

Quality Properties of Husk Tomato Juice and Its Impact in Stirred Probiotic Yogurt

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2019/v7i229962

Editor(s):

(1) Dr. Samaila James, Lecturer, Department of Food Science and Technology, Federal University of Technology, Minna, Nigeria.

Reviewers:

(1) A. Mezaini, Chlef University, Algeria.

(2) Obunukwu Gift Maureen, Imo state University, Nigeria.

(3) Aly Farag El Sheikha, McMaster University, Canada.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/46860>

Original Research Article

Received 02 November 2018

Accepted 29 January 2019

Published 22 February 2019

ABSTRACT

Background: Thinking about something new that is important for consumer health is a great concern to all those interested in the dairy industry and its products. Husk Tomato is a promising nutritious fruit rich in minerals, vitamins, and antioxidant compounds.

Aim: 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) as a novel fermented product.

Methodology: The main components and sugars of fresh HTJ were analyzed using High Performance Liquid Chromatography (HPLC). Antioxidant activity by DDPH (2, 2-diphenyl-1-picrylhydrazil) test as well as total phenolic compounds were also determined. Four treatments of SPY fortified with 20, 30, 40 and 50% (v/v) HTJ & *Lb. casei* FEGY9973 (as probiotic bacteria) were prepared and served as T1, T2, T3 and T4 respectively were compared against control without juice.

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Results: Data revealed that the main compounds of HTJ were Sinapic (8.170 µg/ml), Protocatechuic (6.344 µg/ml) and Cinnamic (4.660 µg/ml). Contents 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. It was found that HTJ had powerful antimicrobial activity against both of *Y. enterocolitica* and *Ent. faecalis*. On the other hand, data revealed that the antioxidant activity of fresh SPY samples were significantly ($p < 0.05$) higher 67.66, 76.93, 69.34 and 69.59% for T1, T2, T3 and T4 in order, compared to control sample (66.57%). While, appearance scores had no significant ($p > 0.05$) for all samples in fresh, 5 and 10 days of storage. All treatment samples possessed acceptable sensory properties.

Conclusion: It could be conducted that SPY fortified with 20 and 30% HTJ had been successfully produced as a healthy dairy product.

Keywords: Husk tomato fruit; probiotic bacteria; minerals content; antioxidant activity; stirred yoghurt; sensory evaluation.

1. 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 [1,2]. Many countries through the world have been detected the magnitude of fermented food as a cheap means of preservation, promoting nutritional quality and consolidating sensory characteristics [3]. Phenolic compounds also had a global attention recently due to antioxidant, anti-inflammatory, anti-mutagenic and anti-clotting power which has been correlated with a declined risk of cardiovascular diseases and cancer development [4]. 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. In Egypt, economical importance of *Physalis* is increase, for achieving a major success in local, Arabic and European markets. Furthermore, 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 [2,5,6]. It has potential medicinal characteristics, such as antipyretic, depurative, diuretic, pectoral, and vermifuge antioxidant and anti-bacterial properties, and high nutritional value owing to its high vitamins content, minerals particularly P, Fe and fibers. Moreover, *Physalis peruviana* is vastly utilized in folk medicine for the treatment of malaria, asthma, hepatitis, dermatitis and rheumatism, reduction of bad cholesterol and glucose level [7,8]. 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.

Fermentation process of LAB is a rich source of bioactive peptides with antioxidant activity, extend the shelf-life and maintain the nutritious components of milk [9,10]. 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 [11]. Lactose intolerant people can consume yogurt without adverse effects [12]. The addition of different fruits to milk may enhance the taste and the therapeutically values of milk products as well. 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 [13].

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 prepare stirred probiotic yogurt and assessed the antioxidant and sensory properties of the final product.

2. MATERIALS AND METHODS

2.1 Materials

Milk: Fresh full fat buffalo's milk 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 were bought from hyper market from Giza, Egypt.

Microbial strains: Different gram positive & negative bacteria, fungi and yeast such as *Bacillus cereus* B-3711, *Bacillus subtilis*, *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 Massachusetts, Amherst 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*, *Penicillium requortii* 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.

2.2 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: 0.1 ml (109 cells /ml) of the tested strains was grown on trypton soya broth media at 37°C for 24 h. 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 [14] 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°C/ 10 sec, cooled to 42°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°C [15]. 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°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 [16]. Sugars in HTJ (Glucose, mannose and sucrose) and in buffalo milk (Lactose & Galactose) were estimated according to Richmond et al., [17] by using High Performance Liquid Chromatography (HPLC), Agilent 1200 series.

2.3 Determination of Husk Tomato Juice Components by High Performance Liquid

Chromatography (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 µm; 5 µm) 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 [16].

Total phenolic content and Radical Scavenging Capacity Assay (RSA): Total phenolic compounds were determined according to Zheng and Wang [18] 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 (2, 2-diphenyl-1-picrylhydrazil) [19].

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).

2.4 Statistical Analysis

Statistical analysis of obtained data was carried out using analysis of variance (ANOVA) and Duncan tests with the Statistical Analysis System SAS [20]. A probability of $P < 0.05$ was used to establish the statistical significance.

3. RESULTS AND DISCUSSION

Chemical composition and Sugar contents of Husk Tomato Juice (HTJ) and buffalo milk samples: Table 1 showed some chemical composition and sugars contents of HTJ and buffalo milk. Husk Tomato Juice had 20.23, 0.98, 0.43 and 2.38 % for total solids, total protein, fat and ash contents respectively. Also, same Table exhibited that Sucrose, Glucose and Mannose contents of the juice were 3.55, 1.14 and 1.06 mg/100ml, respectively. Our results were varied with that found by El-Sheikha et al., [21] who pointed that raw juice contained glucose (2.28 g/L), fructose (2.31 g/L) and sucrose (2.81 g/L) which were lower than in lime juice (6 g/L glucose, 6.1 g/L fructose and 4.8 g/L sucrose). El-Sheikha et al., [21,22] stated that the chemical composition of *physalis* juice were 89.34, 1.02 and 5.58% for moisture, protein and ash, respectively. On the other side, Sharoba and Ramadan [23] declared that golden berry fruit contained 21.00, 16.40, 1.08, 0.84 and 0.32% for dry matter, water soluble dry matter, ash, protein and oil respectively. Also, Yıldız et al., [24] illustrated that the composition of *Physalis peruviana* L. were 18.67, 14.17, 2.98, 1.66, 0.18 and 13.86 %, for dry matter, water soluble dry matter, ash, protein, oil and carbohydrate respectively. While the contents of buffalo milk

were 16.39, 3.97, 6.21 and 0.80 % for total solids, total protein, fat and ash respectively. Lactose and galactose contents of buffalo milk were 30.65 and 0.751 mg/L. Data of buffalo milk chemical composition and sugar contents was in accordance with Abbas et al., [25]. They cleared that the chemical composition of buffalo milk was 16.50, 3.64, 6.30, and 0.78 % for TS, TP, fat and ash contents respectively.

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 (Table 2). As it expected most of them considered as phenolic and antioxidant components. Main compounds were Sinapic (8.170 µg/ml), Protocatechuic (6.344 µg/ml), Cinnamic (4.66 µg/ml) and Ferulic (3.090 µg/ml), Caffeic (0.153 µg/ml), Quercetin (0.077 µg/ml) which considered as flavonols. Same line had been shown by El-Sheikha et al., [22] who clarified the major phenolic compounds of fresh apricot juice. They were ferulic, salicylic and chlorogenic acids, where the predominant compound was resorcinol. Also, El-Sheikha et al., [21,26] were recognized about 12 phenolic fractions in the methanolic extract of fresh *Physalis* juice. The predominant compounds were phenols followed by ferulic acid. They declared that slight decrease in polyphenol compounds was inversely proportional to the non-enzymatic browning reactions which increased slowly throughout the cold storage time. While, Deng et al., [27] isolated some antioxidant fractions like Syringic acid 6-O-β-glucuronopyranoside, Quercetin-3-O-β-D-glucopyranoside and Caffeic acid.

Minerals compounds and antioxidant activity: Some mineral contents in fresh HTJ appeared in Table 3. The content of calcium (62.14 mg/100 ml), iron (1.766 mg/100 ml), potassium (893 mg/ml), magnesium (11.27 mg/ml), sodium (17.436 mg/100 ml) and traces of selenium (1.03 ppm), respectively. These data were close to results which found by El-Sheikha et al. [22]. They conducted that potassium, phosphorus, calcium, sodium, magnesium, zinc, copper, iron and manganese were 1196, 587, 70, 35, 19, 2.4, 1.5, 1.2 and 0.6 mg/100 ml juice, respectively. Ramadan and Moersel [1] and Sharma et al., [28] displayed that *Physalis peruviana* pulp contained essential minerals such as iron, calcium, phosphorus and zinc. These minerals have potential health effect on human. The same table reflected also the total phenol contents

which were 319.15 GAE/100 gm and the percent of DPPH was 68.88%. Our results were higher than the results by Yıldız et al., [24] who demonstrated the antioxidant capacity and total phenolic content in *Physalis peruviana* L. fruit were 57.67% and 145.22 mg GAE/100 g, respectively.

Valdenegro et al., [29] and Yıldız et al., [24] assayed the antioxidant capacity and total phenolic content in golden berry fruit and they ranged from 57.67 % & 5.84 mmol DPPH/100 g and 145.22 mg & 2.46 g GAE/100 g, respectively. As well Ramadan and Moersel [30] 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. faecalis*. While, the lowest activity was recorded against *A. niger* and *List. monocytogenes*. 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. The antimicrobial activity of HTJ could be due to the phenolic compounds which had been found in the juice as mention before by HPLC analysis.

Also, the results mentioned that the activity of HTJ was not affected by pasteurization, it was obvious that pasteurized HTJ were more effective against *Y. enterocolitica*, which had diameter 22 mm of inhibition zone. Otherwise, *A. niger* and *List. Monocytogenes* had less inhibition zone reached to 6 and 7 mm, respectively. The diameter zone of inhibition ranged from 16 to 20 mm and 7 to 22 mm for both of gram positive and negative bacteria respectively, while the diameter zone of fungi recorded 6 to 17 mm. Our results in the same trend with EL Sheikha et al., [22] who showed that metabolic extract of juice had significant antimicrobial activity against *E. coli* O15:H7 and *B. subtilis*. In addition Nathiya and Dorcus [31] confirmed our results by testing the antibacterial effect of *Physalis minima* Linn fruit. They pointed that the antibacterial impact against *Bacillus cerues*, *Enterobacteraero genes* and *Staphylococcus aureus* was greater with maximum inhibition zone (10.0 mm ± 0.5).

Staphylococcus aureus was established to be more susceptible with a maximum inhibitory zone of 9.0 mm ± 1.0.

3.1 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. The decrease in antioxidant activity in control samples was higher than treatment samples either fresh or stored. So, the antioxidant activity could be affected by the mechanical process and the loss of vitamin C due to auto-oxidation. Lourens-Hattingh and Viljoen, [32] Correia et al., [33] displayed fermentation and post-acidification products produced during storage such as organic acid derivative and milk protein proteolysis could be possible sources of DPPH inhibitors. Same line was found by Shori and Baba, [34] when added *Azadirachta indica* which had high ratios of vitamin C and riboflavin to yogurt showed higher antioxidant effect compared to plain yogurt after 28 days of storage. Presented results were in accordance with Nguyen and Hwang [35].

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 two favorable flavor were T1 and T2 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., [36] 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 texture; this may be due to the weakness of the casein network.

Table 1. Chemical composition and sugar contents of husk tomato juice and buffalo milk

Items	Husk tomato juice	buffalo milk
Total Solids (TS) %	20.23	16.39
Total Protein (TP) %	0.98	3.98
Fat %	0.43	6.21
Ash %	2.38	0.80
Lactose mg/L	-	30.65
Galactose mg/L	-	0.751
Sucrose g/100ml	3.55	-
Glucose g/100ml	1.14	-
Mannose g/100ml	1.06	-

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

Compounds	Retention time	Concentrations ($\mu\text{g/ml}$)
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

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

Minerals Content (mg/100 ml juice)	
Calcium	62.14
Iron	1.766
Potassium	893
Magnesium	11.27
Sodium	17.436
Selenium(ppm)	1.03
Antioxidant activity	
DPPH (%)	68.88
Total Phenolic Content	
Total phenolic content as Gallic acid equivalent (GAE)/100gm	319.15

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))	
<i>Bacillus cereus</i> (G+)	19	15
<i>Bacillus subtilis</i> (G+)	18	16
<i>Staphylococcus aureus</i> (G+)	17	15
<i>Pseudomonas aeruginosa</i> (G-)	18	16
<i>Listeria monoytogenes</i> (G+)	6	7
<i>Escherichia coli</i> (G-)	12	10
<i>Yersinia enterocolitica</i> (G-)	25	22
<i>Salmonella typhmirus</i> (G-)	22	19
<i>Enterococcus faecalies</i> (G+)	25	20
<i>Aspergillus niger</i>	7	6
<i>Aspergillus flavus</i>	12	11
<i>Pencillium requfortii</i>	18	17
<i>Saccharomyces cerevisiae</i>	17	15

(G+): Gram positive bacteria, (G-): Gram negative bacteria

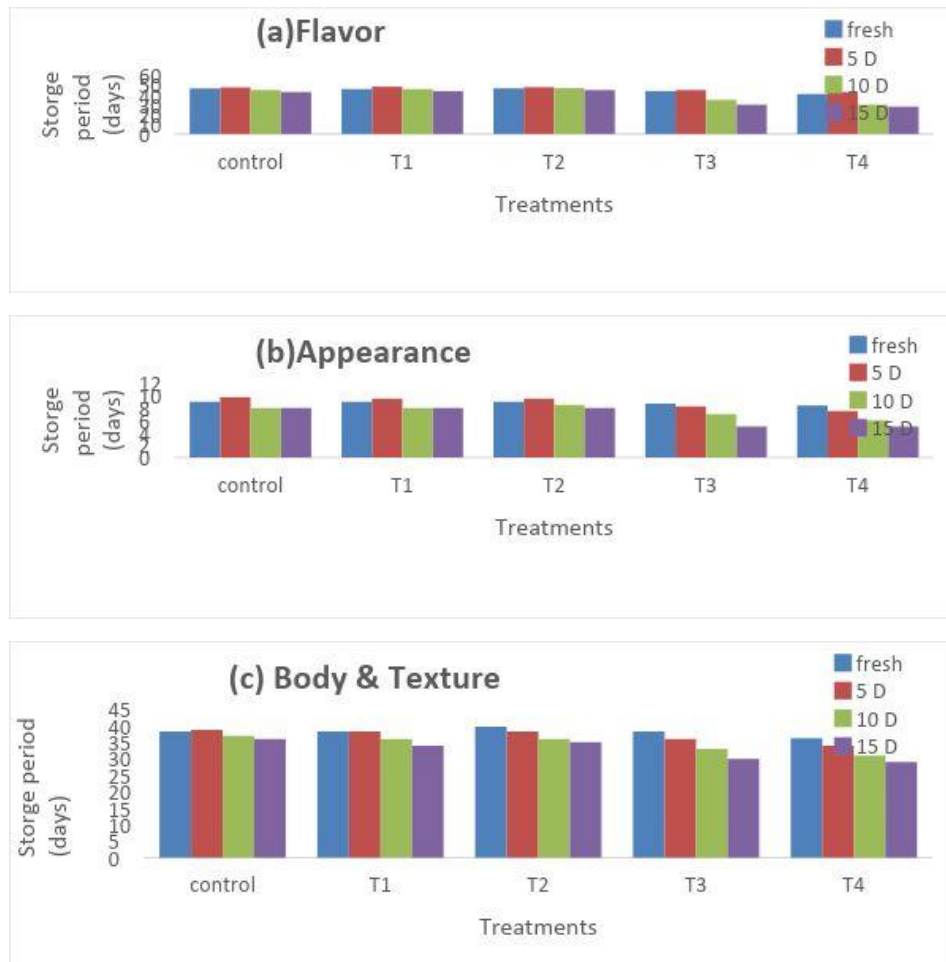


Fig. 1. Sensory evaluation of stirred probiotic yogurt during storage
(a) Flavor (b) Appearance (c) Body & texture
 T1 = 20% HTJ; T2= 30% HTJ; T3= 40% HTJ; T4=50% HTJ

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

Storage period (Days)	Treatment samples				
	Control	T1	T2	T3	T4
Fresh	66.57Ca	67.66Ba	67.93Ba	69.34Aa	69.59Aa
5	66.48Ca	66.61Cab	67.63Ba	68.78ABb	69.9Aa
10	65.39Cb	66.7Bab	66.79Bb	68.2Abc	68.79Ab
15	64.96Dc	65.43Cb	66.56BCb	67.9Bc	68.34 Ab

T1 = 20% HTJ; T2= 30% HTJ; T3= 40% HTJ; T4=50% HTJ

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

4. CONCLUSION

Producing a novel healthy dairy product has got consumer admire is the principle aim of this study. Fortifications with two ratios of Husk Tomato Juice (20 and 30%) in stirred probiotic yoghurt were more favorable to panelists. Also, addition of this juice enhanced the antioxidant and sensory properties of the dairy product.

5. 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 product can be spreadable in the local markets. So, this paper has second part including some important parameters for the juice and the end product as well.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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