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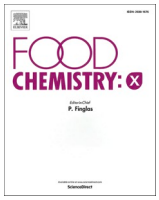

The synergistic potential of orange peel extract: A comprehensive investigation into its phenolic composition, antioxidant, antimicrobial, and functional fortification properties in yogurt

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ABSTRACT

The study explores the potential of orange peel extract (OPE) as a versatile natural resource, focusing on its phenolic composition, antioxidant, and antibacterial properties, as well as its application in fortifying yogurt. Analysis revealed significant concentrations of phenolic compounds in OPE. OPE exhibited notable antibacterial efficacy against pathogenic bacteria, particularly marine *Escherichia coli*, with synergistic effects observed when combined with Amikacin. Incorporating OPE into yogurt led to changes in chemical composition, enhancing total proteins, fat, and ash content. Fortified yogurt showed increased antioxidant activity and potential anti-cancer properties against HCT116 cell lines. In conclusion, OPE emerges as a rich source of bioactive compounds with diverse applications, from its antioxidant and antibacterial properties to its potential in fortifying functional foods like yogurt. This comprehensive exploration provides valuable insights into the multifaceted benefits of OPE, paving the way for its utilization in various industries and health-related applications.

Introduction

The peril of antibiotic resistance is global, seen in recent declines in antibiotic efficacy. Excessive use in humans and animals has led to increased resistance, resulting in longer hospital stays, higher costs, and mortality rates (Prestinaci et al., 2015). Protecting global health security requires promoting prudent an-tibiotic use and developing effective new antibiotics (Terreni et al., 2021). Addressing this challenge finds promise in natural resources.

The consumption of functional food products, attributed to the presence of healthy bioactive ingredients and their potential to mitigate the risk of chronic diseases and disorders, has recently garnered attention from both the scientific and industrial communities. A key step in developing these health-promoting products is the exploration of new, safe, abundant, and cost-effective dietary sources for manufacturing and fortifying food formulations (Gharibzahedi & Chronakis, 2018). Yogurt, a widely consumed dairy product globally (Saint-Eve et al., 2006) is primarily produced by fermenting fresh or reconstituted milk with lactic acid bacteria. It is favored by consumers for its beneficial effects on improving the intestinal environment and enhancing body immunity. There is a growing interest in utilizing fruit processing wastes as functional food ingredients, given their richness in dietary fiber, with most beneficial bioactive compounds retained in these by-products (Balasundram et al., 2006). Moreover, waste products, such as fruit peels, generated during the processing of agricultural commodities, could serve as practical and economical sources of active antioxidants, potentially replacing synthetic alternatives (Reddy et al., 2007).

The disposal of citrus fruits results in a substantial amount of waste, encompassing peels, seeds, and pulp, constituting 40–50 % of their total weight (Saini et al., 2022). Repurposing this waste has the potential to alleviate its environmental impact. Citrus peels are abundant in bioactive compounds, including phenols, carotenoids, and ascorbic acid (Saini et al., 2022). The mandarin (*Citrus reticulata Blanco*), a member of the Rutaceae family, has been identified by Costanzo et al. (2020) as

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having more antioxidants in its peel than pulp, suggesting its potential use as a dietary supplement. Citrus peel boasts higher polyphenol content than edible components, establishing it as a crucial source of bioactive phenolic compounds, particularly flavonoids. Flavones, isoflavones, and anthocyanins are among the flavonoid compounds present in citrus (Diaz-Uribe et al., 2022).

The present study aimed to unveil the diverse potential of orange peel extract, ranging from phenolic composition and antibacterial efficacy to yogurt fortification.

Materials and methods

Preparations of orange peels extract (OPE)

Orange fruits were washed with distilled water and carefully peeled. The peels were subsequently air-dried in a ventilated oven at 40 °C for 48 h and then ground to a fine powder. Fifty grams of orange peel powder was mixed with 250 mL of 70 % ethanol. The mixture was kept in the dark at room temperature (25 °C) for 24 h before undergoing filtration through Whatman No. 1 filter paper. The resulting extract solution was concentrated using a rotary evaporator under reduced pressure at a temperature of 50 °C. The resulting extracts were stored at −20 °C for subsequent analysis.

Total Phenolic Content (TPC), total flavonoids (TF), and antioxidant activity were determined using DPPH and FRAP assays, following the methodologies outlined by (Arnous et al., 2002; Arnous et al., 2002; Benzie & Strain, 1996; Brand-Williams et al., 1995), respectively.

HPLC analysis

The phenolic and flavonoid components of Citrus reticulata Peel were assessed through High-Performance Liquid Chromatography (HPLC) using an Agilent 1100 system (Santa Clara, CA, USA). A 25 μL volume of the extract was injected, following a previously reported methodology (Gazwi et al., 2022). For the determination of phenolic components, a C18 column (125 × 4.60 mm, particle size 5 μm) coupled with a UV/Vis detector at a 250 nm wavelength was employed. Chromatograms were generated and analyzed using the Agilent ChemStation software. A mobile gradient phase comprising methanol [A] and acetic acid in water (1:25) [B] was utilized to effectively separate the phenolic acid components. The gradient program initiated at 100 % B for three minutes, transitioned to 50 % eluent A for 5 min, followed by 2 min of 80 % A, and concluded with 5 min of 50 % A. Detection occurred at a wavelength of 250 nm. Similarly, the flavonoid components were identified using the same HPLC system and a C18 column (250 × 4.6 mm, 5 μm). The UV/Vis detector was set at a 360 nm wavelength. An isocratic elution procedure of acetonitrile (A) and 0.2 % (v/v) aqueous formic acid (B) in a 70:30 ratio was employed for this purpose.

Test microorganism

The pathogenic bacterial strains used in this investigation included *Bacillus subtillus* (NIOF B15), *Staphylococcus aureus* (NIOF-B16), *Escherichia coli* (NIOF-B17), *Enteroco ccus faecalis* (NIOF-B21), *Pseudomonas aeruginosa* (NIOF-B23), *Vibrio fluvialis* (NIOF-B24), *Vibrio damsela* (NIOF-B29), *Bacillus cereus* (NIOF-B33) and *Salmonella typhimurium* (NIOF-B35) (Abdel-Hameed et al., 2023). These selected marine pathogenic strains were obtained from the NIOF Microbiological Lab (National Institute of Oceanography and Fisheries, Red Sea branch, Egypt). The pathogenic strains were maintained on nutrient agar slants and the slants were folded with 25 % glycerol and stored at −4 °C for long preservation

Agar-well diffusion assay

The antibacterial activity was assessed using the agar-well diffusion assay technique. An antibacterial susceptibility test for orange peels extract (OPE) was conducted against the chosen pathogens. In a petri dish filled with 20 mL of Muller Hinton agar media (comprising g/L: beef extract 2.0; acid hydrolysate of casein 17.5; starch 1.5; and agar 17.5), the surface of the agar plate was inoculated by evenly spreading 0.1 mL of a bacterial suspension containing 10^5 CFU/mL across the entire agar surface. Subsequently, a sterile cork borer was used to aseptically create an 8 mm diameter well, into which 100 uL of orange peels extract (OPE) was added. In the agar wells of control plates, we introduced DMSO (0.5 %) (obtained from R&M Marketing, Essex, UK) as a negative control, followed by incubating the plates at 37 °C for 24 h (Gazwi et al., 2022).

Minimum inhibitory concentration (MIC)

We utilized a tetrazolium microplate assay to ascertain the minimum inhibitory concentrations (MICs) of the test organisms. For this experiment, a 96-well clear microtiter plate was employed. In each well of the 96-well plate, we introduced a suspension of freshly isolated bacteria (0.1 mL) at a concentration of 5×10^5 CFU/mL. We then prepared different concentrations, ranging from 15 to 0.25 mg/mL, of the test extract by serial dilution with Muller–Hinton broth (Becton Dickinson, Sparks, MD, USA). Subsequently, 200 μL of each concentration was added in triplicate to the wells, and the plates were incubated for 18–24 h at 37 °C ± 0.5. After the incubation period, we added 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) with a concentration of 0.2 mg/mL to each well, followed by incubation at 37 °C for 30 min. The bacterial suspension without the extract served as the positive control, while the corresponding solvent blank (DMSO) was used as the negative control. To determine the percentage reduction of the dye (indicating the inhibition of bacterial growth), we measured the absorbance at 570 nm relative to a reference wavelength of 650 nm, which was achieved by introducing DMSO to the spectrophotometer (Pourali et al., 2017).

Time-Kill assay

Based on the preliminary findings, orange peels extract (OPE) exhibited the highest level of antimicrobial activity against marine *E. coli* (NIOF-B17). To further investigate the bactericidal effects of orange peels extract (OPE) on *E. coli* (NIOF-B17), a time-kill test was conducted. A bacterial culture with a concentration of 5×10^6 CFU/mL was introduced into Mueller Hinton broth (MHB) containing the extract at four times 4 × MIC, 2 × MIC, MIC 1/2 × MIC, and ¼ × MIC. These cultures, along with untreated ones, were incubated at 37 °C. Subsequently, samples were taken at various time points (0, 2, 4, 6, 8, 10, 12, and 24 h) and cultured on Tryptic Soy Agar (TSA) plates. Additionally, the control incubation was conducted with 1 % DMSO. The surviving colony-forming bacteria were enumerated, and log10 CFU/mL was calculated. The results were analyzed by creating a time-kill curve, which plotted log CFU/mL against time (minutes) (Saeloh & Visutthi, 2021).

Estimation of orange peel extract (OPE) antibacterial activity

The antimicrobial activity of the extract was assessed by comparing it with certain standard commercial antibiotics, following the disc diffusion method as described by Bauer et al. (1966). Approximately 250 mg of the extract was impregnated onto sterile discs (prepared by punching Whatman No.1 filter paper into discs) and then dried under sterile conditions at room temperature. For the comparative study, four standard commercial antibiotic discs were utilized: 20/10 mg/disc of Amoxicillin/Clavulanic acid, 5 mg/disc of Ciprofloxacin, 30 mg/disc of Chloramphenicol and 30 mg/disc of Amikacin (Oxoid Ltd, England). These discs were placed on the surfaces of plates that had been inoculated with *E. coli* (NIOF-B17) (at a McFarland scale rating of 1) as the target strain. The plates were subsequently incubated at 37 °C overnight. Following incubation, the inhibition zone around each disc was

measured in millimeters (mm). The assays were conducted in three replicates (Abd-Elnaby et al., 2016).

Synergistic activity

Orange peels extract (OPE) was assessed in combination with and 5 mg/disc of Ciprofloxacin using the standard disc diffusion method against the selected marine *E. coli* (NIOF-B17). Antibacterial activity was examined on an agar plate using discs that were prepared by combining Ciprofloxacin (5 mg/disc) with varying concentrations of selected extract (250, 500, 750, and 1000 μg/mL). To gauge the effectiveness of orange peels extract (OPE) and Ciprofloxacin combination, the size of the inhibition zone was measured following a 24-hour incubation period at 37 °C.

Manufacturing the fortified yoghurt with OPE

Yogurt was crafted from fresh cow's milk with a 5 % fat content. The milk was heated to 90 °C for 5 min, then cooled to 40 °C before being divided into four equal portions. The first portion served as the control (C), while the remaining three portions were treated with orange peel extract (OPE) at concentrations of 0.25 %, 0.5 %, and 1 % (v/v), denoted as T1, T2, and T3, respectively. Subsequently, all milk sections were inoculated with 3 % (v/v) of a commercial yogurt culture *Lactobacillus delbrueckii* subsp. *Bulgaricus and Streptococcus thermophilus* (YO-MIX 260 LYO 500 DCU Danisco, France).The mixture was poured into clean dry plastic cups and incubated at 42–45 °C until coagulation, the cups were then placed in the refrigerator at 4 °C and stored at 4 °C for 0 days (fresh), 7 days, and 14 days before analysis. Various physico-chemical, microbiological, and sensory tests were conducted on the samples.

Physico-chemical analyses

The moisture, protein, and ash content of the samples were assessed in accordance with the procedures outlined in AOAC (2000). The nitrogen content, obtained through the Kjeldahl technique, was utilized to calculate the protein content in milk and various combinations of yogurt containing OPE using the formula (T.N. × 6.38). The moisture content was determined by subjecting samples to drying until a constant weight was achieved at 105 ± 1 °C. Ash content was determined by incinerating samples at 625 °C in a muffle oven. Fat percentage in different samples was determined using the Soxhlet extraction method over 6 h with petroleum ether (Abd-Elnaby et al., 2016).

Acidity in all samples was determined following the AOAC (2000) method. Ten grams of yogurt were mixed with 10 mL of distilled water and titrated with 0.1 N NaOH using a phenolphthalein indicator, with titratable acidity expressed as lactic acid g/L. pH values were measured at room temperature using a pH meter (Adwa, waterproof pH meter, Romania).

Water-holding capacity (WHC)

The Water Holding Capacity (WHC) of yogurt containing OPE was assessed following the methodology outlined by Akalın et al. (2012).

Microbiological analysis

The viable counts of *L. delbrueckii* subsp. *bulgaricus* in yogurt containing OPE were determined using the standard plate count method, as per the procedure outlined by Dhawi et al. (2020). *Lactobacillus bulgaricus (L. bulgaricus)* was enumerated using MRS agar culture media. The plates were incubated in anaerobic conditions at 42 °C for 48 h or 37 °C for 72 h to enumerate *L. bulgaricus*. The results were expressed as the logarithm of colony-forming units per gram (cfu/g). Mold and yeast enumeration followed the standard methods for examining dairy products.

Sensory evaluation of yoghurt samples

The assessment of the acceptability of yogurt containing OPE involved a 14-day storage period at 4 °C, with panelists comprising postgraduates and staff members from Minia University, Faculty of Agriculture, following the methodology outlined by Ibrahim et al. (2020). The sensory evaluation included flavor, which was assigned 45 points, body and texture with 30 points, appearance with 15 points, acidity with 10 points, and an overall acceptance score of 100 points for yogurt containing OPE.

Antitumor activity using in vitro MTT assay cell lines

The cell line included in the study is colorectal carcinoma colon cancer (HCT-116). The inhibitory effects of yogurt containing OPE on cell growth were examined using the MTT test, following the methodology outlined by Etaiw et al. (2018). The HCT-116 cell line was sourced from ATCC through the Holding Company for Biological Products and Vaccines (VACSERA) in Cairo, Egypt.

Statistical analysis

All data were presented as mean ± standard deviation (SD) based on three replicates. Statistical analyses for color values, antibacterial activity, and cytotoxic activity were conducted using one-way analysis of variance (ANOVA). For the remaining data, SPSS Statistics 22.0 was employed with a two-way ANOVA to assess significant differences among treatment means and across different storage periods. The means of the results were further compared using the Tukey test, considering a significance level of 5 % ($P < 0.05$).

Result and discussion

Phenolic compounds and scavenging ability in orange peel extract (OPE)

Phenolics, products of plants' secondary metabolism, are widely recognized for their biological effects, particularly their antioxidant properties. These compounds serve as antioxidants, exerting both biological and chemical effects, primarily through their ability to scavenge free radicals. In Table1, the considerable ($p < 0.05$) phenolic content, encompassing both total phenolic content (TPC) and total flavonoids (TF), is depicted in the OPE. The concentrations of TPC and TF in OPE were determined to be 174.29 ± 0.072 mg GAE/g and 57.2 ± 0.147 mg QE/g, respectively. As shown in Table 1, OPE possessed strong antioxidant activity measured as FRAP and DPPH, as shown in Table 1.

HPLC analysis

The HPLC chromatograms of OPE revealed numerous polyphenolic compounds, including seven phenolic acids and six flavonoids, as detailed in Table2 and Figs. S1and S2. Among these compounds, the most abundant phenolic substances identified were Salicylic acid (12.66 μg/g), Syringic acid (10.54 μg/g), and Cinnamic acid (7.065 μg/g). Additionally, the characterized flavonoids comprised Luteolin (15.74 μg/g), Quercetin (10.14 μg/g), and Naringin (2.55 μg/g).

Table 1
Total phenolic content, total flavonoid, antioxidant activity (FRAP and DPPH) in orange peel extract (OPE).

Parameter	OPE
Total phenolic (mg GAE/g)	174.29 ± 0.07
Total flavonoid (mg QE/g)	57.2 ± 0.15
FRAP (μM Trolox/mg extract)	795.87 ± 0.08
DPPH (IC50 μg/mL)	59.4 ± 0.89

Data are presented as mean ± SD.

Table 2
HPLC analysis of OPE.

Components	RT (min)	Conc μg/mg
Phenolic Compounds		
Catechol	4.0	5.22
Syringenic	5.0	10.54
Cinnamic	7.0	7.065
Caffeic	8.0	2.33
Pyrogallol	9.4	3.14
Ferulic	11.0	6.87
Salicylic	12.0	12.66
Flavonoids Compounds		
Naringin	4.6	2.55
Rutin	5.2	1.47
Quersestin	6.9	10.14
Kampferol	8.1	1.46
Luteolin	9.0	15.74
Apegenin	10.0	2.33

The phenolic composition identified here aligns with earlier investigations into the phenolic makeup of Citrus reticulata peels (Zefang et al., 2016). Nonetheless, the quantity of individual phenolic compounds appeared to differ marginally compared to those reported in other studies (Wang et al., 2008). This variance may arise from differences in the solvents utilized for extraction, extraction duration, or the specific cultivar, maturity stage, and environmental conditions (Wang et al., 2008).

Our investigations reveal that ferulic acid is one of the predominant phenolic acids present in the extract, consistent with the findings of Kelebek (2010). The concentrations of ferulic and caffeic acids in our citrus samples differed from those reported by Wang et al. (2007). Additionally, cinnamic acid levels were significantly elevated, aligning with the results of Ye et al. (2011).

Several studies have highlighted the antioxidant activity of cinnamic acid (Sánchez-Maldonado et al., 2011), indicating that tangerines are a rich source of antioxidants and antibacterial agents. Numerous studies have explored the antioxidant potential of phenolic and flavonoid compounds derived from diverse plant sources (Shehata et al., 2021). Their demonstrated efficacy in reducing oxidative stress both in vitro and in vivo positions them as promising candidates for the development of natural antioxidants (Shehata et al., 2021). Furthermore, these compounds exhibit potential in mitigating various chronic diseases, including cardiovascular diseases (Razavi-Azarkhiavi et al., 2016), cancer, diabetes, and neurodegenerative disorders.

Biological activity of orange peel extracts (OPE)

Anti-microbial activity

In recent times, there has been a worldwide focus on extracting valuable compounds from agricultural byproducts for their application in food preservation. Despite the fact that citrus peels are not consumed directly, they are known to contain substantial biological activities, including anti-cancer properties, antioxidant, and antimicrobial (Shehata et al., 2021). In this study, we selected nine distinct strains of marine pathogenic bacteria, which included *B. subtillus, S. aureus, E. faecalis, E. coli, P. aeruginosa, V. fluvialis, V. damsela, B. cereus, and S. typhimurium*. The orange extract exhibited an antibacterial impact against the majority of the tested strains, resulting in average inhibition zones ranging between 10 and 18 mm (as detailed in Table S1). The extract displayed potent antibacterial activity against *E. coli*, resulting in a notable inhibition zone of 18.0 ± 0.1 mm (Fig. S3). It exhibited moderate antibacterial activity against *S. aureus, E. faecalis, P. aeruginosa, V. damsela,* and *B. cereus* with inhibition zones measuring 12.0 ± 0.1, 12.0 ± 0.2, 14.0 ± 0.6, 14.0 ± 0.1, and 16.0 ± 0.4 mm, respectively. However, it showed a comparatively weaker effect against *B. subtillus* and *S. typhimurium*, resulting in inhibition zones of 10 mm. Interestingly, the orange extract demonstrated ineffectiveness against *V. damsela*. In the case of the negative control (DMSO), there was no observable zone of inhibition. Other studies have previously indicated that extracts obtained from sweet orange and lemon peels display significant antimicrobial activity against pathogens (Shehata et al., 2021). Dubey et al. (2011) documented robust antibacterial activity associated with orange peel extract against a spectrum of microorganisms including *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Shigella flexneri, and Pseudomonas aeruginosav*. In order to assess the susceptibility of the tested strains to the orange extract, we calculated the values for minimum inhibitory concentration (MIC), which are presented in Table S1. The orange extract exhibited the lowest MIC for *E. coli* (1.75 ± 0.02 mg/mL). The MIC values for *S. aureus, P. aeruginosa, E. faecalis, V. damsela,* and *B. cereus* were 12.0 ± 0.02, 8.5 ± 0.01, 10.5 ± 0.06, 4.5 ± 0.05 and 2.5 ± 0.01 mg/mL, respectively. On the other hand, the MIC values against *B. subtillus* and *S. typhimurium* were 14.5 ± 0.02, and 15.00 ± 0.01 mg/mL, respectively.

Bacterial kinetics assay for the effect of orange peel extract on E. coli (NIOF-B17)

The results, as illustrated in Fig. S4, led to the generation of a time-kill curve that plotted the logarithmic number of CFU/mL against incubation time. Notably, at a concentration of 4 times the MIC (4 × MIC), the orange peel extract exhibited a reduction in the viable *E. coli* population between 5 and 24 h of incubation. Killing time studies showed that plant extracts and bacteria had variable levels of time-dependent microbial inhibition. Consequently, plant secondary metabolites and the reaction to microbial infection might be indicators of antibacterial capabilities (Dabija et al., 2018).

Estimation of the orange extract antibacterial activity

The extract from orange peel was assessed for its antibacterial effectiveness against marine *E. coli* (NIOF-B17) by comparing it to commercially available standard antibiotics (Fig. S5). The disc containing the extract displayed an inhibition zone of 18 mm, while the antibiotic discs containing 30 mg/disc of Tetracycline, 10 mg/disc of Streptomycin, 20 mg/disc of Chloramphenicol and 30 mg/disc of Amikacin showed inhibition zones of 10 mm, 12 mm, 16 mm and 26 mm, respectively.

Synergistic impact of orange peel extract

Fig. S6 and Table S2 present the findings of an investigation into the synergistic effects of orange-peel extract and Amikacin against a specific pathogen known as marine *E. coli.* Amikacin, at a concentration of 30 mcg, demonstrated a moderately effective response against the tested *E. coli*. In contrast, when compared to concentrations of 250 and 750 μg/mL, the combined action of antibiotics and orange peel extract exhibited significantly enhanced antibacterial activity against the selected pathogen at a concentration of 1000 μg/mL. This increased activity was evident in the size of the inhibition zone, which ranged from **26 ± 0.2** to **33 ± 0.1** mm (as indicated in Table S2). The observed synergy between orange peel extract and Amikacin has led to the hypothesis that their combined mechanism is responsible for this heightened effectiveness.

Yoghurt fortified with OPE: New insights into its functional properties

Chemical composition of functional yoghurt

The chemical composition of the yogurt samples, including total proteins, total solids (TS), fat, and ash contents, is presented in Table 3. The current findings indicate a significant ($p \leq 0.05$) enhancement in Total proteins, Total Solids, Fat, and Ash values upon the addition of orange peel extract (OPE), with the most substantial increase observed at 1 % OPE (T3), followed by 0.5 % OPE (T2). Conversely, protein content exhibited insignificant changes with the addition of OPE. The alterations in total solids and ash contents among treatments were likely attributed to the incorporation of OPE. Similar trends in total solids were

Table 3
Chemical composition (%) of yoghurt fortified with different concentrations of OPE (0.25 0.5, and 1 % of yoghurt).

Treatments	Total proteins	Total solids	Fat	Ash	Carbohydrate
C	3.61 ± 0.06	13.35 ± 0.06	3.27 ± 0.03	0.70 ± 0.01	5.78 ± 0.07
T1	3.66 ± 0.08	13.34 ± 0.08	3.40 [a] ± 0.06	0.71 ± 0.01	5.58 ± 0.05
T2	3.75 ± 0.08	13.40 ± 0.08	3.47 [a] ± 0.03	0.71 ± 0.01	5.47 ± 0.17
T3	3.84[a] ± 0.04	13.46 ± 0.11	3.63 [a] ± 0.03	0.72 ± 0.01	5.27[a] ± 0.12

observed when incorporating peanut skin extract and fenugreek and moringa seed flour into buffalo yogurt (Dhawi et al., 2020). This impact can be attributed to the elevated total solids in samples with added OPE (1 % > 0.5 % > 0.25 % > control sample). Furthermore, given that OPE is a polyphenol-rich extract, it interacts with milk proteins to form stable complexes, thereby enhancing the physicochemical properties of the resulting product. Consequently, the addition of plant extracts, particularly those rich in polyphenols, may facilitate increased retention of whey. This dual role involves augmenting total solids and forming stable complexes between polyphenols and proteins. Various studies have reported a comparable effect on the syneresis rate of yogurt by incorporating polyphenol-rich extracts and increasing total solids (Hamed et al., 2021).

The presented values represent means ± standard deviation (SD) based on a sample size of n = 3. Significance among values within a column is denoted by distinct superscripts, indicating a significant difference ($p \leq 0.05$). C, control yoghurt; T1, T2, T3: yoghurt containing 0.25, 0.5, and 1 % OPE, respectively.

Analysis of pH, acidity and WHC total phenolic content and antioxidant activity yoghurt

The pH serves as an indicator of organic acid presence, with acidification being the primary mechanism in yogurt fermentation (Zainoldin & Baba, 2009). The recommended pH range for yogurt is pH 4.6 or lower.The pH values of yogurt across various treatments exhibited a descending order (T3 > T2 > T1) compared to the control (C) both initially and after 14 days of storage. This decrease can be attributed to lactic acid production by the starter culture (Zhong et al., 2018). Functionally, yogurt with Orange Peel Extract (OPE) displayed lower pH and higher acidity after 14 days of storage than the control, aligning with (Zhong et al., 2018). These pH and acidity changes correlate with the growth and activity of yogurt starter and probiotic bacteria, as affirmed by microbiological evaluation (Table 4). The rise in acidity during storage was more pronounced in control yogurt (C) compared to OPE-fortified yogurt, suggesting an impact of the added extract on the growth and acidity of the utilized starter (Dabija et al., 2018).

The application of OPE at varying concentrations influenced the Water Holding Capacity (WHC) of yogurt (Table 4). The WHC of yogurt containing OPE significantly decreased ($P < 0.05$) with increasing OPE concentration. For instance, WHC values for treatments C, T1, T2, and T3 were 63.1 ± 0.49, 62.4 ± 0.41, 61.8 ± 0.69, and 61.8 ± 0.38 at the beginning of storage, and 60.8 ± 0.35, 59.7 ± 0.26, 58.9 ± 0.54, and 59.1 ± 0.38 at the end of storage, respectively ($p < 0.05$). Similar findings were reported by (Karaca et al., 2012), indicating decreased WHC in fruit-added yogurts with concentrated fruit. The reduced WHC might be attributed to higher acidity, affecting both soluble protein complexes and micelle-bound structures (Xu et al., 2015).

Table 4 illustrates a significant increase ($p < 0.05$) in the phenolic content of yogurt with the addition of OPE. Throughout storage, phenolic content steadily increased with OPE, reaching at least twice the content of the control by the end of the storage period.

The antioxidant activity of OPE was assessed using DPPH scavenging activity. Increasing OPE concentration significantly enhanced the DPPH scavenging activity of yogurt ($p < 0.05$) (Table 4). The DPPH scavenging activity of control yogurt (C) was notably lower than that of OPE-fortified yogurt. Adding OPE at different levels (0.25 %, 0.5 %, and 1 %) to the tested yogurt increased antioxidant activity from 11.0 ± 1.1 in control yogurt (C) to 48.3 ± 0.6, 62.6 ± 2.2, and 72.2 ± 0.7 in yogurt fortified with 0.25 %, 0.5 %, and 1 % OPE (T3), respectively, at zero days of storage (Table 4). The DPPH scavenging activity of yogurt containing OPE increased significantly ($p < 0.05$) during storage periods at refrigerator temperature up to 14 days (Table 4). The enhanced antioxidant activity is attributed to the presence of flavonoids and phenolic boosted by OPE. Yogurt containing the highest percentage of extract (T2 and T3) exhibited increased antioxidant activity by 74.6 ± 2.3 and 77.5 ± 1.2, respectively, presenting promising results for enhancing yogurt biological activity through the enrichment with orange (*Citrus reticulata* L.) by-products.

This augmentation in the bioactivity of yogurt enriched with OPE is primarily linked to the number of phenolic compounds added through the extract (Ahmed et al., 2021). Such evidence supports the assertion that the incorporation of OPE enhances the bioactive properties and antioxidant activity of yogurt, thereby potentially contributing to its health benefits. Similar findings have been reported in other publications, demonstrating the positive impact of different plant extracts on yogurt bioactivity. The fortifying yogurt with natural plant extracts rich in bioactive compounds, such as phenolics, has the potential to enhance the health benefits of dairy products, given their high daily demand.

Means with the same superscripts (a, b, c, d) in each column do not differ significantly ($P < 0.05$). C, control yoghurt; T1, T2, T3: yoghurt containing 0.25, 0.5, and 1 % OPE, respectively.

Table 4
Effect of different OPE concentrations on pH, acidity, and WHC, Total phenolic content, and antioxidant activity of yoghurt during storage at 4 °C for 14 days.

Properties	Yogurt Samples	Storage (Days)		
		Zero	7	14
pH	C	4.61[a] ± 0.02	4.33[a] ± 0.02	4.25[a] ± 0.03
	T1	4.57[b] ± 0.02	4.57[b] ± 0.01	4.48[b] ± 0.01
	T2	4.57[b] ± 0.01	4.49[c] ± 0.01	4.44[c] ± 0.01
	T3	4.54[b] ± 0.02	4.44[d] ± 0.01	4.36[d] ± 0.01
Acidity	C	0.81[a] ± 0.006	0.89[a] ± 0.009	0.90[a] ± 0.003
	T1	0.84[b] ± 0.009	0.89[a] ± 0.012	0.93[b] ± 0.01
	T2	0.86[c] ± 0.006	0.94[b] ± 0.009	0.95[b] ± 0.01
	T3	0.85[bc] ± 0.006	0.91[ab] ± 0.006	0.95[b] ± 0.01
WHC	C	63.1[a] ± 0.49	61.6[a] ± 0.53	60.8 [a] ± 0.35
	T1	62.4[a] ± 0.41	61.3[a] ± 0.24	59.7[b] ± 0.26
	T2	61.8[a] ± 0.69	60.3[b] ± 0.64	58.9[c] ± 0.54
	T3	61.8[a] ± 0.38	60.3[b] ± 0.50	59.1[bc] ± 0.38
Total phenolic content	C	117.69[a] ± 1.3	136.15[a] ± 2.22	150.00[a] ± 4.23
	T1	156.41[b] ± 3.6	170.00[b] ± 1.60	177.69[b] ± 2.0
	T2	171.02[c] ± 1.8	183.33[c] ± 2.28	196.92[c] ± 0.89
	T3	200.26[d] ± 2.2	247.43[d] ± 4.99	274.61[d] ± 4.07
Antioxidant activity	C	11.0[a] ± 1.1	16.7[a] ± 0.6	17.0[a] ± 2.0
	T1	48.3[b] ± 0.6	58.4[b] ± 2.0	59.6[b] ± 2.0
	T2	62.6[c] ± 2.2	70.6[c] ± 1.9	74.6[c] ± 2.3
	T3	72.2[d] ± 0.7	76.6[d] ± 0.8	77.5[d] ± 1.2

Microbiological quality of yoghurt

The impact of Orange Peel Extract (OPE) addition to yogurt on the population of starter microorganisms during 14 days of storage at 4 °C, including total bacteria count, presence of yeast and molds, and Lactobacilli counts (MRS medium), is presented in Table 5. For the control yogurt (C), the total bacteria count remained relatively stable, ranging from 6.81 ± 0.09 to 6.88 ± 0.03 log CFU/g, with no detection of yeast and molds. *L. bulgaricus* counts ranged from 6.84 ± 0.01 to 6.72 ± 0.05 log CFU/g across the storage periods. Similar findings were reported by (El-Batawy, 2012), who observed a decrease in the growth of yogurt cultures during the cold storage period.

Yogurt fortified with 0.25 % OPE (T1) showed an initial increase in bacterial count from 7.10 ± 0.05 to 7.34 ± 0.07 log CFU/g, representing an approximate 3.38 % increase, gradually decreasing to 7.10 ± 0.05 by day 14. Similar to the control yogurt (C), no yeast and molds were detected, while Lactobacilli counts fluctuated slightly between 6.92 ± 0.02 and 6.95 ± 0.02 log CFU/g. Treatment T2 demonstrated an increase in bacterial count from 7.09 ± 0.03 to 7.40 ± 0.10 log CFU/mL over 14 days, showing an approximate 4.37 % increase. As observed in other treatments, no yeast and molds were detected, whereas Lactobacilli counts ranged between 6.99 ± 0.01 and 7.29 ± 0.13 log CFU/g. For T3, there was an increase in bacterial count from 7.36 ± 0.04 to 7.54 ± 0.03 log CFU/g by day 7, showing an approximate 2.44 % increase, later slightly decreasing by day 14. Yeast and molds were not detected in these samples, while Lactobacilli counts ranged between 7.23 ± 0.07 and 7.47 ± 0.09 log CFU/g. Overall, treatments T1, T2, and T3 showed varying effects on bacterial counts over the storage duration. However, yeast and molds were not detected across all treatments, indicating a consistent absence throughout the experimental period.

El-Batawy (2012) reported similar observations, noting a decline in the growth of yogurt cultures during cold storage. Moreover, our study detected a gradual reduction in the viability of *L. bulgaricus* in the control yogurt over the cold storage period. This decline may be attributed to the strain's susceptibility to acid development, as documented by Bisar et al. (2015). In contrast, fortifying yogurt with OPE significantly bolstered the viability of the *L. bulgaricus* throughout the storage duration, aligning with findings by Bisar et al. (2015). In general, the food industry targets bacterial populations exceeding 106 probiotics per gram at the time of consumption, as recommended by Salem et al. (2005).

Means with the same superscripts (a, b, c) in each column do not differ significantly ($P < 0.05$). C, control yoghurt; T1, T2, T3: yoghurt containing 0.25, 0.5, and 1 % OPE, respectively.

Table 5
Effect of different OPE concentrations on microbiological properties (Log CFU/mL) of yoghurt during storage at 4 °C for 14 days.

Properties	Yogurt Samples	Storage (Days)		
		Zero	7	14
Total count of bacteria	C	6.88^{a} ± 0.03	6.83^{a} ± 0.08	6.81^{a} ± 0.09
	T1	7.13^{b} ± 0.09	7.34^{b} ± 0.07	7.10^{b} ± 0.05
	T2	7.28^{c} ± 0.01	7.40^{b} ± 0.10	7.09^{b} ± 0.03
	T3	7.33^{c} ± 0.07	7.54^{b} ± 0.03	7.36^{c} ± 0.04
Yeast & molds	C	ND	ND	ND
	T1	ND	ND	ND
	T2	ND	ND	ND
	T3	ND	ND	ND
L. bulgaricus	C	6.84^{a} ± 0.01	6.87^{a} ± 0.02	6.72^{a} ± 0.05
	T1	6.92^{ab} ± 0.02	6.93^{a} ± 0.01	6.92^{b} ± 0.02
	T2	6.99^{b} ± 0.01	7.27^{b} ± 0.15	7.29^{a} ± 0.13
	T3	7.23^{c} ± 0.07	7.47^{b} ± 0.09	7.26^{b} ± 0.05

Sensory evaluation

The assessment of sensory attributes is pivotal in gauging consumer approval of dairy products, including yogurt. Table 6 presents the sensory acceptance of yogurt fortified with OPE. Generally, the plain yogurt sample (C) demonstrated the highest ratings for tested descriptors (flavor, body and texture, appearance, and overall acceptability) at both 1 and 14 days of cold storage compared to OPE-fortified yogurt samples (T1, T2, and T3). As the cold storage duration extended, the various treatments exhibited diminishing scores in sensory evaluation. Despite a decline in all tested descriptors towards the end of cold storage, panelists expressed substantial acceptability for the diverse yogurt samples. The observed decline in these descriptors may be attributed to increased acidity at the final storage time, impeding the formation of aromatic components (Basiri et al., 2018). Additionally, it could be partly elucidated by the decrease in yogurt acetaldehyde concentration as cold storage progresses (Granato et al., 2010).

These findings propose the potential to create yogurt with notable antifungal and antibacterial properties while preserving the sensory attributes of fermented milk. Furthermore, the inclusion of orange peel extract enhanced the flavor profile of the end product and concealed defects through the natural evolution of aroma. Gahruie et al. (2015) stressed that, despite the health advantages associated with fiber, formulations surpassing 3 % fiber content are generally met with low consumer acceptance. In light of these outcomes, it is evident that orange peel extract can be introduced to yogurt at levels of 0.25 % and 0.5 % to achieve products with the highest ratings for all assessed characteristics.

Means with the same superscripts (a, b) in each column do not differ significantly ($P < 0.05$). C, control yoghurt; T1, T2, T3: yoghurt containing 0.25, 0.5, and 1 % OPE, respectively.

Table 6
Sensory evaluation of yoghurt fortified with orange peel extract during storage at 4 °C for 14 days.

Properties	Yogurt Samples	Storage (Days)		
		Zero	7	14
Flavour (50)	C	46.4^{a} ± 0.64	43.0^{a} ± 1.04	40.6^{a} ± 0.71
	T1	44.3^{a} ± 1.33	41.5^{a} ± 0.29	39.1^{ab} ± 0.70
	T2	45.7^{ab} ± 0.72	41.8^{ab} ± 0.92	39.5^{ab} ± 0.93
	T3	41.5^{b} ± 0.87	38.9^{b} ± 0.94	37.8^{b} ± 0.73
Body & texture (40)	C	35.83^{a} ± 0.44	32.50^{a} ± 0.50	35.17^{a} ± 0.60
	T1	35.63^{a} ± 0.49	32.10^{a} ± 0.67	35.50^{a} ± 0.50
	T2	35.83^{a} ± 0.44	35.50^{b} ± 0.29	32.50^{a} ± 0.50
	T3	35.17^{a} ± 0.60	33.83^{c} ± 0.17	32.10^{a} ± 0.67
Appearance (10)	C	8.37^{a} ± 0.37	8.10^{a} ± 0.10	7.67^{a} ± 0.20
	T1	8.47^{a} ± 0.26	7.50^{b} ± 0.25	6.97^{a} ± 0.20
	T2	8.67^{a} ± 0.20	7.57^{b} ± 0.30	6.97^{a} ± 0.55
	T3	8.37^{a} ± 0.26	7.53^{b} ± 0.29	6.50^{a} ± 0.40
Overall acceptability (100)	C	90.30^{a} ± 1.28	86.60^{a} ± 1.23	80.77^{a} ± 0.70
	T1	88.27^{ab} ± 1.65	83.50^{b} ± 0.21	78.00^{ab} ± 0.62
	T2	89.53^{ab} ± 1.39	83.20^{b} ± 1.17	78.53^{b} ± 0.28
	T3	85.70^{b} ± 1.17	81.27^{b} ± 0.66	76.07^{b} ± 1.21

Cytotoxic activity

Increasing attention is directed towards functional foods that enhance consumer health, especially those abundant in phytochemicals recognized for their pivotal role in diminishing the occurrence of chronic diseases, such as cancer. This study utilized MTT-based assays to evaluate the in vitro cytotoxic impacts of yogurt fortified with OPE, as elaborated in Table 7 and Fig. S7. The concentration inducing a 50 % reduction in the cell monolayer was designated as the cytotoxicity half-maximal inhibitory concentration (IC50), with doxorubicin serving as the standard anticancer drug for comparative analysis. The results indicate varying degrees of inhibition of the HCT116 cell line by the control and three yogurts containing orange peel extract (C, T1, T2, and T3). The cytotoxic activity of yogurt fortified with OPE increased with higher OPE concentrations (Table 7). Specifically, the yogurt samples containing 0.5 % (T2) and 1 % (T3) orange peel extract exhibited potent activity (IC50 = 15.29 ± 2.92 μg/ml and 16.26 ± 2.75 μg/ml, respectively) against the HCT116 cell line, while the control yogurt (C) demonstrated moderate activity (IC50 = 47.45 ± 1.31 μg/ml) (Table 7).

The increase in OPE concentration correspondingly decreased the relative viability of the HCT116 cell line (Fig. **S7**). Yogurt samples containing various concentrations of OPE (T1, T2, and T3) demonstrated a dose-dependent effect, with the yogurt sample containing 1 % OPE (T3) exhibiting a more pronounced impact on reducing cell line viability compared to the control and other treatments.

The anticancer activity observed in the fortified yogurt is attributed to the presence of phenolic substances derived from OPE. These findings align with Zhao et al. (2016), who highlighted the abundance of phytochemical compounds inhibiting angiogenesis in pancreatic and breast cancers. Zaki and Naeem (2021) supported previous findings by confirming the cytotoxicity of OPE, attributing it to its phytochemical composition. Additionally, various plant-derived compounds are believed to possess cytotoxic properties against cancer cells and may act as chemopreventive agents in human cancer development (Kobayashi et al., 2002). In their research, Im et al. (2014) discovered that citrus peels contain a substantial amount of total phenolic compounds and exhibit potent antioxidant and anticancer properties.

Table 7
Cytotoxic activity of yoghurt fortified with OPE against HCT-116 cell line.

	IC50 (μg/mL)
Doxorubicin	9.18 ± 0.12
C	47.45 ± 1.31
T1	39.87 ± 7.25
T2	16.26 ± 2.75
T3	15.29 ± 2.92

IC50 (μg): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), and ≥100 (non-cytotoxic). C, control yoghurt; T1, T2, T3: yoghurt containing 0.25, 0.5, and 1 % OPE, respectively.

Conclusion

The comprehensive investigation into orange peel extract (OPE) highlights its versatile potential across various applications. Rich in phenolic compounds like salicylic acid and quercetin, OPE demonstrates robust antioxidant activity and significant antimicrobial effects, particularly against E. coli. Fortifying yogurt with OPE enhances its functional properties, increasing total proteins, solids, and fat content while affecting pH and water holding capacity. The fortified yogurts exhibit elevated phenolic content and antioxidant activity, indicating potential health benefits. Microbiological assessments confirm stability, and sensory evaluations show acceptable levels of palatability, especially at lower OPE concentrations. Additionally, cytotoxic assays reveal promising anti-cancer properties in OPE-fortified yogurts, suggesting synergistic effects. This underscores OPE's potential as a functional ingredient in developing health-promoting dairy products, contributing to the expanding field of functional foods and paving the way for further research and product development.

CRediT authorship contribution statement

Asmaa Hussein Zaki: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation. **Hanaa Salem Saleh Gazwi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Moaz Mohamed Hamed:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation. **Salma Mohamed Galal:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Awatif Musallam Almehmadi:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Funding acquisition, Conceptualization. **Areej Abdulhamid Almuraee:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Amal Fahad Alqurashi:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Funding acquisition. **Eman Elhossainy Yassien:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101458.

References

Abdel-Hameed, S. M., Abd Allah, N. A. R., Hamed, M. M., & Soltan, O. I. A. (2023). Papaya fruit by-products as novel food ingredients in cupcakes. *Annals of Agricultural Sciences, 68*, 60–74.

Abd-Elnaby, H., Abo-Elala, G., Abdel-Raouf, U., Abd-elwahab, A., & Hamed, M. (2016). Antibacterial and anticancer activity of marine Streptomyces parvus: Optimization and application. *Biotechnology & Biotechnological Equipment, 30*, 180–191.

Ahmed, I. A. M., Alqah, H. A. S., Saleh, A., Al-Juhaimi, F. Y., Babiker, E. E., Ghafoor, K., … Fickak, A. (2021). Physicochemical quality attributes and antioxidant properties of set-type yogurt fortified with argel (Solenostemma argel Hayne) leaf extract. *LWT, 137*, Article 110389.

Akalın, A. S., Unal, G., Dinkci, N., & Hayaloglu, A. A. (2012). Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. *Journal of Dairy Science, 95*, 3617–3628.

AOAC. (2000). *Official methods of analysis* (17th Ed.). Gaithersburg, MD, USA: The Association of Official Analytical Chemists.

Arnous, A., Makris, D. P., & Kefalas, P. (2002). Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *Journal of Food Composition and Analysis, 15*, 655–665.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*, 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Basiri, S., Haidary, N., Shekarforoush, S. S., & Niakousari, M. (2018). Flaxseed mucilage: A natural stabilizer in stirred yogurt. *Carbohydrate Polymers, 187*, 59–65.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*, 493–496.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Bisar, G. H., El-Saadany, K., Khattab, A., & El-Kholy, W. M. (2015). Implementing maltodextrin, polydextrose and inulin in making a synbiotic fermented dairy product. *British Microbiology Research Journal, 8*, 585–603.

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*, 25–30.

Costanzo, G., Iesce, M. R., Naviglio, D., Ciaravolo, M., Vitale, E., & Arena, C. (2020). Comparative studies on different citrus cultivars: A revaluation of waste mandarin components. *Antioxidants (Basel, Switzerland), 9*(6). https://doi.org/10.3390/antiox9060517

Dabija, A., Codină, G. G., Ropciuc, S., Gâtlan, A.-M., & Rusu, L. (2018). Assessment of the antioxidant activity and quality attributes of yogurt enhanced with wild herbs extracts. *Journal of Food Quality*.

Dhawi, F., El-Beltagi, H. S., Aly, E., & Hamed, A. M. (2020). Antioxidant, antibacterial activities and mineral content of buffalo yoghurt fortified with fenugreek and Moringa oleifera seed flours. *Foods, 9*, 1157.

Diaz-Uribe, C., Vallejo, W., De la Hoz, T., Florez, J., Muñoz-Acevedo, A., Zarate, X., & Schott, E. (2022). Theoretical and kinetic study of the singlet oxygen quenching reaction by hesperidin isolated from mandarin (Citrus reticulata) fruit peels. *Chemical Papers, 76*, 169–178. https://doi.org/10.1007/s11696-021-01825-2

Dubey, N. K., Ravindra Shukla, R. S., Ashok Kumar, A. K., Priyanka Singh, P. S., & Bhanu Prakash, B. P. (2011). Global scenario on the application of natural products in integrated pest management programmes. In *Natural products in plant pest management* (pp. 1–20). Wallingford, UK: CABI.

El-Batawy, O. I. (2012). Production and properties of low-fat set yoghurt made with Jerusalem artichoke powder. *Egypt Journal Food Science, 40*, 77–90.

Etaiw, S. E. H., Fayed, T. A., El-bendary, M. M., & Marie, H. (2018). Three-dimensional coordination polymers based on trimethyltin cation with nicotinic and isonicotinic acids as anticancer agents. *Applied Organometallic Chemistry, 32*, Article e4066.

Gahruie, H. H., Eskandari, M. H., Mesbahi, G., & Hanifpour, M. A. (2015). Scientific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellness, 4*, 1–8.

Gazwi, H. S. S., Shoeib, N. A., Mahmoud, M. E., Soltan, O. I. A., Hamed, M. M., & Ragab, A. E. (2022). Phytochemical profile of the ethanol extract of Malvaviscus arboreus red flower and investigation of the antioxidant, antimicrobial, and cytotoxic activities. *Antibiotics, 11*, Article 1652.

Gharibzahedi, S. M. T., & Chronakis, I. S. (2018). Crosslinking of milk proteins by microbial transglutaminase: Utilization in functional yogurt products. *Food Chemistry, 245*, 620–632.

Granato, D., Branco, G. F., Cruz, A. G., Faria, J. A. F., & Shah, N. P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety, 9*, 455–470.

Hamed, A. M., Taha, S. H., Darwish, A. A., & Aly, E. (2021). Antioxidant activity and some quality characteristics of buffalo yoghurt fortified with peanut skin extract powder. *Journal of Food Science & Technology, 58*, ,, 2431–2440.

Ibrahim, A., Awad, S., & El-Sayed, M. (2020). Impact of pomegranate peel as prebiotic in bio-yoghurt. *British Food Journal, 122*, 2911–2926.

Im, S. J., Kim, J. H., & Kim, M. Y. (2014). Evaluation of bioactive components and antioxidant and anticancer. *Natural Product Communications, 9*, 483–485. https://doi.org/10.4317/jced.524

Karaca, O. B., Saydam, I. B., & Güven, M. (2012). Physicochemical, mineral and sensory properties of set-type yoghurts produced by addition of grape, mulberry and carob molasses (Pekmez) at different ratios. *International Journal of Dairy Technology, 65*, 111–117.

Kelebek, H. (2010). Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (Citrus paradisi) cultivars grown in Turkey. *Industrial Crops and Products, 32*, 269–274.

Kobayashi, T., Nakata, T., & Kuzumaki, T. (2002). Effect of flavonoids on cell cycle progression in prostate cancer cells. *Cancer Letters, 176*, 17–23.

Pourali, P., Badiee, S. H., Manafi, S., Noorani, T., Rezaei, A., & Yahyaei, B. (2017). Biosynthesis of gold nanoparticles by two bacterial and fungal strains, Bacillus cereus and Fusarium oxysporum, and assessment and comparison of their nanotoxicity in vitro by direct and indirect assays. *Electronic Journal of Biotechnology, 29*, 86–93.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health, 109*, 309–318.

Razavi-Azarkhiavi, K., Iranshahy, M., Sahebkar, A., Shirani, K., & Karimi, G. (2016). The protective role of phenolic compounds against doxorubicin-induced cardiotoxicity: A comprehensive review. *Nutrition and Cancer, 68*, 892–917.

Reddy, M. K., Gupta, S. K., Jacob, M. R., Khan, S. I., & Ferreira, D. (2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. *Planta Medica, 73*, 461–467. https://doi.org/10.1055/s-2007-967167

Saeloh, D., & Visutthi, M. (2021). Efficacy of Thai plant extracts for antibacterial and anti-biofilm activities against pathogenic bacteria. *Antibiotics, 10*, Article 1470.

Saini, R., Sharma, N., Oladeji, O. S., Sourirajan, A., Dev, K., Zengin, G., & Kumar, V. (2022). Traditional uses, bioactive composition, pharmacology, and toxicology of Phyllanthus emblica fruits: A comprehensive review. *Journal of Ethnopharmacology, 282*, Article 114570. https://doi.org/10.1016/j.jep.2021.114570

Saint-Eve, A., Lévy, C., Martin, N., & Souchon, I. (2006). Influence of proteins on the perception of flavored stirred yogurts. *Journal of Dairy Science, 89*, 922–933. https://doi.org/10.3168/jds.S0022-0302(06)72157-9

Salem, M. M. E., Fathi, F. A., & Awad, R. A. (2005). Production of probiotic ice cream. *Polish Journal of Food and Nutrition Sciences, 14*, 267.

Sánchez-Maldonado, A. F., Schieber, A., & Gänzle, M. G. (2011). Structure-function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. *Journal of Applied Microbiology, 111*, 1176–1184.

Shehata, M. G., Awad, T. S., Asker, D., El Sohaimy, S. A., Abd El-Aziz, N. M., & Youssef, M. M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. *Current Research in Food Science, 4*, 326–335.

Terreni, M., Taccani, M., & Pregnolato, M. (2021). New antibiotics for multidrug-resistant bacterial strains: Latest research developments and future perspectives. *Molecules*, 26.

Wang, Y. C., Chuang, Y. C., & Hsu, H. W. (2008). The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chemistry, 106*, 277–284.

Wang, Y. C., Chuang, Y. C., & Ku, Y. H. (2007). Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. *Food Chemistry, 102*, 1163–1171.

Xu, W., He, S., Ma, Y., Zhang, Y., & Wang, R. (2015). Effect of the heat-induced whey proteins/κ-casein complex on the acid gelation of yak milk. *RSC Advances, 5*, 8952–8956.

Ye, X. Q., Chen, J. C., Liu, D. H., Jiang, P., Shi, J., Xue, S., et al. (2011). Identification of bioactive composition and antioxidant activity in young mandarin fruits. *Food Chemistry, 124*, 1561–1566.

Zainoldin, K. H., & Baba, A. S. (2009). The effect of Hylocereus polyrhizus and Hylocereus undatus on physicochemical, proteolysis, and antioxidant activity in yogurt. *World Academy of Science, Engineering and Technology, 60*, 361–366.

Zaki, N. L., & Naeem, M. M. M. (2021). Antioxidant, antimicrobial and anticancer activities of citrus peels to improve the shelf life of Yoghurt Drink, Egypt. *Egyptian Journal of Food Science, 49*, 249–265.

Zefang, L., Zhao, Z., Hongmei, W., Zhiqin, Z., & Jie, Y. (2016). Phenolic composition and antioxidant capacities of Chinese local pummelo cultivar's peel. *Horticultural Plant Journal, 2*, 133–140.

Zhao, X., Wang, Q., Yang, S., Chen, C., Li, X., Liu, J., & Cai, D. (2016). Quercetin inhibits angiogenesis by targeting calcineurin in the xenograft model of human breast cancer. *European Journal of Pharmacology, 781*, 60–68.

Zhong, J., Yang, R., Cao, X., Liu, X., & Qin, X. (2018). Improved physicochemical properties of yogurt fortified with fish oil/γ-oryzanol by nanoemulsion technology. *Molecules, 23*, 56.