect of an environmental factor, light intensity, on antioxidant activities in tomatoes was investigated by growing tomato plants in 100%, 50%, and 25% natural light. Results indicated variation within the same light intensity as well as between different light intensities. TAA in samples from similar treatment were significantly different from each other (Table 19, Table 21, Table 23). Significant differences observed within each treatment imply that other factors, such as plant to plant variation and fruit location relative to the plant (i.e., top of plant or bottom), may have affected the amount of antioxidant activities observed.

Differences were also observed between all three treatments in terms of TAA for fresh weight. Tomatoes from 100% light (2.949 µmol TE/g fw), 50% light (2.435 µmol TE/g fw), and 25% light (1.749 µmol TE/g fw) were significantly different from each other (Figure 7). Differences observed in TAA fresh weight could be due to how the shadings were set up. Per observation, plants under the shade were growing much taller, greener, and producing more fruits than plants in 100% light. Shading materials seemed

to have helped retain soil moisture throughout the day, preventing plants from drying, allowing them to grow healthier, and producing more fruit. Additionally, tomatoes that were picked from the shade were not as ripe as those from 100% light. Degree of ripeness of tomatoes when picked has been found to affect antioxidant capacity (Arias et al. 2000); therefore lower TAA in tomatoes grown in the shade should not be unexpected.

Although many variables, i.e. soil, seeds, and water, were taken into account in setting up the experiment, moisture retention in the shades was not expected, adding another variable to the experiment. Thus, future experimentation could include growing tomato plants indoors under conditions where moisture can be controlled. Additionally, plants in the shade were producing more fruit than full light. Would plants that have more tomatoes to send its resources be spreading itself thin among the fruit? Will those fruits end up having less nutrients because nutrients have to be shared among too many? Limiting the number of flowers allowed to set could control this variable. This would limit the number of fruit produced and thus rule out variability in resource allocations within plants. Lastly, as mentioned earlier, tomatoes that were analyzed across three treatments were picked after certain number of days post anthesis, not based on their ripeness. Since ripeness plays an important role in lycopene activity (Arias et al. 2000), analyzing tomatoes that are at similar ripeness stages, in addition to days post anthesis, would generate a more comparable analysis of those tomatoes.

Overall despite the variations in antioxidant activities, tomatoes and tomato products did exhibit antioxidant activities and are proven to be rich sources of antioxidants. Although statistical differences were observed between different types of tomato products, whether the degree to which these statistical differences are biologically

different should be further investigated. Research has been ongoing to study the correlation between consumption of antioxidant-rich fruit and vegetables and diseases, especially the antioxidant lycopene, which is high in processed tomato products, on cardiovascular diseases. Biddle et al. (2012) demonstrated that higher dietary lycopene intake is associated with longer cardiac event-free survival compared to lower lycopene intake. Consumption of n-3 polyunsaturated fatty acids (PUFA)-enriched tomato juice indicated stronger positive amelioration of cardiovascular disease risk factors, suggesting a synergistic action of n3 PUFA and tomato juice (Garcia-Alonso and Jorge-Vidal 2011). A study on the protective effect of consumption of lycopene-rich tomato paste on erythema demonstrated 40% lower ultraviolet light-induced erythema formation at 10 weeks (Stahl et al. 2001). Moreover, consumption of tomato juice has been associated with reduced inflammation in overweight and obese females, suggesting a useful approach for reducing the risk of inflammatory diseases associated with obesity (Ghavipour et al. 2012).

This current investigation has demonstrated that fresh tomatoes such as cherry and grape tomatoes are rich sources of antioxidants, on a fresh weight basis, compared to roma and slicer tomatoes. Results also indicated that commercial products such as tomato paste and sauce can be important sources of antioxidants. Despite the variations in antioxidant activities, tomatoes are still important source of antioxidants and therefore should prove valuable to dietitians and consumers concerned about the dietary intake of antioxidants.

APPENDIX

Average moisture content of all samples analyzed. Moisture content was calculated by using the formula (fresh weight – dry weight) / (fresh weight) x 100. Number in parenthesis represents the number of batches that was sampled. Each batch consisted of three measurements.

Tomato Sample	Moisture Content		
	Low	High	Average
Cherry (6)	89.138	93.793	92.251
Diced (4)	89.616	93.537	91.883
Grape (5)	91.612	94.803	93.193
Paste (8)	68.984	91.839	81.172
Puree (1)	91.324	91.378	91.327
Roma (5)	94.422	97.439	95.441
Sauce (12)	83.665	95.618	90.458
Slicer (8)	93.886	96.749	94.986
Soup (4)	78.129	87.329	82.483

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