Quality properties of Husk Tomato Juice and its impact In Stirred Probiotic Yogurt

ABSTRACT: Husk Tomato is a promising nutritious fruit rich in minerals, vitamins, and antioxidant compounds. The objective of this study is to evaluate Husk Tomato Juice (HTJ) constituents, its antimicrobial activity and antioxidant properties. Then utilize it for producing stirred probiotic yogurt (SPY). The main components of fresh HTJ were analyzed using HPLC and antioxidant activity by DDPH test as well as total phenolic compounds. Four treatments of SPY fortified with 20, 30, 40 and 50% (v/v) HTJ were prepared and served as T₁, T₂, T₃ and T₄ respectively. The probiotic bacteria used was *Lb. casei* FEGY9973. Data revealed that the main compounds of HTJ were Sinapic (8.170μg/ml), Protocatechuic (6.344 μg/ml) and Cinnamic (4.660 μg/ml). The content of Fe, Mg, Na and Ca were 1.766, 1.627, 7.436 and 12.14 mg/100 g. Antioxidant activity and total phenolic compounds of fresh HTJ were 68.88% and 319.15 ppm respectively. On the other hand, data revealed that the antioxidant activity of fresh SPY samples were 67.66, 76.93, 69.34 and 69.59% for T₁, T₂, T₃ and T₄ in order, compared to control sample (66.57%). The samples possessed acceptable sensory properties. So, it could successfully produce SPY fortified with 20 and 30% HTJ as a healthy dairy product.

Key Words: Husk Tomato Fruit, Probiotic bacteria, Antioxidant activity, Stirred yoghurt.

INTRODUCTION:

Nowadays, consumers are gradually shifting towards the consumption of natural fruit juice-based-beverages because of their high nutritional values, medicinal importance, and good calorific source over synthetic beverages (Ramadan and Mörsel, 2011 & Hemalatha et al., 2018). Husk tomato (*Physalis peruviana*, *L*.) is an auspicious fruit which known also as golden berry, cape gooseberry, tomatillo and ground cherry are gaining publicity in various markets and regions. It has potential medicinal characteristics, antioxidant properties, high nutritional value owing to its high vitamins content, minerals particularly P, Fe and fibers. (Shabana, 2016). In Egypt; Husk tomato 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 (Mustafa, 2009 and Hemalatha et al., 2018). Yogurt is a coagulated milk product produced from fermentation process by lactic acid bacteria (LAB). It is a healthy food due to its high digestibility and bioavailability (Gahruie et al., 2015 and Muniandy et al., 2016). Probiotic bacteria can tolerate acid & bile, survive in the intestinal tract and exert several beneficial effects on human health. They produce short-chain fatty acids and improve the

intestinal microbial balance, resulting in the inhibition of bacterial pathogens, decreasing of colon cancer risk, promoting the immune system and lowering serum cholesterol levels (Gustaw et al., 2011). Lactose intolerant people can consume yogurt without adverse effects (de Vrese et al., 2015). Presenting a verity of fruit-flavored yoghurt has significantly participated to the consumption of yoghurt from all ages. Fruits may be added to yoghurt formulae as single or blends in the form of refrigerated, frozen, canned fruit, juice or syrup (Matter et al., 2016). Therefore, the objective of this study is to evaluate the quality properties of Husk Tomato as a natural juice. Then utilized this juice in addition to a probiotic strain to prepared stirred probiotic yogurt and assessed the antioxidant and sensory properties of the final product.

MATERIALS AND METHODS: Materials:

Milk: Fresh full fat buffalo's milk (Total solids 16.39, total protein 3.97, fat 6.21 and ash 0.80 %) was purchased from Faculty of Agriculture's farm, Cairo University, Giza. Egypt.

Lactic acid strains:

Lactobacillus delbrueckii subsp. bulgaricus Lb-12 DRI-VAC, provided by Northern Regional Research Laboratory. Illinois, USA; while Streptococcus thermophilus CH-1 and Lactobacillus casei FEGY 9973 (as probiotic strain) were obtained from Chr. Hansen's Lab., Denmark.

Husk Tomato fruits: Fine grade of Husk Tomato (*Physalis peruviana*, *L.*) fruits (Total solids 20.23, total protein 0.78, fat 0.43 and ash 1.38 %) were bought from hyper market from Giza, Egypt.

Microbial strains:

Bacillus cereus B-3711, Bacillus subtilus, Aspergillus flavus 3357 and Saccharomyces cerevisiae
Y-2223 were provided by the Northern Regional Research Laboratory Illinois, USA (NRRL).

Listeria monocytogenes 598 was provided by the Department of Food Science, University of
Massashusetts, Ambert MA, USA. Escherichia coli 0157: H7 and Staphylococcus aureus were
isolated and serologically identified by dairy microbiological Lab., National Research Centre.

Yersinia enterocolitica and Salmonella typhamirum were obtained from Hungarian National
Collection of Medical Bacteria, OKI, Gyaliut 2-6, H-1966 Budapest, Hungary. Aspergillus niger,
Pseudomonas aeruginosa, Pencillium requfortii J5 were obtained from Department of
Microbiology, Swedish University of Agricultural Sciences. Candidia albicans were provided by
the Institute of Applied Microbiology, University of Tokyo, Japan.

Methods of preparation and analysis:

Preparation of Husk Tomato Juice (HTJ): The outer cover had been removed from all fruits and well washed. After that, they put in a blender and well mixed till they were completely homogenized. The produced juice had filtered to separate seeds and skin by cheesecloth. The juice was heated to 85°C/10 min then cooled to 4 °C for utilizing in analyzing its components and preparing probiotic yogurt.

Assessment the antimicrobial activity of fresh Husk Tomato Juice:

0.1ml (10⁹ cells /ml) of the tested strains was grown on trypton soya broth media at 37°C for 24h. For mold and yeast, the strains were grown in malt extract broth at 25°C for 72h, and then spread on the entire surface of the Petri dish using a sterile swab. The disc diffusion method as (Randazzo et al., 2016) was used to determine the antimicrobial activity. The inhibition zones around the paper discs (mm) were measured. Another test of antimicrobial activity was assessment using pasteurized HTJ at 65°C/15 min.

Preparation of Stirred Probiotic Yogurt (SPY):

Fresh buffalo milk with 7% sugar was pasteurized at 75 $^{\circ}$ C/ 10sec ,cooled to 42 $^{\circ}$ C, 2% starter cultures (1:1%) and 1% *Lb. casei* FEGY9973 were added respectively. The fermented milk was rested till complete coagulation within 3 hours at 42 $^{\circ}$ C. (**Marshall and Rawson 1999**). The curd was divided to five portions; the first was control (without juice) while the second, third, fourth and fifth treatments had been blended with HTJ at ratios of 20, 30, 40 and 50% (v/v) and served as T₁, T₂, T₃ and T₄. All samples were put in sterilized bottles near flame, and then stored in refrigerator at (5±2 $^{\circ}$ C) and analyzed when fresh and after 5, 10 and 15 days of cold storage.

Chemical Composition:

Total solids, total protein and ash contents of both HTJ and buffalo milk were determined according to **AOAC (2012).** Sugars in HTJ (Glucose, mannose and sucrose) and in buffalo milk (Lactose & Galactose) were estimated according to **Richmond et al.**, **1987** by using HPLC, Agilent 1200 series.

Determination of Husk Tomato juice components by HPLC:

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was Eclipse XDB-C18 (150 X 4.6 μ m; 5 μ m) with C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programmer was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5

min and 0% B to 100% B in 5 min. The injection volume was 50 μl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 μm Acrodisc syringe filter (Gelman Laboratory, MI) before injection.

Determination of Minerals of Husk Tomato juice (HTJ):

Sodium (Na), potassium (K), magnesium (Mg), iron (Fe), calcium (Ca) and phosphorus (P) were determined with Optima 2000DV inductively coupled plasma spectrometer with full PC control (Perkin Elmer) according to **AOAC (2012).**

Total phenolic content and Radical Scavenging Capacity Assay (RSA):

Total phenolic compounds were determined according to **Zheng& Wang (2001)** using Folin-Ciocalteu reagent and expressed as milligrams of Gallic acid equivalents (GAE) / 100 gm.

The Antioxidant activity of the extract was measured in terms of radical scavenging ability (RSA) using the stable free radical DPPH (**Pothitirat** *et al.*, **2009**).

Sensorial evaluation:

Sensory attribution was carried out by 25 members in Dairy department, National Research Centre. Samples were evaluated in intervals at fresh, 5, 10 and 15 days of storage period. The evaluation degrees were included flavor (50), appearance (10), body and texture (40).

Statistical Analysis:

Statistical analysis of obtained data was carried out using analysis of variance (ANOVA) and Duncan tests with the Statistical Analysis System (SAS, 2004). A probability of P < 0.05 was used to establish the statistical significance.

RESULTS AND DISCUSSION

Sugar contents of Husk Tomato Juice (HTJ) and buffalo milk samples:

Table (1) showed some sugars contents of HTJ and buffalo milk. Data exhibited that Sucrose, Glucose and Mannose contents of the juice were 6.55, 1.14 and 1.06 mg/100ml, respectively. While, lactose and galactose contents of buffalo milk were 30.65 and 0.751 mg/L. Data of buffalo milk sugars was in accordance with **Abbas** *et al.*, 2017.

Table (1): Sugar content of Husk Tomato Juice and buffalo milk.

Items	Husk Tomato Juice	buffalo milk	
Lactose mg/L	-	30.65	
Galactose mg/L	-	0.751	
Sucrose g/100ml	6.55	-	
Glucose g/100ml	1.14	-	
Mannose g/100ml	1.06	-	

Some components of fresh Husk tomato juice by HPLC analysis:

Fresh HTJ were analyzed for its constituents using HPLC. Different components were found at various ratios (Table2). Most of them considered as phenolic and antioxidant components.

Main compounds were Sinapic (8.170μg/ml), Protocatechuic (6.344μg/ml), Cinnamic (4.66μg/ml) and Ferulic (3.090μg/ml). In agreement, **El-Sheikha** *et al.*, (2010) were recognized about 12 phenolic fractions in the methanolic extract of fresh *Physalis* juice. The predominant compounds were phenols followed by ferulic acid. While, **Deng** *et al.*, **2016** isolated some antioxidant fractions like Syringic acid 6-*O*-β-glucuronopyranoside, Quercetin-3-*O*-β-D-glucopyranoside and Caffeic acid.

Table (2): Some main components of fresh Husk Tomato Juice (HTJ) using HPLC.

Compounds	Retention time	Concentrations (µg/ml)
Sinapic	33.0	8.170
Protocatechuic	9.6	6.344
Cinnamic	42.4	4.660
Ferulic	31.6	3.090
P-hydroxybenzoic	14.7	1.341
Rosmarinic	39.5	1.052
Chrysin	51.3	0.856
Apigenin-7-glucoside	38.5	0.128
Caffeic	20.8	0.153
Quercetin	43.1	0.077
Syringic	22.3	0.069
Vanillic	23.9	0.063
Chlorogenic	20.0	0.020
Rutin	35.8	0.080
P-coumaric	36.3	0.060

Minerals compounds and antioxidant activity:

Some mineral contents in fresh HTJ appeared in Table (3). The iron content was 1.766 mg/100g, while Magnesium was 1.6mg/100g. Calcium, potassium and sodium contents were 12.14, 0.53 and 7.436 mg/100g resp. **Ramadan and Moersel in 2011 and Sharma** *et al.*, **2015** displayed that *Physalis peruviana* pulp contained essential minerals (iron, calcium, phosphorus and zinc. Theses minerals have potential health effect on human. The same table reflects also the total phenol contents which were 319.15 GAE and the percent of DPPH was 68.88 %.

Table (3): Some minerals, antioxidant activity and total phenolic contents of fresh Husk Tomato Juice.

Minerals and Vitamins	Concentration
Ca (mg/100g)	12.14
Fe(mg/100g)	1.766
K%	0.53
Mg(mg/100g)	1.627
Na(mg/100g)	7.436
Se(ppm)	1.03
DPPH (%)	68.88
Total phenolic content as Gallic acid equivalent	319.15

Valdenegro *et al.*, (2013) and Yıldız *et al.*, 2015 assayed the antioxidant capacity and total phenolic content in golden berry fruit and they ranged from 57.67 % & 5.84 mmol DPPH/100g and 145.22 mg & 2.46 g GAE/100 g, respectively. As well **Ramadan and Moersel (2008)** illustrated that phenolic compounds are very important for their contribution to the sensory quality of fruits (color, astringency, bitterness and flavor) and technological processes.

Assessment of the antimicrobial activity of Husk Tomato juice:

The Husk Tomato Juice was screened for its antimicrobial activity against eight bacterial and five fungal strains Table (4). The results indicated that HTJ had various degrees of inhibition against the growth of some pathogenic and fungi. The highest antimicrobial activity of HTJ was found against *Y. enterocolitica* and *Ent. faecalies*. While, the lowest activity was recorded against *A. niger* and *List. monoytogenes*. The diameter zone of inhibition ranged from 18 to 25, 6 to 25 and 7 to 17 mm for gram positive & negative bacteria and fungi, respectively. These results came in accordance with a study by **Nathiya and Dorcus (2012)** who tested the antibacterial activity of *Physalis minima L* fruit. They found that the antibacterial activity against *Bacillus cereus*, *Enterobacter aerogenes* and *Staphylococus aureus* was greater with maximum inhibition zone (10.0 mm ± 0.5). *Staphylococcus aureus* was found to be more susceptible with a maximum inhibitory zone of 9.0mm ± 1.0. The results were in the same trend with **EL Sheikha** *et al.*, (2008) who indicated that metabolic extract of juice had significant antimicrobial activity against *E. coli* O15:H7 and *B. subtilus*.

Table (4): Antimicrobial effect of Husk Tomato juice against pathogenic strains.

Pathogenic strains	Husk Tomato juice	pasteurized Husk Tomato juice
	Diameter of inhibition zone (mm)	
Bacillus cereus	19	15
Bacillus subtilus	18	16
Staphylococcus aureus	17	15

Pseudomonas aeruginosa	18	16
Listeria monoytogenes	6	7
Escherichia coli	12	10
Yersinia enterocolitica	25	22
Salmonella typhmirum	22	19
Enterococcus faecalies	25	20
Aspergillus niger	7	6
Aspergillus flavus	12	11
Pencillium requfortii	18	17
Saccharomyces cerevisiae	17	15

Yogurt properties:

Antioxidant Activity of stirred probiotic yoghurt during storage:

Table (5) illustrated antioxidant activity of stirred probiotic yoghurt when fresh and after 5, 10 and 15 days. It was observed that treatment samples which contained HTJ had higher antioxidant activity than control samples in all storage periods. Antioxidant activity was in directly proportional to the ratio of the juice in all treatments. So, treatment (T₄) had significantly (p<0.05) the highest antioxidant activity (70.59%) compared to other samples and control. This activity could be due to the phenolic compounds which existed in Husk Tomato Juice. Presented results were in accordance with Lourens-Hattingh and Viljoen, 2001; Correia *et al.*, 2004. Shori and Baba, (2013) and Nguyen and Hwang (2016).

Table (5): Antioxidant activity (%) of stirred probiotic yoghurt when fresh and during storage.

Storage period	Treatment samples				
(Days)	Control	T ₁	T ₂	T ₃	T ₄
Fresh	66.57 ^{Ca}	67.66 ^{Ba}	67.93 ^{Ba}	69.34 ^{Aa}	69.59 ^{Aa}
5	66.48 ^{Ca}	66.61 ^{Cab}	67.63 ^{Ba}	68.78 ^{ABb}	69.9 ^{Aa}
10	65.39 ^{Cb}	66.7 ^{Bab}	66.79 ^{Bb}	68.2 ^{Abc}	68.79 ^{Ab}
15	64.96 ^{Dc}	65.43 ^{Cb}	66.56 ^{BCb}	67.9 ^{Bc}	68.34 Ab

 T_1 = 20% HTJ T_2 = 30% HTJ T_3 = 40% HTJ T_4 =50% HTJ

Data expressed as mean of 3 replicates. Means in the same row showing the same capital letters are not significantly different ($p \le 0.05$). Means in the same column showing the same small letters are not significantly different ($p \le 0.05$)

Sensorial evaluation:

Data in Fig. (1), represented sensorial evaluation of stirred probiotic yoghurt fortified with different ratios of Husk Tomato juice. No doubt that HTJ gave the new stirred yoghurt a special taste that all panelists favorite it. The most favorable flavor were T_1 and T_2 treatments which contained 20 and 30% HTJ compared with other treatments. Flavor scores gradually increased among the first week for all treatments then decreased till the end of the experiment. During

storage period, the higher ratios of HTJ (40 and 50%) had lower scores for flavor when fresh and after 15 days. This could be due to the high acidity of this juice combined with starter culture. On the other hand, appearance for all samples was insignificant (p>0.05) except after 15 days of storage period for T₃ and T₄; there were low scores of appearance. Body & texture for control, T₁ and T₂ samples had high scores against the other treatments. Control samples had significantly (p< 0.05) gained higher scores for body & texture than treated samples because the juice diluted the stirred probiotic yoghurt according to the ratio of the juice. In comparison with present study; **Abou Ayana** *et al.*, **(2012)** noticed the changes in sensory evaluation of fruity probiotic fermented milk. They showed that the total scores gradually increased in all examined treatments in the first week, and then decreased till the end of storage. Also, they observed negative impact emerged with adding fruit pulp on body and

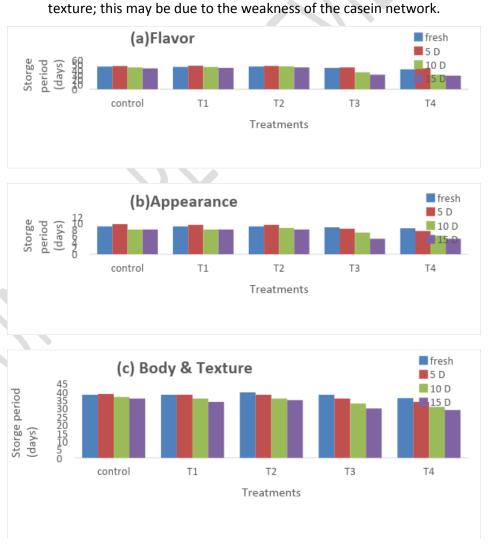


Fig (1): Sensory evaluation of stirred probiotic yogurt during storage.

(a) Flavor (b) Appearance (c) Body & texture.

 T_1 = 20% HTJ T_2 = 30% HTJ T_3 = 40% HTJ T_4 =50% HTJ Future Trends:

Husk Tomato should magnetize great interest because of their nutritional and industrial characteristics. Because of its unique storage properties, wherein the fruits can be kept for a long time, Husk Tomato could be an appropriate plant for various food applications. The development of sufficient agro-technical methods can make this fruit a promising profitable new crop for arid regions. Consequently, further studies must be taken in consideration for applied this fruit in food and dairy processing. Therefore, a novel healthy products can be spreadable in the local markets.

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