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Effect of Adding Heat-Stressed Tomato Juice on Some Buffalo Milk Properties

Ali M. Abd-Elkader [a,b], Mohamed Salama [c] and Shimaa Y. Oraby [d]*

[a] *Agricultural Botany Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*
[b] *College of Resources and Environment, Huazhong Agricultural University, Wuhan, Hubei, China.*
[c] *Dairy Department, National Research Centre, 33th El- Bohouth St., Dokki, P.O. 12622, Giza, Egypt.*
[d] *Soil Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.*

Abstract

Abiotic stresses especially heat, greatly affect the growth and development of tomato fruits and thus affect the final components of these fruits that increase the production of heat shock protein, phenolic compounds and antioxidants. These new compounds may enhance food quality under these conditions. Therefore, heat treatment may appears to be one of the most promising methods for postharvest control of quality. The aim of this study is utilization of tomato juice to produce a drink with high nutritive value and enhancing flavour and colour to enhance its acceptability among children and develop a novel product by adding heat-stressed tomato juice into the milk for better nutrition. Tomato juice was collected from different Incubation periods (1, 2, 4 and 8 days) in one bottle. Afterwards, milk formulations with 10, 15, 20 and 25 % tomato Juice were prepared. Characteristics of tomato fruits were assayed included antioxidant activity, total phenolic compounds, ascorbic acid and lycopene. Milk samples were assayed included gross composition, elements and viscosity. Heat stress treatment on tomato fruits resulted in higher contents of ascorbic acid, phenolic compounds, carotenoids and antioxidant activity in tomato juice. The application of add tomato juice to milk formulations leading to increase in the resulting acidity, accompanied by a rise in the viscosity values while decrease fat content. On the same side, the heat treatment led to the production of new proteins. These proteins were added to milk, accompanied by a decrease in the value of the total protein due to adding juice with different ratios.

Key words: Tomato juice; heat stress; heat shock protein; buffalo milk; bioactive compounds.

1. Introduction

Tomato and tomato-based products are considered healthy foods for several reasons. It contains a wide range of beneficial nutrients and antioxidants, including vitamins A and C, lycopene, folic acid, β-carotene and lutein [1]. On the same trend, Igile *et al.* [2] indicated that tomato referred to as a functional food where not only providing basic nutrition but also providing essential elements and vitamins necessary for well-being, preventing chronic diseases, and delivering other health benefits. Based on these compounds that are found in tomato and tomato-based products, consumers have tended to these functional foods with more antioxidants, minerals, vitamins, natural colorants and free of synthetic additives [3]. So there is a major trend for consumers to purchase foods, which provide excellent nutrition and health benefits, especially those that can prevent disease and maintain health. Thus, the development of foods that promote health and well-being is one of the key research priorities of food industry [4].

From a healthy point of view, tomato and tomato-based products include improved eyesight, good stomach health, reduced blood pressure, and relief from diabetes as well as aging skin and urinary tract infections. In addition, tomato increase digestion, stimulate blood circulation, reduce cholesterol levels, improve fluid balance, protect the kidneys, detoxify the body, heals open wounds and sores, prevent premature aging and reduce inflammation and related conditions [5].

Tomato fruits are rich in carotenoids, representing the main source of lycopene in the human diet [6]. During ripening, the concentrations of carotenoids increase by 10-fold due to the accumulation of lycopene that increases as the fruit matures [7]. The

*Corresponding author e-mail: shaimaa_orabi@agr.asu.edu.eg; (Shimaa Y. Oraby).
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lycopene content of tomato varied between 70-130 mg/kg depending on the variety, geographic location, technique of cultivation, climatic conditions and degree of ripeness. Meanwhile, stability of lycopene depends on the manufacturing process and the type of food, which added. Regarding epidemiological studies, lycopene may play an important role in the prevention of diseases like cancer, cataracts, and chronic heart diseases [8, 9].

Regarding heat stress of tomato, it can be used to inhibit ripening processes, induce resistance to chilling injury and decrease external skin damage during storage, in order to extend shelf-life and increase marketability [10]. Heat stress rapidly induces a set of heat stress protection genes like those encoding heat shock proteins (HSPs) and heat shock factors (HSFs), to very high levels [11]. HSPs are involved in regulation of the general heat shock response by stabilizing or renaturing a variety of proteins denatured by heat stress to maintain the cell membrane integrity [12].

The Heat Shock Proteins are considered bioactive compounds and synthesized as a result of high temperature and other stresses in the vegetative tissues of plants [13, 14]. In addition, HSPs can be induced at a particular time during specific stages in plant life cycle such as embryogenesis and germination [15], fruit development and ripening [16] as well as anther and pollen development [17]. These bioactive compounds possess a wide range of physiological properties, including anti-inflammatory, anti-allergenic, vasodilatory, antithrombotic, cardio-protective, antimicrobial, and antioxidant effects [18].

Over the past decades, addition of tomato juice to milk maybe some changes occur in the final product. After heating milk, it easily coagulates in response to citric, ascorbic and tartaric acids that are contained in tomato juice. Moreover, tomato fruit contains pectin, which can prevent milk from coagulation [19]. Recently, it is given a considerable attention for promoting this hopeful crop; production and quality to meet the gradual request of local fresh markets, medicinal purposes, developing processing industry [20]. Moreover, it is friendly considered to dairy products for their nutritional characteristics and attractive colour and appearance. In addition, tomato is used in the production of many popular dishes for instance, functional processed cheese [9], yogurt [20] and ice cream [3].

Therefore, the of this study is utilization of tomato juice contains bioactive compounds to produce a drink with high nutritive value and enhancing flavour and colour to enhance its acceptability among children and develop a novel product by adding heat-stressed tomato juice that contains HSPs into the milk for better nutrition and good health.

2. Materials and Methods

Raw materials

Tomato cultivar F123 was grown in open field plot at Ain Shams University, and fruits have been harvested before the full red stage. After washing and drying, fruits were then placed in temperature-controlled chamber at 38 – 40 °C where the heating treatment was done.

Egyptian buffalo raw milk was obtained from the Dairy department, Faculty of Agriculture, Cairo University.

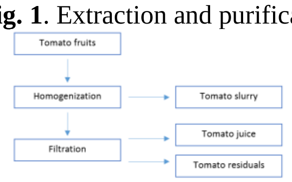
Preparation of tomato juice

As shown in **Fig. 1** and **Fig. 2**; at first, tomatoes with comparable maturity, size, shape and redness were visually selected. Afterwards, the samples were weighted and then homogenized using a kitchen blender (Braun) and filtered using a cheesecloth. The tomato juice and residuals were transferred into a sterilized glass bottle and then stored at 4 °C until further analysis.

Preparation of milk formulations

Tomato juice was collected after different Incubation periods (1, 2, 4 and 8 days), and then all collected tomato juice was mixed in one bottle. After that, milk formulations were prepared by adding 0% (control), 10 %, 15 %, 20 % and 25 % from collected tomato Juice.

Fig. 1. Extraction and purification of tomato juice.



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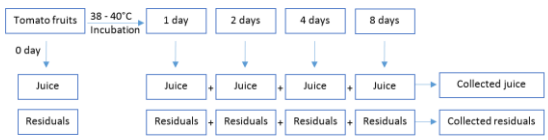

Fig. 2. Schematic diagram of experiment procedure.

METHODS

Gross composition and pH values of samples

Gross composition of milk samples include moisture, total solids, fat, protein and ash contents were determined as mentioned by AOAC [21]. Lactose content was determined by subtracting the percentage of other components (moisture, fat, protein, ash) from 100.
Fruits weight, water content, protein and ash contents of tomato fruits was determined according to the method of AOAC [21].
The pH values of samples were measured by using a laboratory digital pH –meter equipped with glass electrode (Model HI 93 1400, Hanna instruments).

Determination of antioxidant capacity

The measurement of antioxidant capacity was determined according to the method of Ilahi *et al.* [22] with slight modification. A stock solution of 500 ppm of ascorbic acid in methanol was prepared and used as standard antioxidant for antioxidant activity. From the stock solution of ascorbic acid, triplicates of 5 ml solution each of [10, 20, to 80] ppm concentrations were prepared in separate test tubes by using the formula of serial dilution C1V1 = C2V2. Another triplicate set where methanol was used with no ascorbic acid or extract, served as the control. In addition, 5 ml of each of the tomato extracts was pipetted in triplicates into separate test tubes. 1 ml of stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) was added to all test tubes, followed by incubation for 30 minutes in the dark at room temperature. Absorbance was recorded using a UV-Visible spectrophotometer at 517 nm. The difference in absorbance between the test solution and the control (DPPH in methanol) was calculated and expressed as percent inhibition of DPPH radical by using the equation:
Percent inhibition = (Ac-As)/Ac*100)
Whereas, As; is the absorbance of the test solution and Ac; is the absorbance of the control.

Ascorbic acid determination

Determination of ascorbic acid was achieved according to the method as mentioned before by [22]. Standard ascorbic used in the DPPH solution of methonolic DPPH calibration plot (Antioxidant activity) was used, and the ascorbic content was calculated as:
Ascorbic acid content = 100*(V*T)/W
Where: V= ml dye used for titration of aliquot of diluted sample, T= Ascorbic acid equivalent of dye solution expressed as mg/ml of dye, W= gram of sample aliquot that will titrated.

Determination of lycopene

Lycopene in tomato samples was extracted using hexane: ethanol: acetone (2:1:1) (v/v) mixture following the method of Alda *et al.* [23], with some modifications. 1 g of collected tomato juice and 0.1 g of collected residuals were added to 8.0 ml of solvent mixture, then capped and vortexes, followed by incubation in a dark cupboard for 60 minutes. 1 ml of distilled water was added to all samples and vortexes once more then allowed to separation into phases. The cuvette was rinsed with the upper layer of one of the blank samples before using more fresh blank samples to zero the spectrophotometer at 503 nm. Absorbance of the upper layers of the lycopene samples were measured at same wavelength of 503 nm. Lycopene levels of the extracts were calculated as follows:
Lycopene (mg/kg fresh weight) = (A503*537*8*0.55) / (weight of sample g *172)
Where A503= absorbance at 503 nm, 537= the molecular weight of lycopene (g/mole), 8= the volume of mixed solvent, 0.55= the volume ratio of the upper layer to the mixed solvents and 172= the extinction coefficient for lycopene in hexane (mM−1).

Determination of total phenolic content

The total phenolic content in tomato juice was evaluated according to the method [24]. The total phenolics contents of samples were analyzed by the Folin-Ciocalteu colorimetric method. Briefly, the appropriate dilutions of extracts were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue colour was measured at 760 nm. Gallic acid was used as standard, and results were expressed as mean (mg of gallic acid equiv/100 g of edible parts of sample).

Minerals analysis

Minerals were determined after the samples were firstly digested with nitric acid and perchloric acid. Afterward, the filtered aliquots were used for determination of sodium, potassium, calcium, magnesium, phosphorus and iron contents. Potassium and sodium were determined by the Flame photometric method. Iron, calcium and magnesium were determined by atomic absorption spectrophotometric method described by AOAC [21].

Viscosity determination

Viscosity was determined using digital Brookfield Viscometer (Model LVDV-E, Brookfield Engineering Laboratories, Inc. USA), using ULA (ultra low adapter) unit that attached to the viscometer. Milk samples (20 ml/20 °C) were filled in the sample chamber and the spindle was assembled to the viscometer. The speed of spindle was adjusted to 50 rpm. The viscosity reading was recorded as centipoise (cp).

3. Results and Discussion

Characteristics of tomato fruits

Characteristics of tomato fruits are represented in **Table 1**. As expected; there were significant differences in fruit weight, water content, ash, protein, antioxidant activity (DPPH), total phenolic compounds contents and pH between tomato fruits within 0, 1, 2, 4 and 8 days. Their average values were 113.5, 104.7, 102.5, 97.9 and 86.4 g for fruits weight, while they were 95.80, 95.69, 95.29, 95.38 and 95.13 % for water content of tomato fruits during 0, 1, 2, 4 and 8 days, respectively. The average values of ash contents were 0.67, 0.64, 0.62, 0.60 and 0.59 %, while they were 0.80, 0.60, 0.54, 0.58 and 0.55 % for protein contents during 0, 1, 2, 4 and 8 days, in the same order. Regarding antioxidant activity, their average values were 53.32, 72.28, 88.10, 76.53 and 63.27 % whereas they were 0.077, 0.115, 0.123, 0.120 and 0.165 mg/ml for total phenolic compounds contents within 0, 1, 2, 4 and 8 days, respectively. Moreover, **Table (1)** also revealed that the average values of pH of tomato fruits during 0, 1, 2, 4 and 8 days were 4.05, 4.07, 4.15, 4.19 and 4.28, in the same order. These results are in agreement with the results of Ilahy *et al.* [25] who concluded that tomato fruits are rich in vitamins C and E, lycopene, and total phenolic compounds contents. Likewise, our data are in accordance with the results of [26] who found that weight loss increased during storage and was exacerbated by heat treatment and higher storage temperature. They mentioned that fruits heated at 34 °C for 24 h and stored at 4 °C had a final weight loss of 8.8 g/kg, while those stored at 10 °C had a weight loss of 9.8 g/kg. Further, fruits heated at 38 °C for 24 h and stored at 4 °C had a final weight loss of 10.5 g/kg, while those stored at 10 °C had a final weight loss of 11.2 g/kg. Nasir *et al.* [27] reported that tomato possessed a high content of water ranges from 93-95% and total solid about 5.5-9.5%.

On the same side, Mehanna *et al.* [9] reported that the content of free radicals scavenging activity were 80.28, 81.75 and 83.33 % for processed cheese spread with 10, 20 and 30 % tomato juice, respectively. They also mentioned that total phenolic compounds contents were 7.9, 9.8 and 10.1 mg/100 g for processed cheese spread with 10, 20 and 30 % tomato juice, in the same order.

In this regard, Iwahashi and Hosoda [28] delayed the ripening of tomato fruits for several days by heat stress at 37-42°C. They analysed the proteins of tomato fruit pericarp, which were decreased by heat stress. It caused about 23.7 % of the proteins in the pericarp to disappear and about 1.1 % of new proteins to appear.

Acid taste in tomato is attributed to citric and malic acid which constitute together more than 90% of the total pool of organic acid in harvestable fruits [29]. Our data are not in accordance with Ruan *et al.* [30] who reported that a pH of near 6.0 for the pressure-exuded sap of tomato fruits harvested during the active growth phase (25 days postanthesis).

Gross composition of buffalo milk

Gross composition and pH value of buffalo milk are displayed in **Table 2**. The average values of moisture were 83.1, 16.9, 6.2, 3.9, 6.0 and 0.8 % for buffalo milk, in the same order. Moreover, the average values of pH for buffalo milk was 6.62. These data are not in accordance with Kapadiya *et al.* [31] who mentioned that the fat, total solids, protein, lactose and ash contents of buffalo milk were 8.3, 18.4, 4.4, 4.8 and 0.81 %, respectively. On contrary, these results are in agreement with the results of Rasheed, Qazi [32] who found that the fat and protein contents of buffalo milk were 6.2 and 4.0, respectively. Although, our data are not in agreement with the same author regarding ash content and pH where their valuesc were 0.88 % and 6.07, in the same order. Likewise, our data are in accordance with the findings of Abd El-Salam and El-Shibiny [33], where the chemical composition of buffalo milk was close to the results of this study.

Table 2: Gross composition (%) and pH value of buffalo milk

Gross cmposition	Buffalo milk
Moisture	83.1
Total Solids	16.9
Fat	6.2
Protein	3.9
Lactose	6.0
Ash	0.8
pH	6.62

Table 1: Characteristics of tomato fruits during incubation at 38-40 °C within 0, 1, 2, 4 and 8 days

Characteristics	Incubation periods				
	0 day	1 day	2 days	4 days	8 days
fruits weight (g)	113.5	104.7	102.5	97.9	86.4
Water content %	95.80	95.69	95.29	95.38	95.13
Ash %	0.67	0.64	0.62	0.60	0.59
Protein %	0.80	0.60	0.54	0.58	0.55
Antiradical (DPPH) %	53.32	72.28	88.10	76.53	63.27
Total phenolic compounds (mg/ml)	0.077	0.115	0.123	0.120	0.165
pH	4.05	4.07	4.15	4.19	4.28

Collected tomato juice

Table (3) depict the ascorbic acid, lycopene, antioxidant activity contents and pH value of collected juice. From these data, it is noticed that, residuals of tomato fruits are characterized by high content of lycopene, while filtrate was characterized by the lower content of lycopene. Their average values were 168.97 versus 9.62 mg/kg fresh weight, respectively. Moreover, Table (3) showed that ascorbic acid content of tomato juice was 12.95 µg/ml, while antioxidant activity was 75.81 % and pH was 4.13. The findings of the present study are in accordance with findings of Ilahy, Hdider [25] who concluded that tomato fruits are rich in vitamins C and E, lycopene, and total phenolic compounds contents. On the same side, Dewanto *et al.* [34] reported that tomato fruits after heating at 88 °C, the content of vitamin C slightly decreased from 134 –120 µg/g, whereas the content of lycopene markedly increased from 2.01–5.32 mg/g. This was associated to the thermal disruption of cell walls and the consequent release of lycopene, enhancing its bioavailability. Further, Soto-Zamora, Yahia [26] mentioned that heating of 'Rhapsody' tomato fruit in air at 34 °C for 24 h prior to storage at 10 °C for up to 30 days resulted in the least losses in antioxidant content, while fruit colour developed adequately. On the same trend, Mehanna *et al.* [9] found that free radicals scavenging activity contents were 80.28, 81.75 and 83.33 % for processed cheese spread with 10, 20 and 30 % tomato juice, respectively.

Table 3: Ascorbic acid, lycopene, antioxidant activity contents and pH value of collected tomato juice

Parameters	Content
pH	4.13
Antiradical (DPPH) %	75.81
Ascorbic acid (µg/ml)	12.95
Lycopene	
Juice (mg/kg f.w)	9.62
Residuals (mg/kg f.w)	168.97

Elements contents

Tomato is a great source of essential nutrients, which increases its acceptance besides its aesthetic appeal. As shown in **Table 4**; significant differences were recognized between tomato juice and buffalo milk in elements contents. The average contents of elements for tomato juice were about 187, 12, 14, 8, 16 and 0.13 mg/100 ml for potassium, phosphorus, magnesium, calcium, sodium and iron, respectively. The trend was noticeable for elements of buffalo milk; there were 112, 75, 10, 134, 48 and 0.09 mg/100 ml for K, P, Mg, Ca, Na and Fe, in the same order. These data are not in accordance with data of Abd El-Salam and El-Shibiny [33], who mentioned that the average contents of Ca, P, Mg, Na and K of buffalo milk varied between 153–188 mg/100 ml for Ca, while was 93-145 for P and 8-35 for Mg. Meanwhile, the content of Na ranged from 35 to 56 whereas 92 to 155 mg/100 ml for K. On the same side, these results are in agreement with the results of [9] who demonstrated that the content of Na was 16.82, 16.82 and 16.82, while K content was 1.80, 22.50 and 28.0 mg/kg for processed cheese spread with 10, 20 and 30 % tomato juice, respectively.

Table 4: Elements contents (mg/100 ml) of collected tomato juice and milk samples

Elements	Collected juice	Buffalo milk
Potassium (K)	187	112
Phosphorus (P)	12	75
Magnesium (Mg)	14	10
Calcium (Ca)	8	134
Sodium (Na)	16	48
Iron (Fe)	0.13	0.09

Buffalo milk properties

The properties of buffalo milk formulations are presented in **Table 5**. Noticeably, there were significant differences in the results of formulation with 10 % tomato Juice, formulation with 15 % tomato Juice, formulation with 20 % tomato Juice and formulation with 25 % tomato Juice. From these data, it is observed that formulation with 25 % tomato Juice contained higher values of viscosity and acidity than other formulations. Their average values of viscosity were 2.5 versus 1.9, 2.2 and 2.4 cp for formulation with 10, 15 and 20 % tomato Juice, respectively. Moreover, formulation with 10 % tomato Juice had higher contents of fat, protein, total solids and ash than other formulations. These results are in agreement with the results of [9] who reported that when tomato juice added increased, the acidity was increased (pH decreased). The same authors concluded that fat, protein, total solids and ash contents decreased with tomato juice ratios in buffalo milk increased.

Table 5: Effect of adding juice with different ratios (10, 15, 20 and 25 %) on some buffalo milk properties

Parameters	Milk	١٠ %	١٥ %	٢٠ %	٢٥ %
pH	6.71	6.37	6.19	6.07	5.87
Viscosity (cp) at 50 RPM	1.8	1.9	2.2	2.4	2.5
Fat %	6.2	5.6	5.3	5.0	4.7
Protein %	3.9	3.5	3.3	3.2	3.0
Total Solids %	16.9	15.4	14.7	13.9	13.2
Ash %	0.80	0.75	0.72	0.69	0.66

4. Conclusions

The exposure of incompletely ripened tomatoes to heat stress resulted in the tomatoes had higher contents of antioxidants, carotenoids and phenolic compounds. Addition of tomato juice to milk formulations led to produce a good and acceptable milk with high nutritional and healthy product because contains bioactive compounds. Moreover, the addition of tomato juice increased some element contents like iron and magnesium in the final product.

Significant statement

There is a real interest in the development of tomato fruit juice/milk based functional beverages because they possess taste profiles that are attractive to all age groups and because they are healthy and refreshing foods. Besides being delicious, these beverages are highly nutritious. In addition, it is important to pay attention and focus on the usage of heat-stressed tomato juice with other types of milk and its products in the near future.

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