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Antioxidant, Antimicrobial and Anticancer Activities of Citrus Peels to Improve the Shelf Life of Yoghurt Drink

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THE present work aimed to evaluate the antioxidant, antimicrobial and anticancer effects of citrus peels. The quality and storage characteristics of probiotic yoghurt drink samples were investigated which were made with 1, 2 and 3% of citrus peels powder (Orange- Mandarin-Lemon). Sensory analysis, acidity, pH, and bacteria counts, of the yoghurt drink samples, were determined on days 0, 7, 14, and 21 during storage at 4 °C. The results indicated the presence of antioxidant activity of citrus peels due to their high phenolic compounds content, the highest phenolic concentration was found in orange peel (1108 mg/100 g). The flavonoid content of orange peel powder was 518 mg/100 g. The corresponding values for Mandarin and lemon peels were 420 mg/100 g and 860 mg/100 g respectively. The antioxidant activity determined by DPPH in orange, mandarin, and lemon peel powders, were 45.99, 36.10, and 43.60% respectively. Polyphenols were identified in orange, mandarin, and lemon peels. The results revealed that orange, mandarin, and lemon peel powders exhibited an efficient cytotoxicity against human tumor cell lines representing colon carcinoma (HCT116) relative to the positive doxorubicin. The results also showed that lemon (*Citrus limon*), mandarin (*Citrus reticulata*) orange (*Citrus sinensis*) peels had antibacterial activity against all pathogenic bacteria and fungi and the ethanolic extract was higher than the aqueous with all microbes for citrus peels used, and the ethanolic extract of orange peels gave the highest inhibition of pathogenic microbes. The results of the sensory evaluation showed that yoghurt drinks containing 1 and 2% of orange, mandarin, and lemon peel powders concentrations compared with control had no significant differences. It can be concluded that citrus peel may be useful to be applied to the development yoghurt drink with excellent antifungal and antibacterial activities without affecting sensory characteristics of fermented milk.

Keywords: Citrus peel, Yoghurt drink, Anti-oxidant and anti-microbial activities, Cytotoxicity activity.

Introduction

Citrus is a universal term for plants belonging to the family *Rutaceaeis* considered as an important fruit around the world and one-third of the processed crop (Jiang et al*.,* 2014). Peels are generated as the primary citrus byproducts that represent about 50-65% of fruit weight during processing. These byproducts are discarded and considered as a huge load to the environment (Mandalari et al*.,* 2006; Nayak et al*.,* 2015; Wang et al., 2008; Ramful et al*.,* 2011). Citrus processing by-products have a rich source of naturally occurring flavonoids. The peel which represents roughly half the fruit weight contains the highest concentrations of flavonoids in the citrus fruit (Manthley & Grohmann, 2001). Fibers also have beneficial effects on human health. Trumbo et al. (2002) reported that the recommended daily intake of fiber is about 38 g for men and 25 g for women. The extract of Citrus peel contains a lot of phytochemicals including flavonoids, which have antioxidant activity (Rekha & Bhaskar, 2013). Citrus byproducts are promising sources of bioactive ingredients and valuable technological and nutritional properties. These byproducts can be used as ingredients and food additives (Marín et al., 2002; Puupponen-

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Pimia et al., 2002; O'Shea et al., 2012) in the food industry for their cheap valuable component (Galanakis, 2012). Natural products that occur in citrus peels e.g. sugars, flavonoids, carotenoids, folic acid, vitamin C, pectin and volatile oils present are very useful for the food industry and human health. Also, citrus peels are good source of phenolic compounds that can be extracted which employed as natural antioxidants to inhibit oxidation of some foods and can be applied in designing functional foods (Patil et al., 2009; Albishi et al., 2013). Functional foods not only provide nutrients but also have biologically active components that can improve health and can reduce the risk of disease in the body (Mohammadi & Mortazavian, 2011). Fermented food belongs to the present category of functional foods. Reports obtain that probiotics do beneficial effects on the immune system and the gut, reduce side effects after using antibiotics, reduce symptoms associated with irritable bowel syndrome, help reduce lactose intolerance, and have antimicrobial and anticancer characteristics (Fontana et al., 2013). *Lactobacillus* and *Bifidobacteria* spare are the most common types of probiotics (Vasiljevic & Shah, 2008). Probiotics are referred to as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001).

Many health benefits are linked to the consumption of probiotic products such as treatment of diarrhea, reduction of lactose intolerance symptoms, reduction of cholesterol in the blood, treatment of irritable bowel syndrome, and inflammatory bowel disease, anti-carcinogenic properties, synthesis of vitamins, and enhancing immunity. Classified as stirred yoghurt with low viscosity, is a growing area of interest based on its convenience, portability, and ability to deliver all of the health and nutritional benefits of stirred or set yoghurt (Eder, 2003; Thompson et al., 2007).

Prebiotics are classified as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health (Gibson & Roberfroid, 1995).

When prebiotics are combined with probiotics, their relationship is classified as symbiotic. This combination can improve the survival rate of probiotics and provide additional health benefits to the host (Collins & Gibson, 1999). Fiber of various sources is added to dairy products due to its water-holding capacity and its ability to increase the production yield, reduce lipid retention, improve textural properties and structure, and reduce caloric content by acting as a bulking agent (Larrauri, 1999). Citrus fruit peels can be used as sources of functional compounds and preservatives for the newer food products (Singh, et al., 2020). Consumption of foods containing fiber may prevent or decrease gastrointestinal disorders (Elia & Cummings, 2007). Decrease hypertension, hypercholesterolemia, coronary heart disease and cancer (Pereira et al., 2004; Mann, 2007). Both dietary fiber and probiotics are reported to relieve constipation and reduce the incidence of colon cancer (Farnworth, 2008; Kaur & Gupta, 2002).

The main objectives of this study are to investigate the possibility of using probiotic bacteria in the production of yoghurt drink mixed with different levels of citrus peels to contribute to the manufacturing of new functional foods and evaluate the antioxidant, antimicrobial and anticancer effects of citrus peels.

Materials and Methods

Plant materials

Lemon (*Citrus Limon*), mandarin (*Citrus reticulata*), orange (*Citrus sinensis*), cow milk and sucrose were purchased from a local market, Giza, Egypt.

Chemicals, solvents, standards and reagents were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals used were of analytical grade.

Preparation of citrus peel (Orange – Mandarin-Lemon) powder

Lemon, mandarin and orange fruits were washed by running tap water, peeled and their edible portions were carefully separated. The obtained fresh citrus peels were cut into small pieces before the drying processes. Each of fresh lemon or mandarin or orange peel pieces was divided separately into two parts and each part was dried using the following methods, The fresh citrus peels pieces were dried in an air oven (Shellab-Model 1350FX.-Made in the USA) at 40±2 °C for ~ 48 h (lemon, mandarin or orange) dried citrus peels were ground to a fine powder using a mechanical laboratory grinder and the flour obtained was passed through a 35-mesh sieve (0.425 mm), then packaged in polyethylene bags and stored at 4±1°C until required for use.

Preparation of extracts

Citrus peel extracts were prepared according to the method by Yadav et al. (2015) with slight modifications. 4 g of peel powder, stored at 4 °C, was taken in 4 different conical flasks. 20 mL of ethanol, water. The conical flasks were tightly stoppered with plugs of non-absorbent cotton and this was wrapped with aluminum foil as a precautionary measure. The conical flasks were placed in a shaker incubator pre-set at 30°C at 130 rpm for 36 hr for extraction to complete. After the extraction process was completed, the flasks were removed from the incubator and the contents were poured into centrifuge tubes (Tarson Tubes) that were tightly capped and were centrifuged at 4200 rpm at 10°C. The clear liquids were immediately transferred to clean, dry petri-plates and were placed in a tray drier at 35°C to concentrate it up to 80% and to ensure that the solvent used for extraction evaporated. The centrifuge tubes with pellets were discarded. When most of the solvents had evaporated, the extracts were carefully transferred into small Eppendorf tubes and stored at 10°C.

Development of yoghurt drink

The preparation of yoghurt drink was done as the milk-based was heated at 85 °C for 30 min, and then cooled to 42.5 °C. Yoghurt culture was added (1% starter culture), and fermentation was conducted at 42.5 °C for 5 hr. When the pH of yoghurt reached 4.6, the fermentation process was stopped. Then the yoghurt was cool down to 4 °C and stored for 12 hr. The prepared citrus peels (1%,2%,3%) were added to the yoghurt and 10% of sugar. The samples were mixed for 50 s using an electric stirrer and packed in sterilized cups and stored in refrigerator at 4°C.

The inoculants were prepared from lyophilized bacteria cultures of the probiotic microorganisms *Bifidobacterium Bb* 12 and *Lactobacillus acidophilus* LA-5, *Streptococcus thermophilus* (were purchased from Christian Hansen laboratories). The stock culture was prepared with 1g of each culture (containing 1.0×10^7 CFU/g).

Analytical Methods

Determination of total phenolic content

Phenolic compound contents of orange peel powder, mandarin peel powder and lemon peel powder were determined using the Folin-Ciocalteu reagent (Singleton et al., 1999). Gallic acid was used as standard and the results were expressed as mg gallic acid equivalents/100g dry weight orange peel powder, mandarin peel powder and lemon peel powder.

Determination of total flavonoids content

Total flavonoid contents were determined using aluminum chloride (AlCl3) according to the method of Zhishen et al. (1999). The results were expressed as mg quercetin equivalents/g dry weight.

HPLC analysis of phenolic component

Phenolic concentrations of citrus peel were determined by HPLC like the method described by Goupy et al. (1999). As follows: 1g of sample was mixed with methanol and centrifuged at 10000 rpm for 10min ((HERMLE Z206A, Germany) and therefore the supernatant was filtered through a 0.2 µm Millipore after that, its injection into HPLC, using equipped with a variable wavelength detector (Agilant, Germany) 1100. Also, the HPLC was equipped with auto sampler, Quaternary pump degasser and column compartment. Analyses were performed on a C18 reverse-phase (BDS 5 µm, Labio, Czech Republic) packed stainless-steel column (4×250 mm, i.d.), malti wavelength detector set at 330 nm and 280 nm for detection of flavonoids and phenolic compounds, degasser, the column used for fractionation Zorbax OD.4.6x250nm and also the flow rate of mobile phase during the run was 1 mL/min. The column temperature was maintained at 35°C. HPLC method started with the linear gradient at a flow rate of 1.0 mL/min with a mobile phase of water / acetic acid (98: 2 v/v, solvent A) and methanol / acetonitrile (50: 50, v/v, solvent B), starting with 5 % B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50 min, 100% at 55 min. The initial condition was re-established by 5 min wash in both solvents.

Antioxidant activity

Determination of DPPH radical scavenging activity

Free radical scavenging activity was determined by a DPPH radical scavenging assay, according to the modified method of Brand-Williams et al. (1995). The percentage radical scavenging activity (RSA) was calculated using the following formula:

$$\% \text{ RSA} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of samples after the reaction. The free radical scavenging activities of the extracts were expressed as Inhibition Concentration 50 value (IC_{50}). The IC_{50} value was defined as concentration in mg/ml of the sample that inhibits 50% of the formation of DPPH radical.

Assessment of antimicrobial activity of citrus peel (orange – mandarin- lemon) water and ethanol extracts

Antimicrobial activity of water and ethanol extracts of Citrus peel (Orange – Mandarin-Lemon) samples were carried out using five pathogenic bacterial strains, one pathogenic fungal strain and one pathogenic yeast. This method was carried out using the disc diffusion method; the plates were incubated at 37 °C overnight (in case of bacteria) and 28 °C for 3 days in the case of fungi. The inhibition zones were recorded in mm for replicates that were prepared for each treatment according to the method of Kotzekidou et al. (2008).

Pathogenic microorganisms

Bacteria

The bacterial strains used in this work (Gram-positive and Gram-negative) were kindly supplied by the Microbiology Department, Faculty of Agriculture Cairo University. These strains are *Staphylococcus aureus* ATCC 25923, *E.Coli* ATCC25922, *Bacillus cereus* 33018, *Salmonella typhimurium*, ATCC 20231 and *Pseduomones aeruginosa* ATCC 27853. These Cultures were maintained on nutrient agar slants at 4 °C.

Mold

Filamentous food–borne fungus; *Aspergillus niger* was isolated from different spoilage sources (vegetables, fruits, grains) (Rizk et al., 2009).

Yeast

Candida albicans CAIM-22 was obtained from MIRCEN (Microbiology research Center) Ain–Shams University, Cairo, Egypt.

Physicochemical analysis

The physicochemical analyses were determined according to AOAC (2005) methods. The pH and acidity were carried out by AFNOR (1980).

Viscosity

Viscosity was expressed in Pascal sec (Pas) by viscometer using a glass tube and a normalized ball equipped with a chronometer at 25 °C

Microbiology analyses during storages

The coliform bacteria were determined according to the method described by APHA (1976).

Proteolytic bacterial count

Proteolytic bacteria were counted according to the procedure described by Brock et al. (1982) and Difco Manual (1984)

Lipolytic bacterial count

Lipolytic bacteria were counted according to the methods mentioned by Harrigan & McCance (1976) and Difco manual (1984).

Yeast and mold counts

The procedures of Difco-Manual (1984) were applied.

Measurement of potential cytotoxicity by sulphorhodamine B (SRB) assay

Potential cytotoxicity of orange peel powder, mandarin peel powder, and lemon peel powder were tested using the method of Skehan et al. (1990) in Cancer Biology Dep., National Cancer Institute, Cairo Univ., Egypt. Cells for colon (HCT) cancer were plated in a 96-multiwell plate (104 cells per well) for 24 hr before treatment samples to allow attachment of cell to the wall of the plate. Plant and seeds in DMSO (0, 5.0, 12.5, 25.0 and 50.0 μg mL^{-1}) were added to the cell monolayer. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Triplicate wells were prepared for each dose (concentration).

Monolayer cells were incubated with the prepared samples for 48 hours at 37 °C in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulphorhodamine-B stain. Excess stain was washed with 1% acetic acid and at`1tached stained was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and tested sample concentrations were plotted to get the survival curve of each tumor cell line after the cytotoxicity of the specified compound and IC_{50} (dose of the tested samples which reduces survival rate to 50%) were evaluated.

Statistical analysis

The data were analyzed using SPSS program version 19 (2000). Means and standard deviations were determined using descriptive statistics.

Results and Discussion

Polyphenolic compounds (as phenolic acids and flavonoids) are important fruit phytochemicals compounds for their antioxidant activities, their chelation of redox-active metal ions, and inactivation of lipid radical chains and prevention of hydroperoxide conversion into reactive oxy radicals (Cabral de Oliveira et al., 2009). Phenolic content is used as an indicator of antioxidant capacity and as a preliminary screen for any product when planned to utilize as a

natural source of antioxidants in functional foods (Viuda-Martos et al., 2011). Several studies have revealed a positive relationship between TPC and antioxidant activity in many parts of plants and fruits. It's considered that the antioxidant activity of phenolic compounds is due to their high redox potentials, which permit them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Radha et al., 2014). Data in Table 1 illustrated that the highest phenolic concentration was found in orange peel (1108mg/100 g). For mandarin peel was found 930 mg/100 g. On the other hand, lemon peel, phenolic content was (581 mg/100 g). Flavonoids referred to as primary antioxidants and act as radical acceptors and chain reaction breakers. The position and degree of hydroxylation are of primary importance in determining the antioxidant activity of flavonoids (Shahidi et al., 1992). Citrus peels are a rich source of natural flavonoids. Also, phenolic and flavonoid compounds of citrus have high antioxidant activity. Flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are evident for flavonols (Kamran et al*.,* 2009; Hayat et al*.,* 2010; El–Seedi et al., 2012). From the results, the flavonoid content of orange peel powder was 518 mg/100 g. The corresponding value for Mandarin peel was 420 mg/100 g while the flavonoid content of lemon peel was 860 mg/100 g. Wang et al. (2008) analyzed the flavonoid content of different varieties of citrus fruits and found that seven varieties had less than 20 mg flavonoid per gram. The lemon peel had a negligible amount of 21.6 mg/g on dry basis. However, in the presented study, the flavonoid content of lemon peel powder was much higher than those reported previously.

The antioxidant property is linked to the ability of phenolic compounds to scavenge free radicals, break radical chain reactions and chelate metals (Nayak et al., 2015). The total antioxidant capacity of plant extracts is influenced by their chemical composition and antioxidant content. Antioxidants are greatly used as food additives to support the degradation of foods and to improve their shelf life by preventing lipid per-oxidation as well as protect oxidative damage (Kumaran & Karunakaran, 2006). Table 1 revealed that antioxidant activity in orange peel powder, mandarin peel powder and lemon peel powder, were 45.99, 36.10 and 43.60% respectively. The DPPH % activity of dried orange peel extracted was higher than mandarin and lemon peels. The present investigation demonstrated that between the three peel powders studied, the orange peel had higher antioxidant activity than mandarin and lemon peel powders. This could be attributed to the overall bioactive components present in the peels. The antioxidant activity of peel extract might be due to the reduction of superoxide, inactivation of free radicals, or complexion with metal ions or their combination (Karoui & Marzouk, 2013). Casquete et al., (2015) determined the antioxidant capacity of lemon, lime, mandarin and orange peel using (DPPH and ABTS). Regarding DPPH in control samples of lemon, lime, mandarin and orange peel were 80.93, 53.11, 69.02 and 102.39 mg Trolox /100g of peel extracts, respectively. The different levels obtained from these assays may indicate a relative difference in the ability of antioxidant compounds in the extracts to quench aqueous peroxyl radicals (Thaipong et al., 2006).

Polyphenolic compounds of orange peel powder, mandarin peel powder and lemon peel powder

Qualitative HPLC profile of the orange peels, mandarin peels and lemon peels was performed using HPLC. Qualitative HPLC analysis of the major peaks of the orange peels, mandarin peels and lemon peels water extracts were based on the comparison of their retention times with reference standards. The following polyphenols were identified in orange peels, mandarin peels and lemon peels water extract: gallic acid, chlorogenic acid, catechin, Caffeine, Coffeic acid, Syringic acid, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, naringenin, propyl gallate, 4`.7-DihydroxyisoFlavone, quercetin, and cinnamic acid. The major compound in orange peel was naringenin while Coffeic acid and Vanillin were not found in Table 2.

Also, the major compound in mandarin was naringenin while Ellagic acid and Vanillin were not found. However, the major compound in lemon was gallic acid while Caffeine, Coffeic acid, Syringic acid not found Gorinstein et al. (2001) in a comparative analysis of biochemical characteristics of citrus fruits reported the highest total phenolic content in peels of lemons (190 mg/100 g) followed by oranges (179 mg/100 g) and grapefruit (155 mg/100 g). Huge amounts of flavanones and many polymethoxylated flavones are contained in citrus peels which are rare in other plants (Bocco et al., 1998; Rekha & Bhaskar, 2013). Also, Abd El-ghfar et al. (2016) reported that citrus peels have high contents of natural

phenolic, flavonoids, carotenoids and ascorbic acid with a significant antioxidant activity which could be recommended to use as functional ingredients for the food industry.

Cytotoxicity effect of orange peel powder, mandarin peel powder and lemon peel powder

Apoptosis may be a specialized process of cell death that›s a part of the normal development of organs and tissue maintenance but can also occur as a response to varied environmental stimuli, indicating toxicity. Since apoptosis can play a critical role in the development of cancer, the ability of toxins to induce apoptosis appears to be linked to their toxicological effects (Dragan et al., 2001).

Orange peel powder, mandarin peel powder and lemon peel powder were evaluated for their cytotoxicity activities in-vitro disease oriented antitumor screening using sulphorhodamine B (SRB) assay including human tumor cell lines representing colon carcinoma (HCT116). The percentage of the viable cells and the IC_{50} value were measured and were, subsequently, assessed with those of the control, doxorubicin (Fig. 1 and Table 3). The results in Table 3 revealed that orange peel powder, mandarin peel powder and lemon peel powder exhibited efficient cytotoxicity against human tumor cell lines representing colon carcinoma (HCT116) relative to the positive doxorubicin. By increasing the concentration of dried powder of orange peel to 50 µg/mL recorded a high percentage of (HCT116) dead cell 0.753 (live cell of HEPG2 was 0.247), while by the increasing concentration of dried powder of mandarin peel to 50 µg /mL recorded percentage of (HCT116) dead cell 0.684 (live cell of (HCT116) was 0.316), also by increasing concentration of dried powder of lemon peel to 50 µg /mL recorded percentage of (HCT116) dead cell 0.553 (live cell of (HCT116) was 0.447). The highest dead cell percentage recorded by a dried powder of orange peel then mandarin peel then lemon peel may be related to the presence of one or more phenolic compounds. Orange peel was more potent relative to the positive control.

TABLE 1. Total phenolic, total flavonoid contents and antioxidant activity of citrus peels.

Sample	Content of total phenolic compounds (mg GAE /100 g)	Total Flavonoid Content (mg quercetin /100g)	Antioxidant Activity
Orange peel	1108±2.8	518±2.6	45.99±2.1
Mandarin peel	930±2.4	420±1.1	36.10±4.6
Lemon peel	581±3-6	860±2.2	43.60±3.2

TABLE 2. Fractionation of polyphenols and flavonoids components by HPLC (µg/g) for citrus peels.

Components	Conc. (µg/g orange)	Conc. (µg/g mandarin)	Conc. (µg/g lemon)
Gallic acid	1641.85	958.46	867.48
Chlorogenic acid	1152.05	548.44	0.00
Catechin	1627.06	1207.60	469.17
Caffeine	122.90	33.97	0.00
Coffeic acid	0.00	96.54	0.00
Syringic acid	198.47	95.01	0.00
Rutin	1046.71	686.40	309.19
Ellagic acid	216.50	0.00	95.59
Coumaric acid	74.36	60.94	56.70
Vanillin	0.00	0.00	30.95
Ferulic acid	609.42	247.52	70.76
Naringenin	6167.63	3612.51	158.73
Propyl Gallate	1104.64	426.26	217.65
4`.7-DihydroxyisoFlavone	176.77	94.62	94.18
Querectin	438.69	313.74	41.75
Cinnamic acid	20.18	17.56	14.67

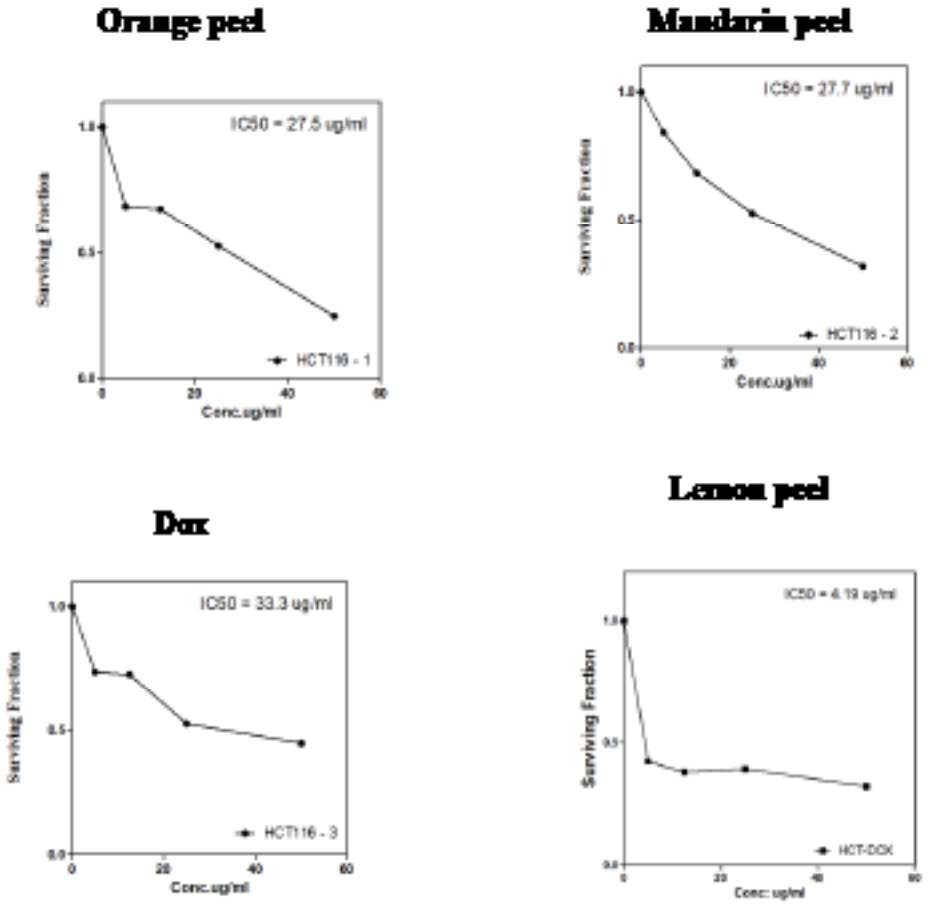

Fig. 1. Cytotoxicity of orange peel powder, mandarin peel powder and lemon peel powder on colon carcinoma cell line (HCT 116).

TABLE 3. Effect of orange peel powder, mandarin peel powder and lemon peel powder on HCT 116 .

CONC. µg/mL	Orange peel HCT 116 -1	Mandarin peel HCT 116 -2	Lemon peel HCT 116 -3	HCT 116 – DOX
0.000	1.000	1.000	1.000	1.000
5.000	0.684	0.842	0.737	0.424
12.500	0.674	0.684	0.726	0.379
25.000	0.526	0.526	0.526	0.393
50.000	0.247	0.316	0.447	0.321

As Fig. 1 showed that dried powder of orange peel showed cytotoxic effects with the IC50 values of 27.5 µg /mL in (HCT116) cell line, while dried powder of mandarin peel showed cytotoxic effects with the IC50 values of 27.7 µg/mL in (HCT116) cell line, and dried powder of lemon peel showed cytotoxic effects with the IC50 values of 33.3 µg/mL in (HCT116) cell line. Im et al. (2014) reported that enzymatic hydrolyzed citrus peels have high total phenolic content and display strong antioxidant and anticancer activities.

Assessment of antimicrobial activity of Citrus peel

Table 4 shows the results of the inhibition of microbial strains by the disc diffusion assay which is expressed in different extracts as Zone against pathogenic strains. The highest antimicrobial activity was obtained with the ethanol extract of the orange peel against *Pseduomone aeruginosa* and *Bacillus cereus* with inhibition zone diameters of 26 mm and 24 mm. The effectiveness of the extracts can be summarized as ethanol extracts effective in orange peels > ethanol extract of mandarin peel > ethanol extract of lemon peel.

TABLE 4. Assessment the antimicrobial activity of Citrus peel (Orange – Mandarin- Lemon) extracts (ethanol, water) 200 uL against some pathogenic microbial strains

Pathogens	***Pseduomones Aeruginosa***	***Salmonella typhimurium***	***Staphylooccus Aureus***	***Bacillus cereus***	***Eschrichia coli***	***Candida albicans***	***Aspergillus niger***	***Fusarium oxysporum***
Extracts	**Inhibition zone (mm)**							
Ethanol extract of orange peel	26	20	21	24	21	22	17	16
Water extract of orange peel	14	12	12	13	ND	13	ND	9
Ethanol extract of mandarin peel	24	17	19	21	16	12	13	14
Water extract of mandarin peel	10	10	9	13	ND	ND	ND	9
Ethanol extract of lemon peel	19	12	14	15	13	11	10	12
Water extract of lemon peel	9	ND	9	9	ND	ND	ND	ND

Citrus peel has antibacterial activity observed by Dorman et al. (2000) and Mandalari et al. (2007). The mandarin peel had greater antimicrobial activity than lemon peel demonstrated by Espina et al. (2011). The antimicrobial activities of Citrus are related to flavonoids and phenols (Viuda-Martos et al., 2008). Pavithra et al. (2009) reported that the active component responsible for the antimicrobial activity of citrus peel oils is a monoterpene. The major attributors for the antimicrobial capacity of citrus peel oils are D-limonene, linalool. Previous work revealed that the inhibitory influence of citrus peel essential oils is owed to the presence of linalool rather than limonene (Fisher & Phillips, 2006). The aqueous extract of the fruit of C. Lemon contained the phytochemicals such as alkaloids, flavonoids, phenols, quinines, terpenoids and carbohydrates. The extract was found to possess promising antimicrobial activity against several bacterial species (Singh et al., 2020). Different studies reported that plant extracts more effective to gram-positive bacteria than gram-negative bacteria, due to the presence of an additional lipopolysaccharide coat, nevertheless (Kalemba & Kunicka, 2003). The results are in agreement with this Citrus species have the antibacterial activity against bacterial strains. It was found Citrus unshiu peel extract inhibited *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* (Min et al., 2014). Shetty et al. (2016) reported that ethanolic extracts of citrus peel are more effective on antimicrobial activity than aqueous extracts. Oikeh et al. (2020) reported that the fresh Citrus peel extract contains more phenolic and better antimicrobial activities against the microbial strains compared to the dry peel extract.

Effect of storage at 4±2 °C on the physical properties (pH) of yoghurt drink with different citrus peels.

The acidity and pH of yoghurt drink supplemented with citrus peel were presented in Table 5 & 6. pH of the yoghurt drink with orange peel 1% stored at 4 °C decreased significantly ($p \leq 0.05$) over the storage period from 4.7 (day 0) to 4.1 (day21), yoghurt drink with orange peel conc. 2% decreased at storage period from 4.6 (day 0) to 4.37 (day 21) but orange peel 3% decreased at storage period from 4.6 (day 0) to 4.46 (day 21). Yoghurt drink with Mandarin peel 1% decreased at storage period from 4.7(day 0) to 4.02 (day 21), in conc.2% decreased at storage period from 4.6 (day 0) to 4.21 (day 21) but in 3% decreased at storage period from 4.6 (day 0) to 4.33 (day 21). Yoghurt drink with lemon peel 1% decreased at storage period from 4.7(day 0) to 3.9 (Day 21), in conc. 2% decreased at storage period from 4.6 (day 0) to 4.13 (day 21) but in conc. 3% decreased at storage period from 4.6 (0day) to 4.20 (day 21) but the control decreased from 4.61(day 0) to 3.65 (day 21)**.**

Titratable acidity (lactic acid) of yoghurt drink samples refrigerated and stored at 4 °C for 21days were increased. The values of yoghurt drinks with citrus peels are slightly lower than titratable acidity values for control yoghurt drinks during the storage period. Decreased pH and increased acidity of the yoghurt during the storage period could be attributed to the starter culture's activity, and the breakdown of lactose into lactic acid so a reduction in pH (Hassan & Amjad, 2010). These results are in line with Kim et al. (1998) who found that acidity was 0.95–0.99% of Yam – yoghurt. Similarly, Gregurek (1999) showed slightly higher acidity in yoghurt prepared with lower amounts of inoculum (post acidification). Collado et al. (1994) who found that the yoghurt drink and yoghurt like products had acidity 0.56% and 0.58%.

Microbiological analysis

Results in Table 7 showed that all samples had adequate amounts of viable lactic acid bacteria until 21 days of storage. So, all samples are satisfactory for three weeks which know the shelf life of the product. Lactic acid bacteria count was decreased during storage. Growth of probiotic bacteria *(Bifidobacterium Bb* 12 and *Lactobacillus acidophilus LA-5*, *Streptococcus thermophilus*) in the yoghurt drink during the 21days storage at 4±2 °C which may be related to the low pH post-acidification. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* live in a mutual stimulation that is related to the growth, acidification, and production of aromatic compounds. After fermentation, organic acid accumulation (e.g., lactic and acetic acid) occurs. Akalin et al. (2004) reported that organic acids are powerful antimicrobial agents

Microbiological Evaluation

The results of the microbial profile of yoghurt drink supplemented with citrus peel which represents zero time of the storage and during 21 days at (4 °C). No growth has been detected of yeast, molds, coliform group, proteolytic and lipolytic bacteria in fermented drink samples so ensuring the hygienic-sanitary safety of the yoghurt drink. This study demonstrated that the addition of citrus peels, contribute to the growth of microorganisms in yoghurt drink Table 7.

TABLE 5. Effect of storage at 4 °C on the physical properties (pH) of yoghurt drink with citrus peels

Storage time	control	Orange peel (pH)			Mandarin peel (pH)			Lemon peel (pH)		
		1%	2%	3%	1%	2%	3%	1%	2%	3%
Zero time	$4.61^{d}\pm$ 0.09	$4.7^{c}\pm$ 0.29	$4.6^{b}\pm$ 0.1	$4.6^{b}\pm$ 0.02	$4.7^{c}\pm$ 0.2	$4.6^{c}\pm$ 0.11	$4.6^{b}\pm$ 0.02	$4.7^{d}\pm$ 0.29	$4.6^{c}\pm$ 0.09	$4.6^{b}\pm$ 0.29
7 days	$4.32^{c}\pm$ 0.11	$4.6^{c}\pm$ 0.26	$4.52^{a}\pm$ 0.08	$4.6^{b}\pm$ 0.09	$4.65^{c}\pm$ 0.19	$4.4^{b}\pm$ 0.14	$4.5^{b}\pm$ 0.1	$4.3^{c}\pm$ 0.29	$4.40^{b}\pm$ 0.05	$4.48^{b}\pm$ 0.29
14 days	$4.02^{b}\pm$ 0.14	$4.4^{b}\pm$ 019	$4.41^{a}\pm$ 0.07	$4.53^{a}\pm$ 0.05	$4.22^{b}\pm$ 0.23	$4.37^{b}\pm$ 0.16	$4.47^{a}\pm$ 0.08	$4.13^{b}\pm$ 0.29	$4.33^{b}\pm$ 0.07	$4.37^{a}\pm$ 0.29
21 days	$3.65^{a}\pm$ 0.07	$4.1^{a}\pm$ 0.13	$4.37^{a}\pm$ 0.09	$4.46^{a}\pm$ 0.06	$4.02^{a}\pm$ 0.17	$4.21^{a}\pm$ 0.19	$4.33^{a}\pm$ 0.09	$3.93^{a}\pm$ 0.29	$4.13^{a}\pm$ 0.08	$4.20^{a}\pm$ 0.29

TABLE 6. Effect of storage at 4 °C on the physical properties (acidity) of yoghurt drink with different citrus peels.

Storage time	Control	Orange peel conc.			Mandarin peel conc.			Lemon peel conc.		
		1%	2%	3٪	1%	2%	3٪	1%	2%	3٪
Zero time	$0.94^{d}\pm$ 0.11	$0.95^{b}\pm$ 0.07	$0.95^{b}\pm$ 0.29	$0.97^{a}\pm$ 0.29	$0.95^{b}\pm$ 0.06	$0.96^{c}\pm$ 0.03	$0.97^{a}\pm$ 0.07	$0.95^{b}\pm$ 0.09	$0.96^{a}\pm$ 0.08	$0.97^{a}\pm$ 0.05
7 days	$1.08^{c}\pm$ 0.09	$0.99^{b}\pm$ 0.15	$0.98^{a}\pm$ 0.29	$0.97^{a}\pm$ 0.29	$1.00^{b}\pm$ 0.07	$1.06^{b}\pm$ 0.08	$0.98^{a}\pm$ 0.14	$1.00^{b}\pm$ 0.16	$1.09^{a}\pm$ 0.05	$0.99^{a}\pm$ 0.17
14 days	$1.27^{b}\pm$ 0.2	$1.04^{a}\pm$ 0.11	$1.01^{a}\pm$ 0.29	$1.00^{a}\pm$ 0.29	$1.09^{a}\pm$ 0.09	$1.08^{b}\pm$ 0.11	$1.03^{a}\pm$ 0.13	$1.17^{a}\pm$ 0.15	$1.13^{a}\pm$ 0.1	$1.07^{a}\pm$ 0.09
21 days	$1.52^{a}\pm$ 0.17	$1.09^{a}\pm$ 0.13	$1.06^{a}\pm$ 0.29	$1.02^{a}\pm$ 0.29	$1.17^{a}\pm$ 0.17	$1.29^{a}\pm$ 0.13	$1.08^{a}\pm$ 0.1	$1.19^{a}\pm$ 0.06	$1.2^{a}\pm$ 0.07	$1.12^{a}\pm$ 0.06

TABLE 7. Growth of probiotic bacteria (*Bifidobacterium Bb 12 and Lactobacillus acidophilus LA-5, Streptococcus thermophilus*) in the yoghurt drink during the 21days storage at 4 °C.

Storage time	control	Orange peel (pH)			Mandarin peel (pH)			Lemon peel (pH)		
		1%	2%	3%	1%	2%	3%	1%	2%	3%
Zero time	6.4×10^{8}	6.5×10^{8}	7.0×10^{8}	7.5×10^{7}	6.4×10^{8}	6.4×10^{8}	6.4×10^{8}	6.2×10^{8}	6.0×10^{8}	6.5×10^{8}
7 days	4.17×10^{7}	5.0×10^{7}	5.7×10^{7}	6.5×10^{7}	4.5×10^{7}	5.17×10^{7}	6×10^{7}	4.8×10^{7}	4.9×10^{7}	5.2×10^{7}
14 days	2.0×10^{6}	2.4×10^{7}	4×10^{7}	5.17×10^{7}	4.0×10^{6}	4.7×10^{6}	5.5×10^{6}	3.5×10^{6}	4.0×10^{6}	4.4×10^{6}
21 days	1.5×10^{5}	2.5×10^{6}	3.5×10^{6}	5×10^{6}	3.0×10^{6}	2.5×10^{6}	2.5×10^{6}	2.5×10^{6}	3.0×10^{6}	3.5×10^{6}

Contamination by yeast and molds is a major problem faced by the dairy industry. Ledenbach & Marshall (2009) noted that yeast and mold can affect changes in sensory characteristics of yoghurt by fermented off-flavors and like yeasty and a gassy appearance.

Sensory evaluation of yoghurt drink

Sensory evaluation of yoghurt drink prepared with different ratios of orange peel powder, mandarin peel powder and lemon peel powder during cold storage periods at (4± °C) after 21 days are shown in Table 8 a, b, c. Form results presented in Table 8 a, b, c, it confirmed that 1 and 2% of orange peel powder, mandarin peel powder and lemon peel powder concentrations and control of fortified fermented milk possessed the best flavor, with no significant difference in between, but significantly differed in comparison with control and 3% of orange peel powder, mandarin peel powder and lemon peel powder concentrations. Also, in the sensory evaluation, there are no significant differences in color and odor between the samples of yoghurt drink fortified with orange peel powder, mandarin peel powder and lemon peel powder 1 and 2%.

As the concentration of orange peel powder, mandarin peel powder and lemon peel powder was increased in fortified fermented milk the score of flavor, color, odor, Viscosity, shape and overall acceptability were decreased. Hence, the overall acceptability was high in the yoghurt drink containing 1 and 2% of orange peel powder, mandarin peel powder and lemon peel powder concentrations. At the beginning of storage, sensory scores of all yoghurt drinks were high, due to their more intense flavor and better consistency. However, after 7 days, the acidity of the yoghurt drinks increased, and the sensory scores of all samples began to decrease. The overall acceptability scores of samples increased during storage for up to 7 days and then decreased. This may be attributed to the development of acidity. In general, yoghurt drinks made with different levels of orange peel powder, mandarin peel powder and lemon peel powder had acceptable during the storage period. These results can be applied to the development of yoghurt drinks with excellent antifungal and antibacterial activities without affecting the sensory characteristics of fermented milk. Also improved the flavor characteristics of the final product and masked the defects by the natural development of aroma. Sendra et al. (2008) found that citrus fiber-enriched fermented milk has good acceptability and is excellent vehicle for a variety of commercial probiotics. According to Gahruie et al. (2015), despite the fiber-related health benefits, it is important to note that consumers do not usually accept formulations with more than 3% fiber. From these results, it could be revealed that orange peel powder, mandarin peel powder and lemon peel powder could be added to yoghurt drinks at levels1% and 2% to obtain products having the best scores for all the evaluated characteristics.

TABLE 8a. Sensory evaluation of fermented milk during 4 °C storage.

Sensory attribute	Treatment	Storage period				LSD
		Zero time	7 days	14 days	21 days	
Flavor	C	9.70±0.48^{Aa}	9.60±0.516^{A}	8.70±0.483^{ABb}	7.80±0.632^{Ac}	0.4832
	M1%	9.8±0.422^{Aa}	9.7±0483^{Aa}	9.10 ±0.737^{Ab}	8.30±0.483^{Ac}	0.4950
	M2%	9.5±0.527^{Aa}	9.4±0.966^{Aa}	8.90±0.994^{ABa}	8.10±0.316^{Ab}	0.6884
	M3%	8.7± 0.483^{Ba}	9.30± 0.675^{Aab}	8.40±0.699^{Bbc}	7.90±0.875^{Ac}	0.6329
	LSD	0.4359	NS	0.6817	NS	
Color	C	9.50±0.527^{ABa}	9.50± 0.527^{Aa}	8.30± 0.4830^{Ab}	7.90±0.5676^{Bb}	0.4784
	M1%	9.60±0.5164^{Aa}	9.50±0.5270^{Aa}	8.70 ±0.9487^{Ab}	8.30±0.4830^{Ab}	0.5879
	M2%	9.60±0.5164^{Aa}	9.50±0.527^{Aab}	8.90±0.8756^{Abc}	8.70±0.8233^{Ac}	0.6401
	M3%	9.10±0.5676^{Ba}	9.40±0.6992^{Aba}	8.80±1.1353^{Aa}	8.00±0.9428^{Bb}	0.7847
	LSD	0.4832	NS	NS	0.6613	
Odor	C	9.70±0.483^{Aa}	9.40± 0.516$^{\mathbf{Aba}}$	8.20± 0.632$^{\mathbf{Ab}}$	7.60± 0.516^{Cc}	0.4903
	M1%	9.8±0.422^{Aa}	9.7±0.483^{Aa}	8.9±0.8756^{Ab}	9.1±0.5676^{Ab}	0.5560
	M2%	9.7±0.483^{Aa}	9.6±0.516^{Aba}	8.8±0.9189^{Ab}	8.9±0.5676^{Ab}	0.5860
	M3%	9.2±0.632^{Ba}	9.2±0.632^{Ba}	8.8±1.0328^{Aab}	8.3±0.6749^{Bb}	0.6917
	LSD	0.4639	0.5285	NS	0.5906	
Viscosity	C	9.40± 0.516^{Aa}	9.0±0.667^{Bab}	8.50±0.527^{Abc}	8.10± 0.567^{Bc}	0.5497
	M1%	9.7 ±0.483^{Aa}	9.4±0.699^{ABab}	8.7±0.6749^{Aabc}	8.9±0.316^{Ac}	0.5129
	M2%	9.5±0.707^{Aa}	9,6±0.516^{Aa}	8.8 ±0.7888^{Ab}	8.5±0.707^{ABb}	0.6239
	M3%	8.7±0.675^{Ba}	9.0±0.666^{Ba}	8.8±0.6325^{Aba}	7.4±0.699^{Cb}	0.6071
	LSD	0.5476	0.5821	NS	0.5393	
shape	C	9.4±0.516^{ABa}	9.3±0.6749$^{\mathbf{Aa}}$	8.7±0.6749^{Ab}	8.0± 0.4714^{Bc}	0.5371
	M1%	9.6±0.516^{Aa}	9.5±0.7071^{Aa}	9.1±0.8756^{Aab}	8.9±0.3162^{Ab}	0.5801
	M2%	9.7±0.483^{Aa}	9.4±0.6992^{Aa}	8.6±0.8433^{Ab}	8.6±0.5164^{Ab}	0.5918
	M3%	9.1±0.568^{Ba}	9.3±0.4830^{Aa}	8.5± 0.7071^{Ab}	8.1± 0.5676^{Bb}	0.5328
	LSD	0.4736	NS	NS	0.4333	
Overall acceptability	C	9.8±0.422^{Ab}	9.6±0.516^{Ab}	8.5±0.7071^{A}	7.30±0.4830^{C}	0.4926
	M1%	9.9±0.316^{Aa}	9.7±0.483^{Aa}	9.0±0.8165^{Ab}	9.1±0.5676^{Ab}	0.5219
	M2%	9.5±0.527^{Ba}	9.7±0.483^{Aa}	8.8±0.7888^{Ab}	8.9±0.5676^{Ab}	0.5476
	M3%	9.0± 0.471^{Cab}	9.2±0.422^{Ba}	8.6±0.6992A^{bc}	8.1± 0.737^{Bc}	0.5434
	LSD	0.4004	0.4304	NS	0.5413	

a, b, c with different lowercase letters in the same row are significantly different ($p < 0.05$).
A, B, C with different uppercase letters in the same column are significantly different ($p < 0.05$).
C: control, M1:mandarin1% ,M2:mandarin2% , M3 :mandarin3%

TABLE 8b. Sensory evaluation of fermented milk during 4 °C storage.

Sensory attribute	Treatment	Storage period				LSD
		Zero time	7 days	14 days	21 days	
Flavor	C	9.7±0.483^{Aa}	9.60±0.516^{Aa}	8.50±0.707^{ABb}	7.80±0.632^{Bc}	0.537
	O1%	9.50±0.527^{a}	8.80±0.632^{Bb}	8.70±0.823^{Ab}	8.50±0.527^{Ab}	0.50
	O2%	9.5±0.527^{Aa}	8.70±0.674^{Bb}	8.40±0.516^{ABb}	7.70±0.67^{4B}	0.547
	O3%	9.70±0.48^{Aa}	8.50±0.527^{Bb}	8.0±0.471^{Bc}	70±0.674^{Cd}	0.4950
	LSD	NS	0.5371	0.5860	0.5722	
Color	C	9.50±0.527^{Aa}	9.60±0.516^{Aa}	8.30±0.483^{Ab}	5.90±2.07^{Cc}	1.0250
	O1%	9.40±0.516^{Aa}	9.20±0.421^{Ba}	8.90±0.737^{Aa}	8.30±0.483^{Ab}	0.5019
	O2%	9.30±0.483^{Aa}	9.10±0.316B^{Ca}	8.90±0.875^{Aa}	7.90±0.567^{ABb}	0.5413
	O3%	9.10±0.567^{Aa}	8.80±0.421^{Ca}	8.70±0.674^{Aa}	7.20±0.421^{Bb}	0.4832
	LSD	NS	0.3857	NS	1.0204	
Odor	C	9.70±0.483^{Aa}	9.40±0.516^{Aa}	8.20±0.632^{Ab}	7.60±0.516^{Bc}	0.4903
	O1%	9.60±0.516^{Aa}	9.30±0.483^{Aa}	8.70±0.823^{Ab}	8.40±0.516^{Ab}	0.5455
	O2%	9.50±0.527^{Aa}	9.30±0.483^{Aab}	8.80±0.788^{Ab}	7.90±0.567^{Bc}	0.5476
	O3%	9.50±0.527^{Aa}	9.10±0.567^{Aab}	8.60±0.966^{Ab}	7.50±0.527^{Bc}	0.6109
	LSD	NS	NS	NS	0.4832	
Viscosity	C	9.40±0.516^{Aa}	9.00±0.666^{Aab}	8.50±0.527^{Abc}	8.10±0.567^{Ac}	0.5197
	O1%	9.40±0.516^{Aa}	9.20±0.421^{Aab}	8.70±0.674^{Ab}	8.10±0.737^{Ac}	0.5455
	O2%	9.10±0.567^{Aa}	9.20±0.421^{Aa}	8.90±0.737^{Aa}	7.10±0.567^{Bb}	0.5306
	O3%	9.20±0.421^{Aa}	8.90±0.316^{Aab}	8.60±0.516^{Ab}	6.40±0.516^{Cc}	0.4088
	LSD	NS	NS	NS	0.5476	
shape	C	9.40±0.516^{Aa}	9.30±0.674^{Aa}	8.70±0.674^{Ab}	8.00±0.471^{Ac}	0.5371
	O1	9.30±0.483^{Aab}	9.40±0.699^{Aa}	8.80±0.788^{Ab}	7.900±0.567^{ABc}	0.5860
	O2%	9.00±0.471^{ABab}	9.30±0.674^{Aa}	8.60±0.843^{Ab}	7.400±0.516^{BCc}	0.5840
	O3%	8.60±0.516^{Bb}	9.40±0.516^{Aa}	8.80±0.788^{Ab}	6.900±0.737^{Cc}	0.5918
	LSD	0.4514	NS	NS	0.5285	
Overall acceptability	C	9.80±0.422^{Aa}	9.60±0.5164^{Aa}	8.50±0.7071^{Ab}	7.30±0.483^{Cc}	0.4926
	O1%	9.20±0.632^{Ba}	9.20±0.421^{Aa}	8.70±0.823^{Aab}	8.40±0.516^{ab}	0.5600
	O2%	9.40±0.516^{Aa}	9.20±0.6325^{Aba}	8.90±0.875^{Aa}	7.90±0.316^{Bb}	0.5620
	O3%	9.40±0.516^{ABa}	9.00±0.471^{Bab}	8.70±0.823^{Ab}	7.70±0.483^{Cc}	0.5371
	LSD	0.4784	0.4688	NS	0.4143	

a, b, c with different lowercase letters in the same row are significantly different ($p < 0.05$).

A, B, C with different uppercase letters in the same column are significantly different ($p < 0.05$).
C: control, O1:orange1% ,O2: orange2% , O3 :orange3%.

TABLE 8c. Sensory evaluation of fermented milk during 4°C storage

Sensory attribute	Treatment	Storage period				LSD
		Zero time	7 days	14 days	21 days	
Flavor	C	9.70±0.483^{Aa}	9.60±0.516^{Aa}	8.70±0.483^{Ab}	7.80±0.632^{Ac}	0.483
	L1%	9.20±0.632^{Ba}	8.80±0.421^{Bab}	8.50±0.707^{Ab}	7.60±1.075^{Ac}	0.678
	L2%	9.00±0.0^{Ba}	8.60±0.516^{Ba}	8.50±0.707^{Aa}	7.60±1.075^{Ab}	0.629
	L3%	8.20±0.421^{Ca}	8.00±0.666^{Ca}	7.50±0.527^{Bb}	7.50±0.527^{Ab}	0.492
	LSD	0.4088	0.4880	0.5580	NS	
Color	C	9.50±0.527^{Aa}	9.50±0.527^{Aa}	8.30±0.483^{Bb}	7.90±0.567^{Ab}	0.478
	L1%	9.10±0.567^{ABa}	8.80±0.421^{ABa}	9.10±0.875^{Aa}	7.60±0.699^{Ab}	0.601
	L2%	8.80±0.632^{BCab}	9.10±0.737^{Ba}	8.20±0.421^{Bbc}	7.90±0.875^{Ac}	0.623
	L3%	8.40±0.516^{Ca}	8.00±0.471^{Cab}	7.80±0.421^{Bbc}	7.50±0.527^{Ac}	0.441
	LSD	0.5108	0.5049	0.5285	NS	
Odor	C	9.70±0.483^{aa}	9.40±0.516^{aa}	8.20±0.632^{Bb}	7.60±0.516^{Ac}	0.490
	L1%	9.40±0.516^{Aba}	9.30±0.674^{Aa}	8.90±0.737^{Aa}	7.90±0.994^{Ab}	0.681
	L2%	9.30±0.483^{aba}	9.20±0.788^{Aa}	9.00±0.666^{Aa}	8.00±0.942^{Ab}	0.671
	L3%	9.20±0.422^{ba}	9.00±0.942^{aa}	8.80±0.918^{ABa}	7.60±0.966^{Ab}	0.765
	LSD	0.4330	NS	0.678	NS	
Viscosity	C	9.40±0.516^{ABa}	9.10±0.737^{Aa}	8.50±0.527^{Ab}	8.10±0.567^{Ab}	0.539
	L1%	9.70±0.483^{Aa}	8.90±0.567^{Ab}	8.70±0.823^{Ab}	7.70±0.483^{Ac}	0.550
	L2%	9.30±0.483^{ABa}	9.30±0.674^{Aa}	8.40±0.516^{Ab}	7.60±0.699^{Ac}	0.545
	L3%	9.20±0.422^{Ba}	9.10±0.567^{Aa}	8.40±0.699^{Ab}	7.60±0.516^{Ac}	0.508
	LSD	0.4333	NS	NS	NS	
shape	C	9.40±0.516^{Aa}	9.30±0.674^{Aa}	8.70±0.674^{Ab}	8.00±0.471^{Ac}	0.537
	L1%	9.40±0.699^{Aa}	9.30±0.^{674Aa}	8.70±0.823^{Aa}	7.900±0.994^{Ab}	0.733
	L2%	9.10±0.567^{Aa}	8.60±0.966^{Bab}	8.50±0.527^{ABab}	8.100±0.875^{Ab}	0.688
	L3%	9.10±0.316^{Aa}	8.30±0.674^{Bb}	8.10±0.567^{Bb}	7.900±0.875^{Ab}	0.582
	LSD	NS	0.688	0.597	NS	
Overall acceptability	C	9.80±0.422^{Aa}	9.70±0.483^{Aa}	8.50±0.707^{Ab}	7.00±1.155^{Bc}	0.680
	L1%	9.40±0.516^{Ba}	9.30±0.483^{ABab}	8.80±0.632^{Ab}	7.900±0.876^{ABc}	0.586
	L2%	9.20±0.422^{BCa}	9.20±0.421^{Ba}	8.90±0.567^{Aa}	8.100±1.101^{Ab}	0.623
	L3%	9.00±0.00^{Ca}	8.60±0.516^{Cab}	8.50±0.707^{Aab}	8.00±1.054^{Ab}	0.622
	LSD	0.358	0.433	NS	0.954	

a, b, c with different lowercase letters in the same row are significantly different ($p < 0.05$).

A, B, C with different uppercase letters in the same column are significantly different ($p < 0.05$).
C: control, O1:lemon1% ,O2: lemon 2% , O3 :lemon 3%

Conclusions

This study may be used as a reference for yoghurt drink manufacturers looking forward to mix novel prebiotics with probiotics. The presence of functional dietary fiber, natural phenolic, flavonoids and antioxidants in orange, mandarin and lemon peel powder permit their application in food processing to get healthy products and used as a natural antioxidants to increase the shelf life of food products. The utilization of food by-products for yoghurt drink production should be encouraged as it promotes consumers' access to healthier foods and reduces the negative effects of organic waste disposal on the environment.

References

Abd El-ghfar, A.M. H., Ibrahim, H. M., Hassan I. M., Abdel Fattah A. A. and Mahmoud, M.H. (2016) Peels of lemon and orange as value-added ingredients: chemical and antioxidant properties. *International Journal of Current Microbiology and Applied Sciences,* 5(12), 777-794.

http://doi.org/10.20546/ijcmas.2016.512.089

APHA (American Public Health Association) (1976) Compendium of Methods for the Microbiological Examination of Foods. (edited by Speck, M. L.), Washington, DC, USA.P,702.

AFNOR (1980) Lait et produits laitiers: méthodes d'analyse1er éd. AFNOR, Paris.

Akalin, A. S., Fenderya, S. and Akbulut, N. (2004) Viability and activity of bifidobacteria in yoghurt containing fructooligosaccharide during refrigerated storage. *International Journal Food Science Technology,* 39, 613-621. http://doi.org/10.1111/j.1365-2621.2004.00829.x

Albishi, T., John, J. A., Al-Khalifa, A. S. and Shahidi, F. (2013) Phenolic content and antioxidant activities of selected potato varieties and their processing by-products. *Journal Functional Foods*, 5, 590–600. https://doi.org/10.1016/j.jff.2012.11.019

AOAC (2005) Association of Official Analytical Chemists. Official methods analysis of the association analytical chemists, 18th ed. AOAC, Washington, DC.

Bocco, A., Cuvelier, M. E., Richard, H., and Berset, C. (1998) Antioxidant activity and phenolic composition of citrus peel and seed extracts. *Journal Agriculture and Food Chemistry*, 46(6), 2123-2129. https://doi.org/10.1021/jf9709562

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Brock, F. M., Forsberg, C. W. and Buchanan-Smith, J. G. (1982) Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. *Applied Enviromental Microbiology*, 44 , 561-9. https://doi.org/10.1128/aem.44.3.561-569.1982

Cabral de Oliveira, A., Valentim, I. B., Silva, C. A., Bechara, E. J. H., Paes de Barros, M., Mano, C. M. and Goulart, M. O. F. (2009) Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. *Food Chemistry,* 115(2), 469-475. https://doi:10.1016/j.foodchem.2008.12.045

Casquete, R., Sonia M. C., Martín, A., Ruiz-Moyano, S., Saraiva, J. A., María G. C. and Paula, T. (2015) Evaluation of the effect of high pressure on total phenolic content, antioxidant and antimicrobial activity of citrus peels. *Innovative Food Science and Emerging Technologies*, 31, 37-44. https://doi.org/10.1016/j.ifset.2015.07.005

Collado, L.S., Mabesa, R. C., Sumague, M. J. V. and Mok, C. K. (1994) Yoghurt like products from rice flour and soy milk. *Philippine Agriculturist Sceintist*, 77(3), 307-319.

Collins, M. D. and Gibson, G. R. (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *American Journal of Clinical Nutrition,* 69, 1052S–1057S. https://doi.org/10.1093/ajcn/69.5.1052s

Difco-Manual (1984) Dehydration Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures, Pub. Difco- Lab., Detroit's Michigan, USA. P, 860.

Dorman, H. J. D. and Deans, S. G. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*, 88(2), 308-316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

Dragan, Y. P., Bidlack, W. R., Cohen, S. M., Goldsworthy, T. L., Hard, G. C., Howard, P. C., Riley, R. T., and Voss, K. A. (2001) Implications of apoptosis for toxicity, carcinogenicity, and risk assessment: fumonisin B1 as an example. *American-Eurasian Journal of Toxicological Sciences,* 61, 6–17. *https://doi.org/10.1093/toxsci/61.1.6*

Eder, R. (2003) Drinkable yogurt beats the bagel. Drug Store News25:42. effects of carbohydrates. *European Journal of Clinical Nutrition*, 61(1), 40–74. https://doi: 10.7767/omz.2003.58.5.42

Elia, M. and Cummings, J. H. (2007). Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates.*European Journal of Clinical Nutrition*, 61(1), 40–74. https:// doi:10.1038/sj.ejcn.1602938

El-Seedi, H. R., El-Said, A. M. A., Khalifa, S. A. M., Göransson, U., Bohlin, L. and Borg- Karlson, A. K. (2012) Biosynthesis, natural sources, dietary intake pharmacokinetic properties and biological activities of hydroxycinnamic acids. *Journal of Agriculture and Food Chemistry*, 60, 10877–10895. https://doi. org/10.1021/jf301807g

Espina, L., Somolinos, M., Lorán, S., Conchello, P., García, D., and Pagán, R. (2011) Chemical composition of commercial Citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control*, 22, 896-902. http://dx.doi.org/10.1016/j. foodcont.2010.11.021

FAO/WHO (2001). Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Report of a Joint FAO/ WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. *Cordoba, Argentina* October: 1-4.

Farnworth, E. R. (2008) The evidence to support health claims for probiotics. *Journal of Nutrition,* 138, 1250-1254. https://doi.org/10.1016/j. foodcont.2010.11.021

Fisher, K., and Phillips, C. A. (2006) The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *Journal of Applied Microbiology*, 101, 1232-1240. http://doi.org/10.1111/j.1365-2672.2006.03035.x

Fontana, L., Bermudez-Brito, M.,Plaza-Diaz, J., Munoz-Quezada S. and Gil, A. (2013) Sources, isolation, characterisation and evaluation of probiotics. *British Journal of Nutrition*, 09, S35–S50. https:// doi:10.1017/S0007114513003127

Gahruie, H.H., Eskandari, M.H., Mesbahi, G. and Hanifpour, M.A. (2015) Scientific and technical aspects of yogurt fortification: A review. *Food Science and Human Wellenss*, 4, 1–8. http:// doi. org/10.1016/j.fshw.2015.03.002

Galanakis, C. M. (2012) Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. Trends Food Science and Technology, 26, 68-87. https://doi:10.1016/j.tifs.2012.03.003

Gibson, G. R., and Roberfroid, M. B. (1995) Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutritional,* 125, 1401–1412. https:// doi:10.1093/ jn/125.6.1401

Gorinstein, S., Martín-Belloso, O., Park, Y.S., Haruenkit, R., Lojek, A., Íž, M., Caspi, A., Libman, I. and Trakhtenberg, S. (2001) Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, 74, 309-315. https:// doi.org/10.1016/S0308-8146(01)00157-1

Goupy, P., Hugues, M.; Boivin, P. and Amiot, M. (1999) Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *Journal of the Science of Food and Agric*ulture, 79, 1625-1634. https:// doi.org/10.1002/(SICI)1097-0010(199909)79:12

Gregurek, L. (1999) Effect of level of starter culture on viability of probiotic bacteria in yoghurt. *Mljekarstvo* (Croatia), 49(1), 3-8. https://doi.orcid. org/0000-0003-4711-0971

Harrigan, W. F. and McCance (1976) Laboratory methods in food and dairy microbiology.452 pp, London, New York, San Francisco: Academic Press.

Hassan, A. and Amjad, I. (2010) Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage. *African Journal of Biotechnology*, 9, 2913-2917. http://www.academicjournals.org/AJB ISSN 1684–5315

Hayat, K., Zhang, X., Farooq, U., Abbas, S., Xia, S., and Jia, C. (2010) Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. *Food Chemistry*, 123(2), 423-429. https://doi.org/10.1016/j. foodchem.2010.04.060

Im, S. J., Kim, J. H. and Min Young Kim, M. Y. (2014) Evaluation of Bioactive Components and Antioxidant and Anticancer. *Natural Product Communications*, 9(4), 483-485. http://dx.doi. org/10.4317/jced.524

Jiang, N., Jin, L. F., Teixeira da Silva, J. A., IsIam, M. Z., Gao, H-W., Liu, Y-Z., and Peng, S. A. (2014) Activities of enzymes directly related with sucrose and citric acid metabolism in citrus fruit in response to soil plastic film mulch. *Scientia Horticulturea*, 168, 73–80. https://doi:10.1016/j.scienta.2014.01.021

Kalemba, D. and Kunicka A. (2003) Antibacterial and an antifungal proprieties of essential oils. *Current Medicinal Chemistry*, 10(10), 813-829. https://doi:10.2174/0929867033457719

Kamran, G., Yosef, G., and Ebrahimzadeh, M. A. (2009) Antioxidant activity, phenol and flavonoid contents of 13 Citrus species peels and tissues. *Pakistan Journal of Pharmacology and Science*, 22(3), 277-281.

Karoui, I. J. and Marzouk, B. (2013) Characterization of bioactive compounds in tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. *BioMed Research International*, 2013, Article ID 345415, 12 pages. https://doi.org/10.1155/2013/345415

Kaur, N., and Gupta, A. K. (2002) Applications of inulin and oligofructose in health and nutrition. *Journal of Bioscience*, 27(7),703-714. https://doi:10.1007/BF02708379

Kim, S. B., Kim, K. Y. and Lin, J. W. (1998). The physiochemical and microbiological properties of yam-yoghurt. Korean *Journal of Dairy Science (Korea)*, 20(3),177-190

Kotzekidou, P., Giannakidis, P. and Boulamatsis, A. (2008) Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in chocolate, Swiss Soc. *Food Science and Technology*, 41, 119-127. https://doi.org/10.1016/j.lwt.2007.01.016

Kumaran, A. and Karunakaran, R. J. (2006) Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food Chemistry*, 97(1), 109-114. https://doi.org/10.1016/j.foodchem.2005.03.032

Larraui J. A. (1999) New approaches in preparation of high dietary fiber powders from fruit by products. *Trends in Food Science and Technology*, 10(1), 3-8.

Ledenbach, L. H. and Marshall, R. T. (2009) Microbiological spoilage of dairy products. In Compendium of the Microbiological Spoilage of Foods and Beverages; 41- 67 pp, Sperber, W. H., Doyle, M. P., Eds.; Springer: New York, NY, USA.

Mandalari, G., Bennett, R. N., Bisignano, G., Trombetta, D., Saija, A., Faulds, C. B., and Narbad, A. (2007) Antimicrobial activity of flavonoids extracted from bergamot (Citrus bergamia Risso) peel, a byproduct of the essential oil industry. *Journal of Applied Microbiology*, 103(6), 2056-2064. https://doi.org/10.1111/j.1365-2672.2007.03456.x

Mandalari, G., Bennett, R. N., Bisignano, G., Saija, A., Dugo, G., Faulds, C. B. and Waldron, K. W. (2006) Characterization of flavonoids and pectin from bergamot (*Citrus bergamia Risso*) peel, a major byproduct of essential oil extraction. *Journal of Agriculture and Food Chemistry*, 54, 197-203. https://doi.org/10.1021/jf051847n

Mann, J. (2007) Dietary carbohydrate: relationship to cardiovascular disease and disorders of carbohydrate metabolism. *European Journal of Clinical Nutr*ition, 61(1), 100–111. doi:10.1038/sj.ejcn.1602940

Manthey, A. and Grohmann, K. (2001) Phenols in citrus peel byproducts: concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *Journal of Agriculture and Food Chemistry,* 49, 3268. https://doi.org/10.1021/jf010011r

Marı´n, F. R., Martınez, M., Uribesalgo, T., Castillo, S., and Frutos, M. J. (2002) Changes in nutraceuticals composition of lemon juices according to different industrial extraction systems, *Food Chem*istry, 78, 319-324. https://doi.org/10.1016/S0308-8146(02)00102-4

Min, K. Y., Kim, H. J., Lee, K. A., Kim, K. T. and Paik, H. D. (2014) Antimicrobial activity of acid-hydrolyzed Citrus unshiu peel extract in milk. *J Dairy Sci.*, 97(4), 1955–1960. http:// doi.org/10.3168/jds.2013-7390

Mohammadi, R., and A. M. Mortazavian. (2011). Review article: Technological aspects of prebiotics in probiotic fermented milks. *Food Rev. Int*. 27: 192–212. https://doi.org/10.1080/87559129.2010.535235

Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Madani, K., Dairi, S., Aoun, O. and Khodir, M. (2015) Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from Citrus sinensis peels. *Food Chemstry*, 187, 507-516. https://doi.org/10.1016/j.foodchem.2015.04.081

Oikeh, E. L., Oviasogie, F. E, and Omoregie, S. E. (2020) Quantitative phytochemical analysis and

antimicrobial activities of fresh and dry ethanol extracts of Citrus sinensis (L.) Osbeck (sweet Orange) peels. *Clinical Phytoscience,* 6, 46. https://doi.org/10.1186/s40816-020-00193

O'Shea, N., Arendt, E. K., and Gallagher, E. (2012) Dietary fiber and phytochemical characteristics of fruit and vegetable by -products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technology*es, 16, 1-10. https://doi.org/10.1016/j.ifset.2012.06.002

Patil, B. S., Jayaprakasha, G. K., Chidambara Murthy, K. N. and Vikram, A. (2009) Bioactive compounds: historical perspectives, opportunities, and challenges. *Journal of Agriculture and Food Chemistry*, 57(18), 8142-8160. https://doi.org/10.1021/jf9000132

Pavithra, P. S., Sreevidya, N. and Verma, R. S. (2009) Antibacterial activity and chemical composition of essential oil of Pamburusmissionis *Journal of Ethnopharmacology*, 124, 151-153. http:// doi.org/10.1016/j.jep.2009.04.016

Pereira, M. A., O'Reilly E. and Augustsson K. (2004) Dietary fiber and risk of coronary heart disease: A pooled analysis of cohort studies. Arch. Intern. Med. polymethoxylated flavones in citrus peel molasses. *Journal of Agriculture and Food Chem*istry, 49, 3268. http://doi:10.1001/archinte.164.4.370

Puupponen-Pimia, R., Aura, A. M., Oskman-Caldentey, K. M., Mylla¨rinen, P., Saarela, M., Mattila-Sandholm, T., and Poutanen, K. (2002) Development of functional ingredients for gut health. *Trends Food Science and Technology*, 13, 3-11. https://doi.org/10.1016/S0924-2244(02)00020-1

Radha, K. K., Babuskin, S, Azhagu, S. B. P., Sasikala, M., Sivarajan, M. and Sukumar, M. (2014) Antimicrobial and antioxidant effects of spice extracts on the shelf life extension of raw chicken meat. *International Journal of food microbiology*, 171,32–40.

Ramful, D., Tarnus, E., Aruoma, O. I., Bourdon, E. and Bahorun, T. (2011) Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food research international* 44, 2088-2099. https://doi.org/10.1016/j.foodres.2011.03.056

Rekha, S. S. and Bhaskar, M, (2013) In vitro screening and identification of anti-oxidant activities of orange (citrus sinensis) peels extract in different solvents. *International Journal of Pharma and Bio Science,* 4 (4),405-412.

Rizk, M. A.; Khalil, M. S.; Abu-Zaid, A. and Magdi, M. (2009) Evaluation of antimicrobial activity of stored fermented soybean products. *Bulletant Facilty of Science, Cairo Univ*., 77(C): 1-17.

Saint-Eve, A., C. Levy, N. Martin, and I. Souchon. (2006) Influence of proteins on the perception of flavored stirred yogurts. *Journal of Dairy Science*. 89, 922–933. https://doi.org/10.1016/j.foodres.2011.03.056

Shahidi, F. Wanasundara, P. K. and Janitha P. D. (1992) Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, 32 (l), 67-103. http://dx.doi.org/10.1080/10408399209527581

Shetty, S. B., Mahin-Syed-Ismail, P., Varghese, S., Thomas-George, B., Kandathil-Thajuraj, P., Baby, D., Haleem, S., Sreedhar, S. and Devang-Divakar, D. (2016) Antimicrobial effects of *Citrus sinensis* peel extracts against dental caries bacteria: An in vitro study. *Journal of Clinical and Experimental Dentistry*, 8 (1), 71-77. http:// doi.org/10.4317/jced.52493

Sendra, E., Fayos, P., Lario, Y., Fernandez lopez, J., Sayas barbera, E. and Perez alvarez, J. A. (2008) Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiology*, 25,13–21. https://doi:10.1016/j.fm.2007.09.003

Singh, N., Jaiswal, J., Tiwan, P. and Sharma, B. (2020) Phytochemicals from Citrus Limon Juice as Potential Antibacterial Agents. *The Open Bioactive Compounds Journal*, 8, 1-6. https://doi:10.2174/1874847302008010001

Singh, B., Singh, J.P., Kaur, A. and Singh, N. (2020). Phenolic composition, antioxidant potential and health benefits of citrus peel. *Food Research International*, 132, 109-114. https://doi.org/10.1016/j.foodres.2020.109114

Singleton, V. L.; Orthofer, R. and Lamuela – Raventos, R. M. (1999) Analysis of total phenols and other oxidation substrates and antioxidations by mean of Folin-Ciocalteu reagent. *Methods Enzym*, 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S. and Boydjournal, M.R. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute*, 82, 1107-1112. https://doi.org/10.1093/jnci/82.13.1107

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H. (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Compostion and Analysis*, 19, 669–675. https://doi:10.1016/j.jfca.2006.01.003

Thompson, J. L., K. Lopetcharat, and M. A. Drake. (2007) Preferences for commercial strawberry drinkable yogurts among African American, Caucasian, and Hispanic consumers in the United States. *Journal of Dairy Science,* 90, 4974–4987. https://doi:10.3168/jds.2007-0313

Trumbo, P., Schlicker, S., Yates, A. and Poos, M. (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. *Journal of the American Dietetic Association,* 102(11), 1621–1630. http://doi:10.1016/s0002-8223(02)90346-9

Turkmen, N., Velioglu, Y. S., Sari, F., and Polat, G. (2007) Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. *Molecules*, 12(3), 484-496. http://doi:10.3390/12030484

Vasiljevic, T., and Shah, N. P. (2008) Probiotics—from metchnik off to bioactives. *International Dairy Journal*, 18, 714–728. http://doi:10.1016/j.idairyj.2008.03.004

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lo´pez, J., and Perez-Alvarez, J. (2008) Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrusparadisi L.) and orange (Citrussinensis L.) essential oils. *Food Control*, 19, 1130-1138. http://dx.doi.org/10.1016/j.foodcont.

Wang, Y. C., Chuang, Y. C. and Hsu, H.W. (2008) The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chem*istry, 106, 277-284. http://doi:10.1016/j.foodchem.2007.05.086

Yadav D., Kaumar, A., Kumar, P., and Mishra, D. (2015) Antimicrobial Properties of black grape peel extracts against antibiotic resistant pathogenic bacteria and toxin production molds. *Indian journal of pharmacology,* 47(6), 663. https://doi:10.4103/0253-7613.169591

Zhishen, J., Mengcheng T., and Jianming, W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals *Food Chemistry*, 64, 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2