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Physicochemical, Antioxidant, Microbiological, and Sensory Properties of Functional Yoghurt Supplemented with Guava Seeds, Leaves, and Pulp Extracts

Doaa F. Hassan[1*], Sahar A. Mohamed[1] and Hisham M. El- Shishtawy[2]
[1]Special Food and Nutrition Research Department, Food Technology Research Institute, Agricultural Research Center, Egypt
[2]Agriculture genetic engineering, Research Institute, Agricultural Research Center, Egypt

PSIDIUM GUAJAVA L., popularly known as guava has many medical benefits especially leaves and it considered as waste and seed was by product of juice industry. The aim of this study to investigate the effect of the phenolic and flavonoid compounds on antioxidant and antimicrobial activity of functional flavored yoghurt supplemented with guava extracts (GE) (seeds, green and red leaves and juice). The results showed that all guava extracts have antioxidant activity using (DPPH, ABTS) methods being (seed 89.18%, green leaves 75.41% and red leaves 71.26%) with high total phenolic compounds (TPC) in green leaves 672.42 and in red 658.90 mg GA/100g. Polysaccharide fraction was higher in red guava leaves extract than green leaves and seed extracts, it consists of inulin (2.04%), sucrose (10.84%), stachyose (0.49%), fructose (0.26%), galacturonic (0.35%), sorbitol (0.14%). Also, guava extracts have antibacterial activity against tested bacteria. Addition of guava extracts to yoghurt especially (pulp, leaves) improved sensory properties, increased total solids and protein content, didn't affect the acidity and pH. All yoghurt samples were protected from growth of spoilage and pathogenic bacteria and improved growth and viability of lactic acid bacteria (LAB). During the cold storage, (TPC) and individual phenolic contents and antioxidant activity of GE-fortified yoghurt gradually and significantly increased ($p<0.05$). Antioxidant activity of GE-fortified yoghurts is highly related to TPC. The results showed that GE can be used as a natural antioxidant ingredient and can be used for the production of functional dairy products to improve nutritional value and biological activity.

Keywords: Guava leaves, seeds, pulp, Physicochemical, Antioxidant, Antibacterial, Yoghurt, sensory properties.

Introduction

Functional foods are foods that are designed to improve health or lower the risk of disease. Foods that contain specific minerals, vitamins, fatty acids, or dietary fibers are examples of functional foods (FAO/WHO, 2001). Yogurt has long been a staple in many people's diets around the world because of its taste and nutrition. Yogurt's health benefits could be boosted by the addition of functional plant resources. Polyphenol concentrations in fermented milks were dramatically raised by the addition of grape pomace extract, which safeguarded the viability of the lactic acid bacteria (LAB). The radical scavenging abilities of yoghurt were improved when phenolic-rich pomegranate juice was added. The addition of green tea powder considerably improved the fermentation and antioxidant activity of yoghurt that had been set (Dos Santos et al., 2017; Jeong et al., 2018 & Du et al., 2022).

In the present research piece, we believe that the employment of Guava extract (GE) in

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the manufacturing of yogurt could enhance the antioxidant activity of the generated product. However, yogurt is a constant changing system due to the huge number of active LAB, so the variation of active ingredients in the system is a key topic worth addressing. Effectiveness of polyphenols depends on their bio accessibility and bioavailability. Free phenolic compounds are prone to oxidation with low stability, but those phenolic compounds covalently bonded to indigestible components such as dietary fiber are hard to be released. Some phenolic chemicals in fruit pomace are generally linked to cell walls. Liberation from matrices can improve the bioavailability of bound phenolic chemicals. Fermentation is one of the food processes which have potential to release the phenolic. Traditionally, yoghurt has been fermented with lactic acid, which has been shown to enhance the extraction of phytochemicals from food products. Plant polyphenols may act as symbiotic agents, according to a recent study. Therefore, they hypothesis that the use of fruit pomace powder rich in phenolic substances in yoghurt manufacturing, the fermentation of LAB, is helpful in releasing free phenolic compounds and increasing the antioxidant activity of the product. Functional flavored yoghurt supplemented with MP was studied in this experiment for its total free phenolic content, phenolic profiles, and in vitro antioxidant activity during cold storage. If you›re interested in learning more about the effects of climate change on the human body (Acosta-Estrada et al., 2104; Sharma and Padwad 2020 & Du et al., 2022) are good places to start.

Guava (*Psidium guajava* L.), belonging to Myrtacea family, believed to have anti-diarrheal, hepatoprotective, hypoglycemic, cholesterol lowering, antibacterial and antioxidant effects. It includes essential phytoconstituents such as tannins, triterpenes, flavonoid: quercetin, pentacyclic triterpenoid: guajanoic acid, saponins, carotenoids, lectins, leucocyanidin, ellagic acid, amritoside, beta-sitosterol, uvaol, oleanolic acid and ursolic acid. The phenolic and terpenoid chemicals in guava that were shown to impede the formation of malignant cells have potential to build a functional food product. Unfortunately, guava seeds are hardly exploited in food business and medicines while they are rich in plant carbohydrates for germination according to Patel et al. (2016).

This tropical fruit is a good source of vitamins A and C and other nutrients, as well as fiber and antioxidants like carotenoids and phenolic compounds. It also contains calcium, potassium and iron. Vitamin C content is four times higher than oranges (228 mg/100 g). It also has lycopene, lutein and zeaxanthin (Chandrika et al., 2009). Vitamin C and beta-carotene make it an excellent source of antioxidants (Akinmoldun et al., 2010). Bioactive chemicals in guava seed meal show great promise. Antioxidant, antibacterial, and anticarcinogenic substances, as well as minerals and functional qualities, can be found in this food. In addition, Fontanari et al. (2008) indicated that novel products based on fibers produced from guava residues may be formulated to prevent disorders connected to the digestive tract and cardiovascular system, particularly those related to obesity. According to Gamal et al. (2011), the proximate composition of defatted guava seeds meal contains 11.52 percent protein, 0.54 percent oil, and 79.62 percent crude fiber, which has yet to be used for any beneficial purpose (Maurya et al., 2016).

In recent years, fermented dairy products fortified with fruits have attracted a lot of attention because of their nutritional and health benefits. Fruit yogurt's nutritional and sensory characteristics have been enhanced with the addition of various fruits and ingredients during manufacture (Cakmaki et al., 2012). Vitamin C, pectin, and calcium and phosphorus are all abundant in guava. Because of its great nutritional value and mild flavor, it's known as the "apple of the plains." It is not uncommon for traditional fermented dairy products to have a wide range of composition, flavor, and texture due to factors such as the fermentation organisms used, the milk used, and the region in which they are produced. More than 3500 of these items have always been popular with customers (Hamad et al., 2017). Foods made from fermented milk are of enormous importance because they offer and retain vast quantities of nutritious foods in a wide variety of flavors, scents, and textures, which enrich the human diet. The aim of this study was to evaluate and compare effects of guava extracts (seeds, green and red leaves and juice) as antioxidant and antimicrobial and their application in manufacturing functional yoghurt and studying its new properties.

Materials and Methods

Materials

Guava *Psidium guajava L* (leaves, seeds, fruits) were obtained from Agricultural Research Center, Giza, Egypt. Buffalo's milk using obtained from Faculty of Agriculture, Cairo University, starter culture for yoghurt manufacture (*Lactobacillius delbrueckii* subsp *bulgaricus* Lb-12-DRI-VACand *Streptococcus thermophiles* CH1) were kindly obtained from the Food Technology Research Institute, Agricultural Research Center, Giza. Microorganisms which have been used in antimicrobial test are (*E. coli, Salmonella typhi, Staph. aureus, Str. mutans and Listeria moncytogenis*) which were kindly provided by microbiology department, Food Technology and Research institute, Agriculture Research Center, Giza, Egypt. All solvents and chemicals (ABTS, DPPH, methanol, ethanol) used were HPLC grades and obtained from Sigma Chemical Co. (USA).

Methods

Guava (Psidium guajava) Leaf Extract

Samples were obtained from Agricultural Research Center, Giza, Egypt, and were harvested at maturity stage, then dried at 40°C under vacuum. Guava leaf powder was prepared by drying and milling of fresh leaves until the whole sample passed through a 0.125 mm sieve. The obtained sample was stored in dark container for further use. The dried guava powder was extracted with water (1:12 w: v) or 48 hours at room temperature, in dark. The final volume after concentration with rotary reach 70 ml from (30g/450 ml) for each one of them. according to the method described by El-Gazzar et al. (2018).

Guava Seed Powder (GSP)

Guava (*Psidium guajava L.*) overripe fruits were purchased from Agricultural Research Center, Giza, Egypt. Pulp was extracted from helicoidal juice extractor (C117, Bajaj process pack maschinen Pvt Ltd) to obtain guava pomace. The seeds were cleaned, washed in running water and were dried in vacuum at 60ºCuntil constant weight. The dried seeds were reduced to fine powder in electric grinder according to method described by Maurya et al. (2016). Extracts of guava seed in water were prepared in ratio of (1:12 w: v), after concentration the final volume reach120 ml from (20g /300ml) according to El-Gazzar et al. (2018).

Preparation of yoghurt fortified with guava extracts

Fresh buffalo's milk containing 6 % fat and 9 % solid-not-fat (SNF) was procured from Faculty of Agriculture Cairo University. Level of guava seed powder (GSP) is 2g/100g (T4) (Maurya et al., 2016), guava pulp 10 % (T1) (Hamad et al., 2017), guava leaves extract 2% (red T2), (green T3) (El-Gazzar et al., 2018). Yoghurt cultures were added at (2% v/v) Sugar at (5 g/100g) was also added in the milk. The preparation was mixed thoroughly and heated at 90 ºC for 10 min followed by cooling and kept for incubation at the experimental temperature (37-42 ºC). After incubation, yoghurt samples were stored at 4ºC for further study according to Mauryaet al. (2016).

Chemical Composition

In all samples total solids, fat, total nitrogen was determined according to AOAC (2000). Titratable acidity in terms of % lactic acid was measured by titrating 10g of sample mixed with 10 ml of boiling distilled water against 0.1 N NaOH using a 0.5% phenolphthalein indicator to an end point of faint pink color. pH of the sample was measured at 17 to 20°C using a pH meter (Corning pH/ion analyzer 350, Corning, NY) after calibration with standard buffers (pH 4.0 and 7.0).

Sensory Evaluation of Functional Yoghurt

Ten experienced panelists from the Special Food Science Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt, assessed the sensory quality of yoghurt fortified with guava extracts. Taste, odour, body and texture, appearance and colour were scored on a scale of 20, 20, 20, 10, and 10 for each sample according to Bodyfelt et al. (1988).

Microbiological Evaluation of Fortified Yoghurt

Lactic acid bacterial (LAB), the total bacterial count (TBC), yeasts and moulds and coliform bacterial counts were enumerated according to IDF (2003) and APHA (1992), respectively.

Antioxidant Activity of Extracts

DPPH Radical Scavenging Activity

An aliquot of each extract (200 µL) was mixed with 50 µL of 1Mm DPPH (prepared with MeOH) and kept in dark for 30 min, and then the absorbance was recorded on spectrophotometer (Beckman DU-7400, U.S.A) at 517 nm. The test is based according to Liu et al. (2009).

ABTS Radical Scavenging Activity

ABTS Radical Scavenging Activity was measured as described by Re et al. (1999) with

slight modifications. Briefly, the ABTS cation radical was prepared by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, which was allowed to stand in the dark at room temperature for 14 h. Before usage, the solution was diluted to achieve an absorbance of 0.700 ±0.025 at 734 nm. The scavenging activity (inhibition %) of the ABTS radical was calculated using the following equation:

$$\text{Inhibition\%} = 1 - (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100$$

Total Phenolic Contents

The measurement of total phenolic contents was based with Chang et al. (2019). An aliquot (50 µL) of each extract was mixed with 1 mL of dd H2O and 0.5 mL of Folin-Ciocalteu's phenol reagent. After adding 2.5 mL of 10% Na_2CO_3 solution and reacting for 20 min, the absorbance was measured at 735 nm. Standard curve was established by using Gallic acid, and the results were expressed as mg Gallic acid equivalent (GAE)/g extract (dry weight, dw).

Total flavonoid contents

An aliquot (250 µL) of each extract of guava was mixed with 1.25 mL of dd H2O and 75 µL of 5% $NaNO_2$ solution and reacted for 6 min; then, 150 µL of 10% $AlCl_3 \cdot H2O$ solution was added and reacted for another 5 min. After 0.5 mL of 1M NaOH solution and 275 µL of MeOH were added to the mixture, the absorbance was read at 510 nm. A standard curve was prepared using (+) catechin; the results were expressed as mg catechin equivalent (CE)/g extract (dw) (Liu et al., 2009).

Fractionation of phenolic components by HPLC

Phenolic fraction of extracts was determined using high performance liquid chromatography with variable wave length detector (Agilent technologies, Germany) 1200 series and an auto sampler, Quaternary pump degasser, and column compartment set to 35°C were also included in the HPLC setup. The experiments were carried out on a stainless-steel packed C18 reverse phase (BDS 5 m, Labio, Czech Republic) column (4250 mm, i.d.).

To assess phenolic acids and flavonoids, samples were prepared according to the method specified by Jakopic et al. (2009). 100 mg of the material was placed in a test tube. For 45 minutes in an ultrasonic bath, 10 mL methanol was used to extract the samples' weights. After 7 min. of centrifugation at 4200 rpm, the materials were collected. Polyamide Chromafil filters, AO-45/25 was used to remove the supernatant and place it in a vial for further examination. Method began with a linear gradient at a flow rate of 1 mL per min. using water/acetic acid (98:2 v/v, solvent A) and methanol/acetonitrile (50:50, v/v) as mobile phases, starting with 5 percent B and increasing B to 30 percent at 25 min., 40 percent at 35 min., 52 percent at 40 min., 70% at 50 min., and 100% at 55 min.. A 5-min. wash in both solvents re-established the original conditions. Chromatograms were shown for phenolic acids and flavonoids using wavelengths of 278 to 332 nanometers. Peak regions were used to identify and quantify all of the components *Fractionation of sugar components by HPLC*

The Agilent programme was used to collect the chromatographic data. A quaternary pump, degasser, and auto injector were included in the Agilent (series 1200) chromatographic system linked to the refractive index detector, aminex-carbohydrate HPX-87 column with deionized water was used to evaluate the samples collected as mentioned above. There was a flow rate of approximately 0.5 mL/min. Detector temperature was kept at 50°C, while the column temperature was kept at 85°C. Retention time criteria were used to detect samples according to Zelinski et al. (2014).

Antibacterial Activity

The disc diffusion experiment was used by method described by Biswas et al. (2013) to test the antibacterial activity of guava extracts. The nutrient broth was used to activate indicator microorganisms, which were then diluted to a final concentration of 10^8 CFU mL^{-1}. Each test microorganism was incubated at the appropriate temperature (37°C for *E. coli* ATCC2592 and *S. aureus* ATCC25923, 40°C for *str. mutans*, *sal. typhi* and 5–6°C for *Listeria moncytogenis*) before aliquots of 25 L of sample (50 mg mL-1) were spotted on the freshly created indicator strain. Following this, the widths of the growth inhibition zones (represented by clear zones) were measured and presented as inhibition zones (mm). As a positive control, 500 mg of Streptomycin was employed, while sterilized water was used as the negative control.

Statistical Analysis

The collected data was analyzed using SPSS (1999) version 16 on a PC (Statistical Package for the Social Sciences). One-way ANOVA (Analysis of variance) tests using Duncan's multiple range tests and p 0.05 was used to determine

significance between various groups SPSS was used to examine the effects of different treatments.

Results and Discussion

Antioxidant Activity of Guava Extract

Free radicals and active oxygen species cause oxidative damage to biomolecules and are implicated in a slew of diseases, while antioxidant components in food play a critical role in maintaining antioxidant defense mechanisms by preventing or scavenging their creation (Stadtman 2006; Gupta et al., 2009). Reactive oxygen species (ROS) and free radicals are produced as a result of oxidative metabolism, which is critical for cell growth but can also cause oxidative stress (Fang et al., 2002). Because some synthetic antioxidants have been found to be harmful, natural antioxidants (free radical scavengers) are preferable (Liu et al., 2005). The two approaches used in this study were analyzed based on their respective mechanisms of operation. An assay known as Trolox Equivalent Antioxidant Capacity (TEAC) measures the ability to scavenge 2,21-azinobis (3-ethylbenzothiazoline-6-sulfonate) radicals (ABTS+) and the DPPH technique. Table 1 shows the antioxidant activity, total phenol, and total flavonoid concentration of guava extract (leaves, seeds and pulp).

The content of total phenol was found higher in green than red (672.42 than 658.90 mg/100mg gallic acid) with significant differences between them. As for total flavonoid content red leave have higher content than green and seed, it was 91.27 than 59.76 and 6.30 mg/100g catachin respectively with significant differences between extracts.

As for antioxidant activity determined by DPPH methods the highest value was observed in seed extract 89.18% then green and red leaves 75.41 and 71.26% resp. and at last pulp or guava juice with 46.52%. the same trend was also found by ABTS methods.

These results are in agreement with the results of Luo et al. (2018; 2019) which indicated that guava leaves polysaccharide (GLP) exhibited good DPPH, OH, and ABTS free-radical scavenging abilities it was 56.38% and 51.73% at 100 μg/mL, respectively. The results obtained were lower than those reported by (Seo et al., 2014; Natitanon et al., 2010) quantified total phenolic compounds using the Folin–Ciocalteu technique Even though the observed variation is not significant, it might be because the HPLC measurement provided only 50–60% of total phenolic content (Natitanon et al., 2010). Kamari & Basha (2014) found that methanol extract had more phenolic than water extract. The phenolic generated during extraction (456.3 mg Gallic/ml). The lowest oxidation states reported the highest values for TEAC and FRAP (3.1 0.1 mM eq Trolox/mg leaf d.w. and 5.4 0.1 mM FeSO4/mg leaf d.w, respectively) and decrease as the oxidation state rises. Daz-de-Cerio et al. (2016a, 2016b) found that significant changes ($p>0.05$) across the three extracts. The lowest oxidation state has the greatest total phenolic component concentration (103 2 mg/g leaf d.w.), followed by the medium and high oxidation states (92.0 0.4 and 87.91 0.04). Maurya et al. (2016) observed that seed extract has (54.58%) antioxidant activity and total phenolic content (25.03%).

Phenolic components fractions by HPLC

Data presented in Table 2 reveled that red leaves extract has high contents of total phenolic components determined by HPLC than green leaves extracts and seed extracts and juice, in red leaves the major components were: ellagic> catechol>catachin>oluropn>chlorogenic>ferulic >pyrogallol>vanillic> caffeine. IN case of green leave extract, the major components were: ellagic >oluropin >chlorogenic >catachin >pyrogallol. On the other hands seed extract and juice of guava have little amount of phenolic components. The finding results are in agreement with Kumari and Basha (2014) observed that phenolic compounds in Psidium guajava extract are gallic acid, vanillic acid, caffeic acid, epicatechin and coumaric. Enhancement of total phenolic soluble in methanol was higher than that of soluble in water. Daz-de-Cerio et al. (2016a, 2016b) HPLC-DAD can accurately measure the concentrations of ellagic acid, quercetin and gallic acid, as well as catechins and gallocatechins, in guava leaves. Guava leaves samples had between 48.1 and 50.6 mg/g leaf dry weight of flavonols, according to Daz-de-Cerio et al. (2016a, 2016b). Second, flavan-3-ols (24.2–24.7 mg/g leaf dry weight), next gallic acid derivatives (14.8–15.8 mg/g leaf dry weight), and then flavanones (0.49)–0.63 mg/g leaf dry weight rounded out the polar compounds.

Flavonoid components fractions by HPLC

Flavonoid fractions in guava extracts were shown in Table 3 and illustrate that red leaves extract has high amount of flavonoid components then green leaves, seed and the latest one was the guava pulp.

TABLE 1. Antioxidant activity for all guavas extracts (%).

Treatments	DPPH %	ABTS%	T. Phenol mg/ 100gm Gallic	T. Flavonoid mg/100ml Catachin
Green leaves	75.41^{b}±0.19	66.94^{b}±0.47	672.42^{a}±1.1	59.76^{b}±0.18
Red leaves	71.26^{c} ±0.14	59.95^{c}±1.24	658.90^{b}±0.74	91.27^{a}±1.70
Seed	89.18^{a}±0.01	88.53^{a}±0.17	ND	6.30^{c}±0.11
Juice	46.52^{d}±4.28	21.61^{d}±0.92	ND	ND

Different superscript (a, b, c...) significantly different between treatments (±) Standard Deviation
ND: Not Detected

TABLE 2. Phenolic components fractions for all guava extracts (mg/100gm).

Phenol component	Red	Seed	Juice	Green
Pyrogallol	291.99	5.69	3.84	203.42
Gallic	61.07	1.14	0.10	38.68
3-OH Tyrosol	22.40	0.22	0.08	21.37
Catechol	837.57	14.81	2.16	871.65
4-Amino benzoic	23.49	0.47	0.21	20.68
Catechein	763.00	14.28	2.03	366.57
Chlorogenic	716.02	16.75	0.00	400.85
P-OH- benzoic	0.00	6.49	2.47	0.00
Benzoic	0.00	8.71	3.31	0.00
Caffeic	228.18	4.35	3.07	0.00
Vanillic	189.66	7.21	1.61	150.84
Caffeine	177.21	9.91	4.18	162.72
Oleuropin	718.72	6.41	4.69	456.93
Ferulic	636.16	7.08	2.62	91.20
Ellagic	2040.29	27.67	0.00	943.31
Coumarin	99.47	3.21	1.85	193.45

TABLE 3. Flavonoid components fractions for all guavas extracts(mg/100gm).

Flavonoid component	Red	Seed	Juice	Green
Rutin	307.27	0.77	0.69	46.56
Naringin	2138.00	18.48	4.86	366.66
Rosmarinic	81.34	0.61	0.58	17.03
Quercetrin	263.38	1.57	0.74	434.61
Apigenin-7-glucose	0.00	0.00	1.37	0.00
Quercetin	98.12	1.86	1.28	67.54
Naringenin	32.17	1.26	0.00	45.14
Kaemp.3-(2-p-comaroyl) glucose	0.00	0.00	0.00	0.00
Kampferol	25.89	0.56	0.00	21.58
Acacetin 7 neo.rutinoside	125.27	2.73	0.00	104.45
Apigenin	8.18	0.00	0.00	3.14

The main components in red leave extracts were: rutin > quercetrin > kampferol.

In case of green leave extract, the main components were: quercetrin > naringin > kampferol was found. Phenolic and flavonoid content in the fruit was 40.13± 2.12 and 18.43±1.22 mg/g of dry weight sample, according to the authors' findings. A gallic acid equivalent (GAE) recovery in the peel of 58.7 4.0 g/kg dry matter was also found. In the pulp, the phenolic content was 1723.06 111.58 mg GAE/100 g dry basis, according to Ribeiro da Silva et al. (2014); in the seeds, the total phenols (TP) ranged between 14.54 and 91.05 mg/100 g of defatted ground seeds in several solvents (Gamal et al., 2011); and the TPC of the stem bark was 1.15 0.12 g GAE/ (Aminu et al., 2012).

Antibacterial Activity of Guava Extracts

The phytochemicals found in the plant extracts have been shown to have a variety of medicinal and physiological effects. Antibacterial action has been shown for polyphenolic tannins (Sanches et al., 2005; Ulubeleu, 2003), which bind to proline-rich proteins and interfere with protein synthesis (Min et al., 2008). In response to microbial infections, plants produce hydroxylated polyphenolic substances known as flavonoids, which have antibacterial properties in vitro (Cowan, 1999). Their capacity to form complexes with extracellular and soluble proteins and bacterial cell walls has been credited (Trease and Evans, 1989). Although mostly employed for their fragrant properties, terpenoids have been discovered to be antibacterial agents (Ulubeleu, 2003).

Data presented in Table 4 illustrate the antibacterial activity of guava extracts It is clear that almost all leaves extract had antibacterial effect against the tested strains. Generally, all red leaves extract had antibacterial effect on *E. coli, Staph. aureus, str. mutans, sal. typhi, Bacilus cereus* and *Listeria moncytogenis*. It is noticeable that seed extract had no effect on *Sal. typhiumiurum, E. coli and Listria*. As for guava juice, no inhibition effect was noticed on Listeria than other bacteria have effect. Chemical components discovered in guava extracts exhibit antibacterial properties, which may explain the antibacterial effects seen in methanol, ethanol, and distilled water extracts. It was shown that guava leaf extract had inhibitory effects against Staphylococcus and Bacillus but no impact on Escherichia and Salmonella. Lin et al. (2002) found that guava

methanolic extracts inhibited the development of 2 Salmonella isolates and enteropathogenic *E. coli.* Biswas et al. (2013) reported that unlike Gram-positive bacteria, Gram-negative bacteria are more resistant to plant-derived antimicrobials, as shown by the failure of guava leaf extract to suppress the development of Pseudomonas aeruginosa. The results from Escherichia coli further reveal that not all gram-negative bacteria are resistant to plant-derived antimicrobials. Some Gram-negative bacteria's resistance may be due to their cell wall construction. Unlike Gram-positive bacteria, Gram-negative bacteria have a thin lipopolysaccharide outer membrane that may prevent plant extract penetration.

A study by Serunjogia and Muwongeb (2018) reveals that the crude extract of guava may be used to treat gram positive bacterial infections, such as *Staphylococcus aureus* and *Streptococcus Pneumoniae*. When extracting phytochemicals against gram-positive *Staphylococcus aureus*, distilled water performed better than other extraction solvents, such as *Escherichia coli, Pseudomonas aeruginosa*, and Streptococcus, in comparison. More methanol didn't make it more effective at killing bacteria, thus 30 percent methanol was better for extracting active phytochemicals from plants.

The result is in the same trend with Das & Goswami (2019) whom observed that the growth of gram positive bacterial and fungal strains was inhibited strongly, whereas gram negative bacterial strain displayed less sensitivity against the antimicrobial (antifungal and antibacterial) property of guava leaf extract. It is rich in phenols, flavonoids and tannins whereas components like alkaloids, flavonoids, saponins and triterpenes are present in comparatively lesser amounts. As polyphenols have strong antimicrobial property, it can be concluded that rich source of phenols, flavonoids and tannins are the probable cause of antimicrobial property of guava leaves.

Total Sugar Fractions in Guava Extracts

Data presented in Table 5 illustrate the sugar fraction of guava extracts free or after hydrolysis. It was shown that red guava leaves extract have the highest content of sugar fraction than green leaves and seed extracts, it was inulin (2.04%), sucrose (10.84%), stachyose (0.49%), fructose (0.26%), galacturonic (0.35%), sorbitol (0.14%), then in the second was green leave extract, it was inulin (2.20%), sucrose (8.23%), galactose (015%), rhaminose (0.12), raffinose (0.26), galacturoinc acid (0.006%). As for seed extract sucrose was 1.83%, arabinose 0.28%, galactose 0.29%, galacturoinc acid 0.19%.

Lin & Lin (2020a; 2020b) used a pre-column derivatization high performance liquid chromatography (HPLC) analysis of GSF3 monosaccharides composition, it was composed of glucuronic acid (3.28%) and galacturonic acid (28.13%), as well as neutral sugar, including galactose (14.88%), mannose (3.96%), glucose (22.99%), arabinose (7.31%), ribose (1.55%), xylose (14.81%), and fucose (1.68%) (Lin & Lin 2020 a: b). In GSF3, there was a lot of xylose and galactonic acid. The researchers say GSF3 has potent anti-inflammatory and immunomodulatory properties. They discovered that GSF3, a new polysaccharide from guava (*P. guajava* L.) seeds, inhibits breast cancer cell development via raising the Bax/Bcl-2 ratio or Fas mRNA levels in target cancer cells. GSF3 is an anti-cancer proteopolysaccharide

TABLE 4. Antimicrobial activity of guava extracts (inhibition zone IZ mm).

Treatments	*Sal. thyphi*	*Str. mutans*	*Staph. aureus*	*Listeria*	*E. coli*
Pulp	6.93^{b}±0.11	7.53^{a}±0.05	7.56^{a}±0.11	ND	6.60^{a}±0.11
Seed	ND	ND	6.46^{c}±0.05	ND	ND
Red leaves	7.73^{a}±0.05	6.56^{b}±0.05	7.63^{a}±0.11	7.43^{a}±0.11	6.36^{b}±0.05
Green leaves	6.58^{c}±0.08	ND	6.93^{b}±0.11	7.13^{b}±0.11	6.66^{a}±0.05

Different superscript (a,b,c...) significantly different between treatments (±) Standard Deviation
ND: Not Detected

TABLE 5. Sugar fraction of guava extracts by HPLC (mg/100gm).

Free sugar	Green leaves	Red leaves	Seed
Inulin	2.205	2.045	0.043
Stachyose	--	0.490	0.014
Sucrose	8.236	10.840	0.117
Galactose	0.099	0.177	0.008
Fructose	0.224	0.269	0.011
Galactouorinc	0.442	0.254	0.003
Sorbitol	0.005	0.007	----
Ribose	0.018	0.021	0.008
Maltose	---	-	----
Glucose	---	--	0.009
Raffinose	0.020	--	0.003
Mannitol	---	---	---
Hydrolysis sugar			
Sucrose	-----	------	1.832
Galactose	0.156	0.567	0.299
Rhaminose	0.0046	0.409	0.175
Fructose	0.124	0.086.91	0.197
Arabinose	----	0.072	0.280
Raffinose	-----	0.357	0.0053
Galacturonic	0.261	---	0.191
Sorbitol	0.0061	----	0.096
Ribose	----	0.145	0.160
Maltose	------		----

Sensory Evaluation of Yoghurt Fortified with Guava Extracts

The organoleptic properties of yoghurt made with guava extract and juice are shown in Table 6 when fresh and during cold storage. In fresh yoghurt samples, T1, T2 and control gained the highest flavor scores followed by T3 and T4 with significant differences between T4 and other treatments. As storage periods progressed, a gradual decrease in flavor scores was observed in all treatments with non-significant differences between treatments.

As for body and texture, fresh samples (control and T1) are characterized by its good body and texture with significant differences between other treatments. As storage period progressed, a slightly decrease was observed in all treatments with significant differences between treatments and T4. Concerning yoghurt color and appearance, there were variations noticed between treatments with significance differences between them either when fresh or during storage period. The overall acceptability of fresh yoghurt samples was higher in T1, T2 and control than T3 and T4. After 7 days of cold storage T1, T2, T3 still have the superiority. Overall acceptability decreased in all treatments and T3 showed the highest scores being with significant differences between treatments.

These results were in agreement with Hamad et al. (2017) they reported that the organoleptic properties of Rayeb milk manufactured from goat's milk and fortified with various amounts of guava pulp were generally improved. Also, Maurya et al. (2016) observed that guava seed powder, yoghurt culture and temperature had significant ($p<0.0001$) positive effect on texture of the yoghurt sample at linear level but guava seed powder, it is clear that there is a positive effect of guava seed powder and yoghurt culture on overall acceptability shows interaction effect of guava seed powder with yoghurt culture, as the level of guava seed powder increased (0.8 to 2 g) the whey separation score decreased. It may be due to the water holding capacity of dietary fiber present in guava seed powder, which absorb the moisture and decrease whey separation. The whey separation was lowest (2.7 %) at 2.4 g, 3% and 39.5ºC of guava seed powder, yoghurt culture and temperature level, respectively.

Chemical Composition of Yoghurt Fortified with Guava Extracts

Data presented in Table 7 show the chemical composition of yoghurt fortified with guava extracts when fresh and during cold storage. Total solid (TS) content ranged from 14.09 to 20.86% in fresh samples and is significantly increased during storage to reach 14.96 to 21.74% with significant differences between treatments. Both protein and fat content showed the same trend. Fresh samples had a T.A of 0.53 to 0.80%, while samples kept in the freezer had a T.A of 0.58-0.79%. The same trend in pH was observed. Ziena and Abd Elhamied (2009) showed that Addition of extract of guava leaf by different concentrations to a functional yogurt, showed significant changes of pH, titratable acidity during cold storage up to 5 days.

The results were in the same trend with Hamad et al. (2017) which used guava pulp in rayeb milk production have slightly increased the dietary fiber content. In the same trend, significant increases in ash, total nitrogen, total protein, WSN and TVFA contents.

Antioxidant Activity of Yoghurt Fortified with Guava Extracts

According to Table 8 of antioxidant activity of yogurt determined by two methods (DPPH and ABTS). The scavenging activity of T3 was the greatest, ranging from 46.55% to 72.41% in fresh yoghurt samples. There were significant differences between treatments. All treatments showed an increase in antioxidant activity after cold storage, with T3 (82.35 %) showing the greatest increase, with significant variations across treatments. The ABTS test behaved in a similar way to the DPPH assay in terms of results. Samples that had been stored for less than a year had radical scavenging activity of 38.60 to 89.08%, which rose with time to 47.69 to 94.95% at the conclusion of storage. There were also substantial variations between T3 and both of the other two antioxidants, T1 and T2, in terms of antioxidant activity. Opposed trend were found by Ziena and Abd Elhamied (2009) that The reducing activity of all samples significantly ($P>0.05$) decreased up to the end of storage period, while the inhibition of ascorbate autoxidation significantly increased with increasing of the amount of phenolic compounds till 300 µg phenolic components /100ml yoghurt followed by a slight decrease.

TABLE 6. Sensory Evaluation of Yoghurt Fortified with Guava Extracts.

Treatment	Flavor (20)		Texture (20)		Color (10)		Overall Acceptability (50)	
	F	E	F	E	F	E	F	E
Control	$19.66^{a}\pm0.57$	$18.66^{a}\pm0.57$	$18.66^{a}\pm0$	$19^{a}\pm0$	$10^{a}\pm0$	$10^{a}\pm0$	49.33^{a}	48.66^{ab}
T1	$20^{a}\pm0$	$18.66^{a}\pm0.57$	$18.66^{a}\pm0.57$	$18^{b}\pm0$	$9.33^{ab}\pm0.57$	$9^{b}\pm0$	50^{a}	49^{a}
T2	$19.33^{ab}\pm0.57$	$19^{a}\pm0$	$19^{a}\pm0$	$18^{b}\pm0$	$7.66^{c}\pm0.57$	$8^{c}\pm0$	49.33^{a}	47.66^{b}
T3	$18.66^{b}\pm0.57$	$19.33^{a}\pm0.57$	$19.33^{a}\pm0.57$	$18.66^{ab}\pm0.57$	$7.66^{c}\pm0.57$	$7.66^{c}\pm0.57$	48.33^{b}	48.66^{ab}
T4	$17.66^{c}\pm0.57$	$18.33^{a}\pm0.57$	$18.33^{a}\pm0.57$	$16.66^{c}\pm0.57$	$8.66^{b}\pm0.57$	$8.33^{c}\pm0.57$	47.66^{b}	47.66^{b}

T1 (yoghurt fortified with guava pulp), T2 (yoghurt fortified with guava red leave extract),
T3(yoghurt fortified with green leaves extract), T4 (yoghurt fortified with guava seed extract)
Different superscript (a,b,c...) significantly different between treatments (±) Standard Deviation

TABLE 7. Chemical Composition of Yoghurt Fortified with Guava Extracts.

Treatment	Acidity		pH		T.S		Protein	
	F	E	F	E	F	E	F	E
Control	$0.65^{b}\pm0.05$	$0.79^{a}\pm0.09$	$4.63^{e}\pm0.02$	$4.45^{a}\pm0.02$	$14.09^{c}\pm0.33$	$16.96^{c}\pm0.46$	$4.26^{bc}\pm0.11$	$4.46^{d}\pm0.46$
T1	$0.50^{c}\pm0.05$	$0.58^{c}\pm0.02$	$4.75^{d}\pm0.05$	$4.58^{a}\pm0.02$	$20.86^{a}\pm1.27$	$20.45^{ab}\pm0.46$	$4.01^{c}\pm0.13$	$6.43^{c}\pm0.34$
T2	$0.80^{a}\pm0.01$	$0.58^{c}\pm0.05$	$4.81^{c}\pm0.01$	$3.94^{a}\pm0.11$	$19.39^{ab}\pm0.78$	$19.58^{b}\pm0.82$	$6.7^{a}\pm0.41$	$9.04^{a}\pm0.07$
T3	$0.53^{c}\pm0.01$	$0.68^{b}\pm0.02$	$5^{a}\pm0.05$	$4.49^{a}\pm0.01$	$19.29^{ab}\pm0.71$	$21.74^{a}\pm1.25$	$5.23^{b}\pm0.46$	$7.42^{b}\pm0.37$
T4	$0.53^{c}\pm0.02$	$0.68^{b}\pm0.02$	$4.9^{b}\pm0.01$	$4.64^{a}\pm0.03$	$17.66^{b}\pm1.62$	$20.78^{ab}\pm0.87$	$5.26^{b}\pm0.46$	$7.11^{b}\pm0.14$

T1 (yoghurt fortified with guava pulp), T2 (yoghurt fortified with guava red leave extract), T3(yoghurt fortified with green leaves extract), T4 (yoghurt fortified with guava seed extract).Different superscript (a,b,c...) significantly different between treatments (±) Standard Deviation

TABLE 8. Antioxidant Activity of Yoghurt Fortified with Guava Extracts .

Treatments	DPPH		ABTS	
	F	E	F	E
Control	$53.50^{e}\pm1.18$	$70.44^{d}\pm0.42$	$46.95^{c}\pm0.75$	$51.90^{d}\pm0.47$
T1	$46.55^{d}\pm1.93$	$60.45^{e}\pm0.51$	$44.01^{d}\pm2.31$	$69.87^{c}\pm0.17$
T2	$53.97^{c}\pm0.65$	$73.54^{c}\pm0.90$	$60.04^{b}\pm0.54$	$92.61^{b}\pm0.56$
T3	$72.41^{a}\pm0.65$	$82.35^{a}\pm0.06$	$89.08^{a}\pm1.39$	$94.95^{a}\pm1.37$
T4	$64.52^{b}\pm0.47$	$77.26^{b}\pm0.51$	$38.60^{e}\pm0.74$	$47.69^{e}\pm1.12$

T1 (yoghurt fortified with guava pulp), T2 (yoghurt fortified with guava red leave extract),
T3(yoghurt fortified with green leaves extract), T4 (yoghurt fortified with guava seed extract)
Different superscript (a,b,c...) significantly different between treatments (±) Standard Deviation

Jiménez-Escrig et al. (2001) found substantial quantities of dietary fiber, indigestible fraction, and phenolic chemicals in *Psidium guajava* peel and pulp. The estimated extractable phenol concentration correlated with radical scavenging activity/ferric reduction power. These bioactive substances contributed greatly to the guava fruit's strong antioxidant activity. Musaa et al. (2015) reported that the antioxidant activity of pink guava (red leaves extract) related to the primary flavonoid component is kaempferol, vitamin C, and lycopene content. According to Rahmawatia and Suntornsuk (2016) the antioxidant activity in yoghurt produced from fermented buffalo, goat, and cow milk rose during production, but dropped or remained unchanged during storage. Consequently, they advised customers to use these activities within three weeks of purchase or before the product's expiration date.

Hamad et al. (2017) Antioxidant activity percentages in Guava Rayeb Milk were greater than those discovered in the control Rayeb. Rayeb milk's anti-oxidant properties improved as the amount of guava pulp in the formula rose. Samples that include 15 percent guava pulp had a 75.11 percent increase in the antioxidant activity of the fresh samples.

Microbiological Properties of Yoghurt Fortified with Guava Extracts

Data shown in Table 9 revealed that the total bacterial count (TBC) of fresh control yoghurt was higher than other treatments being 367 x 10^5cfu/ml while it ranged from 15 to 60 x 10^5cfu/ml inT1, T2, T3 and T4 with non-significant differences between all treatments. At the end of storage period, it increased significantly only in treatments T1 and T3 to reach 100X10^5cfu/ml in T1 and 47x10^5cfu/ml in T3. T2 and T4 were significantly different, with 163, 48, and 40 x105, respectively. Because of the hygienic circumstances, no coliform bacteria, moulds, or yeasts were found in any of the samples, whether they were fresh or in cold storage. Hoque et al. (2007) evaluated that a flavonoid molecule called quercetin-3-Oalpha- l-arabinopyranoside (guaijaverin), isolated from leaves, inhibited the development of Streptococcus mutans, a pathogen associated with plaque formation.

TABLE 9. Microbiological Properties of Yoghurt Fortified with Guava Extracts.

Treatments	T. C 10^3 cfu/ml		Coliform	M&Y
	F	E		
Control	136	367	ND	ND
T1	10	100	ND	ND
T2	38	60	ND	ND
T3	47	15	ND	ND
T4	40	60	ND	ND

T1 (yoghurt fortified with guava pulp), T2 (yoghurt fortified with guava red leave extract), T3(yoghurt fortified with green leaves extract), T4 (yoghurt fortified with guava seed extract)
Different superscript (a,b,c...) significantly different between treatments(±) Standard Deviation
ND: Not Detected.

Conclusions

Guava extract (seed, green and red leaves, and juice) is added to GE yoghurt as a natural taste and antioxidant element, which is rich in health-promoting chemicals, as part of this study's objective. The content of total phenolic compounds in the enriched yoghurt rose considerably after it was fermented by LAB. It was shown that all GE yoghurts had high antioxidant qualities and were stable in cold storage by detection of DPPH and ABTS free radicals, compared with regular yoghurts. Because of its bioactive components and antioxidant properties, we believe guava extracts have a lot of potential as a functional yoghurt ingredient. DPPH and APTS techniques found that all guava extracts were very potent antioxidants. Guava extracts' antioxidant properties were mostly due to the presence of phenolic compounds.

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Conflict of interests

The authors declare no conflict of interest.

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دراسة الخواص الفيزوكيمائية و المضادة للاكسدة و الميكروبات و الخواص الحسية للزبادي المدعم بمستخلصات الجوافة (البذور، الاوراق و اللب الداخلي)

دعاء فتحي حسن[١]، سحر أحمد محمد[١] و هشام محمد الششتاوي[٢]

١قسم الاغذية الخاصة والتغذية- معهد بحوث تكنولوجيا الاغذية- مركز البحوث الزراعية- مصر

٢ معهد بحوث الهندسة الوراثية- مركز البحوث الزراعية- مصر

تعتبر الجوافة من أهم الفواكهه في مصر حيث تكثر زراعتها وتعتبر اوراق اشجارها من اهم النواتج الثانوية لما لها من فوائد صحية عديدة لذلك تهدف هذة الدراسة الى دراسة تاثيراضافة مستخلصات الجوافة (اوراق و بذور و اللب الداخلي) على خواص الزبادي من حيث الخواص المضادة للاكسده و الميكروبات ودراسة محتوى هذة المستخلصات من الفينولات و الفلافونيد الكلية. وكانت النتائج كما يلي:

وجد انه لكل المستخلصات محل الدراسة نشاط مضاد للاكسدة و كان أعلاهم في البذور ٨٩.١٨٪ يليه مسخلص الاوراق الخضراء ٧٥.٤١٪ ثم مستخلص الاوراق الحمراء ٧١.٢٦٪ وهذا النشاط راجع الى ارتفاع محتوى هذة المستخلصات من الفينولات الكلية و التى قد تصل الى ٦٧٢.٤٢ ملليجم جاليك لكل ١٠٠جم في مستخلص الاوراق الخضراء و ٦٥٨.٩٠ملليجم جاليك لكل ١٠٠ جم لمسخلص الاوراق الحمراء.

وعند تفريد السكريات الكلية لهذة المستخلصات وجد ان مستخلص الاوراق الحمراء و الخضراء الاعلى في المحتوى ومن أهم السكريات التي تم تحديدها : الانيولين ٢.٠٤٪, سكروز ١٠.٨٤٪, ستكيوز ٠.٤٩٪, فركتوز ٠.٢٦٪, جلاكتوبوريونيك ٠.٣٥٪و سوربيتول ٠.١٤٪. ووجد أن لكل المستخلصات التي تم دراستها في البحث تأثير مضاد لنشاط الميكروبات السالبة و الموجبة للجرام المرضة و المسببة لفساد الاغذية.

عند تدعيم الزبادي بمستخلصات الجوافة كان لها تأثير ايجابي على الخواص الحسية خاصة مستخلص الاوراق(الخضراء و الحمراء و اللب الداخلي) وزيادة الجوامد الكلية و البروتين . مع عدم تغيير في الحموضة . وقد لوحظ أن التدعيم بمستخلصات الجوافة زاد من قدرته الزبادي المضادة للاكسدة والميكروبات وزيادة فترة الحفظ لاكثر من ١٥ يوم تحت تخزين المبرد. لذلك يهدف البحث الى زيادة استخدام المواد المضادة للاكسدة الطبيعة في المنتجات الغذائية للحصول على منتجات لبنتية وظيفية تساعد في الحفاظ على الصحة.

الكلمات الدالة: : أوراق الجوافة, البذور, اللب الداخلي, الخواص الفيزوكيمائية, النشاط المضاد للاكسدة, النشاط المضاد للميكروبات , الخواص الحسية للزبادي