

Development of Functional Synbiotic Flavored Fermented Skim Milk Drinks Supplemented with Doum (*Hyphaene thebaica* L.) and Carob (*Ceratonia siliqua*) Fruits Powder for Nutritional, Antimicrobial and High Antioxidant Activities

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Abstract

The present study was carried out to make new healthy synbiotic flavored fermented skim milk drinks (SFFSD) supplemented with either Doum (*Hyphaene thebaica* L.) or Carob (*Ceratonia siliqua*) fruits powder for nutritional, antioxidant and antimicrobial activities. The HPLC analysis showed higher phenolic and flavonoid content in Doum extract than that of Carob. The antimicrobial activity of the methanolic extracts of both Doum and Carob against foodborne pathogens showed that the most sensitive indicators were *Bacillus cereus*, *C. albicans* and *S. aureus*, followed by *T. mentagrophyte* and *E. coli* wherein, the diameter of clear zones was, 29, 27, 24, 23 and 13 mm, respectively. While no effect was noticed against *Aspergillus flavus*. On the other hand, the methanolic extract of Carob fruit exhibited only a weak antibacterial effect against *B. cereus* (20 mm). Six treatments of SFFSD were made using both Doum or Carob fruit powder as prebiotic and *Lactobacillus paracasei* as probiotic bacteria. The added levels from Doum were 2%, 4% and 6%, while that added from Carob were 5%, 10% and 15%. Adding Doum and Carob powder increased the containing of minerals; Ca, K, Mg and iron compared with control. Both viscosity and viability of *L. paracasei* for the SFFSD were increased with increasing the added levels from either Doum or Carob, compared with the control. Samples of SFFSD containing Carob powder, show superior sensory for all parameters and total score points during storage period, especially at a level of 10%. It seems evident that samples

supplemented with Doum powder were also acceptable.

Keywords

Antimicrobial, Carob Fruits, Doum Fruits, Phenolic Compounds, Prebiotic and Probiotic

1. Introduction

Recently, healthy foods with functional properties and high content of nutraceuticals have been concerned [1] [2]. Fermented food has a health benefit as it contains living lactic acid bacteria, which could cure some intestinal disorders, decrease the risk of cancer, lower blood cholesterol and improve digestion of lactose, especially for people who have lactose intolerance [3]. Moreover, the lactic acid fermentation of yogurt makes it easily digestible [4] and increases the bioavailability of calcium in intestine [5]. Fermentation and proteolysis of milk by various microorganisms lead to releasing of bioactive peptides which has beneficial biological activities, such as antioxidant and inhibition of the angiotensin-converting enzyme [6]. Yogurt is a good source of essential amino acids, vitamins, and minerals but no fibers. However, fibers can act as stabilizers, fat replacers, prebiotic agents, functional ingredients and nutraceuticals when added into yogurts [3] [7]. A probiotic is defined as a viable microbial dietary supplement, which beneficially affects the host through its effects in the intestinal tract, or as it is a live microbial food ingredient that is beneficial to health and used widely in fermented dairy products. While, prebiotic is not digestible food ingredient with carbohydrates origin, especially oligosaccharides and it can selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon as it may use it as a source of energy [8]. A prebiotics has many health benefits, including reducing cancer risk in the large intestine, enhancing the immunity and increasing mineral absorption, especially calcium. Prebiotics are found in several vegetables and fruits and are considered as functional food components. Prebiotics when added into foods can improve sensory characteristics such as taste, texture, and mouthfeel, hence it is used in many applications like dairy products and bread. Prebiotics, e.g., inulin is found naturally in many plants, so it may be used as ingredients in functional foods [8] [9]. Probiotics and prebiotics together may be combined to introduce synbiotic products which can provide consumers with health benefits [10]. Synbiotics improve absorption of minerals, optimize assimilation of nutrients and prevent incidence of diarrhea. Also, synbiotic have antimicrobial, anticancer, anti-allergic and immune-stimulating properties. Probiotic bacteria along with prebiotics cause the release of antibacterial substances such as bacteriocins and retarding growth of pathogenic bacteria [9]. Low-fat and non-fat fermented dairy products such as yogurt, have gained consumer acceptance as it is good for health as it decreases

the risks that connected with obesity and coronary heart diseases [11]. Besides, the therapeutic properties of yogurt also have high nutritive benefits, as it is a rich source of proteins, carbohydrates, vitamins, phosphorous and calcium [12]. Proteins in yogurt are of excellent biological quality, because the nutritional value of milk proteins is well preserved during the fermentation process [13]. Fortification of yogurt with plant sources such as fruits, vegetables and different cereals provides phytochemical antioxidants, as well as protein-bound polysaccharides, which help to reduce blood glucose levels through increasing serum insulin [3] [14]. Hence supplementation of physiochemical active ingredients such as dietary fibers, phytosterols, antioxidants, and isoflavones can use as functional tools in yogurt [3] [15].

Doum fruit (*Hyphaene thebaica* L.), belonging to the family of *Arecaceae* [16], is widespread in the Sahel and tends to grow in areas where groundwater is present and is found along the Nile River in Egypt, Sudan [17] and Western India [16]. Doum fruit is registered as one of the most beneficial and useful plants in the world [18]. It has a taste like gingerbread and has an oblong, yellow-orange apple sized fruit with red outer skin, a thick, spongy and rather sweet and fibrous fruit pulp [19]. Doum is a common beverage consumed in traditional places in Egypt. The aqueous extract is rich in flavonoids and polyphenolic compounds [20] [21]. It also possesses and exhibits antioxidant; which is important in health and disease prevention [16] [22] [23]. The bioactive phenols in Doum have the ability to act as efficient free radical scavenging and stabilizing food against oxidative deterioration [24] [25]. Doum has health promotion for consumers through a reduction in cholesterol and [23] [26] [27] [28]. It also has anticancer [20] and antimicrobial activities [16] [24] [25].

Doum fruit contains high levels of essential minerals such as potassium, sodium, calcium, magnesium, and phosphorus, as well as, it contains B-complex vitamins, carbohydrates, and fiber, which is essential for good nutrition [29] [30] [31]. Doum contains high levels of protein (2.17% - 7.05%) [30] [31] [32], ash (4.00 and 6.55 g/100g), amino acids and fatty acids, which if properly utilized can improve human nutrition and health. It is a good source of essential amino acids, especially in the flesh such as phenylalanine, leucine, valine and isoleucine, moreover, it is considered as a rich source of palmitic acid. The flesh had a high content of unsaturated fatty acid especially oleic acid and linoleic acid [28]. The powder of Doum is often dried and then added to food as a flavoring agent [19], stabilizer [23] [33] and as functional food used in some dairy products like frozen yogurt [34] [35], whey beverages [36] and ice cream manufacture [37].

Carob (*Ceratonia siliqua*) is one of the important crops in western Asia and North Africa, it belongs to the *Leguminosae* family and it is mainly cultivated in Mediterranean and Aegean regions. Carob is being used by humans as food source and medicinal purposes. The modern food industry starts to discover the great potential of this plant because of its nutritional value and medicinal properties. It has an excellent antioxidant capacity along with other important medi-

cinal activities [38] [39]. The pods of the carob fruit have long been used in human nutrition, including sweets, biscuits and processed drinks, because of its high sugar content and low price [40]. The gum in it is a galactomannan, a valuable natural food additive for products such as ice cream, sweets and soups [41]. Carob has chemical and medicinal properties; it contains phytoconstituents, the aqueous extracts of its fruits inhibit lipid peroxidation, and inflammation and enhance cholesterol efflux. Also, it has antibacterial, antifungal, antiviral, anticancer and antidiabetic activities [38] [39]. Carob extracts have various health-promoting effects mainly attributed to its high phenolic contents [39] [42], and anti-proliferative activity [43].

Carob pod has been used as feed and food mainly due to its richness in sugars contents (40% - 60% of sugars) [44], which is mainly composed of sucrose (32% - 38%), fructose (5% - 7%) and glucose (5% - 6%) [39] [45], and this makes its flour might be considered as a natural sweetener [44]. Also contains dietary fibers (27% - 50%) and minerals (potassium, sodium, iron, copper, manganese and zinc) and low amounts of protein (3% - 4%) and lipids (0.4% - 0.8%) [45]. In addition, Carob is containing phenolic acids, flavonoids, etc., which have functional properties and provide health benefits to the human body. Moreover, Carob pulp has similar nutritional, functional and organoleptic properties to cocoa, but low-fat content [39].

The aim of this study: 1) Investigation of the phenolic and flavonoids content, antioxidant and antimicrobial activity of both Doum and Carob fruits powder, 2) Making synbiotic flavored fermented skim milk drinks (SFFSD) with a new flavor, functional properties and health benefits by adding either doum or carob fruits powder as prebiotic and adding *L. paracasei* as probiotic, 3) Studying some chemical, organoleptic properties, minerals, viscosity and assessment the viability of the probiotic bacteria for the synbiotic flavored fermented skim milk drinks and probiotic control during the storage period.

2. Materials and Methods

2.1. Materials

Fresh skim buffalo's milk was obtained from Dairy Processing Pilot Plant, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. Dome and Carob palm fruits powder were purchased from local market in luxor, Egypt. Commercial grade sugar cane was purchased from local market in Fayoum. The isolate of lactic acid bacteria (LAB) that used in this study as probiotic culture is LAB13 which previously identified as *Lactobacillus paracasei* (Accession No. HQ177096.1) [46] [47] [48], was obtained from the culture collection of Agricultural microbiology department, Faculty of Agriculture, Fayoum University, Egypt. The pathogenic bacterial and fungi indicators used in this study were supported by Prof. Khaled Elbanna, Prof. of Microbiology and Biotechnology, Department of Microbiology, Fayoum University, Fayoum, Egypt. All chemicals and reagents used for this study were of analytical grades and were obtained

from Sigma and Merck Companies.

2.2. Methods

2.2.1. Preparation of Doum and Carob Fruits Powder and Determination of the Total Phenolic Content

The main powder of Doum and Carob were sieved (100 mesh) to obtain the fine powder, then sealed in bags and stored at $5^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until use.

The Folin-Ciocalteu colorimetric method was used to determine total phenolic content (TPC) according to the methods described by Singleton, *et al.* [49]. The total phenolic content was determined by comparing with a standard curve, prepared using Gallic acid at a wave length of 765 nm ($10 - 200 \mu\text{g/ml}$; $Y = 0.025X + 0.2347$; $R^2 = 0.9986$), where, Y, is the absorbance and X, is concentration of Gallic acid in $\mu\text{g/ml}$. The mean of at least three readings was calculated and expressed as mg of Gallic acid equivalents (mg GAE)/100g of fruit powder.

2.2.2. Total Flavonoids Content and HPLC Analysis of Phenolic Compounds

The total flavonoids content (TFC) was determined according to Barros *et al.* [50]. The sample (100 μL) of each extracted solution was mixed with 1.25 ml and 75 μL of distilled water and NaNO_2 solution (5%), respectively, then after 5 min.; 150 μL of a 10% $\text{AlCl}_3 \cdot \text{H}_2\text{O}$ solution was added. After more 6 min; 500 μL of 1 M NaOH and 275 μL of distilled water were added to the reaction mixture. The solutions were mixed well, and the intensity of the pink color was measured at 510 nm, using spectrophotometer (Chrome Tech model: CT-2400 UV/Vis). The standard curve was made using (+)-Catechin (20 - 100 $\mu\text{g/ml}$) and the results were expressed as mg of (+)-Catechin equivalents (CEs)/g of fruit powder.

HPLC analysis of the phenolic extracts was carried out as described by Ydjedd *et al.* [51]. The separation of phenolic acids and flavonoids were performed with an Agilent 1260 Infinity series HPLC system equipped with Column Zorbax C18 (5 μm , 4.6 mm \times 150 mm, Agilent) with mobile phase flow rate of 0.5 ml/min. The temperature of column was controlled at 25°C . Injection volume was 20 μL , the detection wavelengths were set at 280 nm. Prior to each run, the HPLC system was allowed to warm, and the baseline was monitored until it was stable before sample analysis.

2.2.3. Determination of Radical Scavenging Activity

The free radical scavenging activity of the methanolic extracts of both Doum and Carob fruit powder were measured using, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to Pacôme *et al.* [52] and Smetanska *et al.* [53]. The scavenging or inhibition percentage was calculated according to the following equation:

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

where: Abs. is absorbance at 515 nm.

Measurement was performed at least in triplicate. Inhibition of coloration was

expressed as a percentage, and the effective concentration 50% (EC₅₀) was obtained from the inhibition curve.

2.2.4. Activation of LAB Culture

Activation of *Lactobacillus paracasei* (Accession No. HQ177096.1) was first done in MRS broth medium and then plotted in agar MRS medium, which was prepared according to Oxoid [54]. More activation occurred in 10% (w/v) sterilized skim milk and incubated at 37°C for 24 h under anaerobic conditions to obtain at least 10⁶/ml viable counts.

2.2.5. Preparation of Methanolic Extracts and Determination of the Antimicrobial Activity and Minimum Lethal Concentrations (MLC)

To prepare the methanolic extracts, dried powder (50 g) of either Doum or Carob was soaked in 500 ml of methanol/water (80:20) and shaken at room temperature for 24 h. Then, the mixture was filtered through Whatman paper No. 1 and evaporated to dryness in a rotary evaporator at 45°C. Stock solutions (200 mg/ml H₂O) of each extract were prepared for further work.

Antimicrobial activity of the methanolic extracts of Doum and Carob fruits powder was evaluated against *Bacillus cereus* DSM 31, *Staphylococcus aureus* (ATCC8095) as Gram-positive pathogenic bacteria, and *Escherichia coli* (ATCC 25922) as Gram-negative. In addition, all extracts were assessed against the molds and fungi such *Trichophyton mantigrophytes*, *Aspergillus flavus*, and *Candida albicans* (ATCC 10231). Both stock cultures of bacterial strains, fungi and candida yeast were maintained on nutrient agar and potato dextrose agar (PDA) slants, respectively and stored at 4°C until use.

Antimicrobial activity of Doum and Carob extracts was determined by agar well diffusion method according to Torres *et al.* [55], Assiri *et al.* [56] and Elbanna *et al.* [57]. For this, the sterilized potato dextrose agar (used for yeast and fungi) and Mueller Hinton agar (used for bacteria) were poured into sterilized petri dishes, then left to solidify at room temperature. All agar plates were swabbed from fresh microbial cultures. Wells of 9 mm in diameter were created in the center of agar plates using a sterile cork borer and 200 µl of each tested extract were pipetted to the wells. Plates with yeast and fungi were incubated at 28°C for 24 - 72 h, while pathogenic bacteria were incubated at 37°C for 24 h. The antimicrobial activity was assessed by measuring the clear zones (mm) around each well. Methanol free of extracts was used as a control. The antibacterial activity of antibiotics was assessed by the agar disk diffusion method according to Bauer *et al.* [58] by measuring the diameter (mm) of clear zones around each well. Antibiotics of Gentamycin (30 µg), Chloramphenicol (30 µg), Augmentin (30 µg), and Fluconazole (100 µg) were used as standards antibiotics for comparison.

The minimum lethal concentrations (MLCs) of Doum and Carob extracts were assessed against tested microorganisms according to the dilution method described by Elbanna *et al.* [57]. For this, serial two-fold concentrations of each extract (2 - 128 mg/ml) were pipetted into tubes containing 4 ml of LB or potato

dextrose broth media (PD) for pathogenic bacteria or fungi, respectively. For pathogenic bacteria, each tube was inoculated with 0.4 ml (0.5 McFarland medium) of a standardized suspension of bacterial species containing 1×10^6 cell/ml. For pathogenic fungi, serial two-fold concentrations of each extract were pipetted into tubes containing 4 ml of PD broth medium. Each tube was inoculated with 1×10^6 of prepared spores. Subsequently, all inoculated tubes were incubated at appropriate temperature and time for each microorganism. After the incubation period, 0.1 ml from each tube were sub-cultured on LB agar or PDA plates and incubated at appropriate temperature and time for each microorganism. The lowest concentration of tested extracts which gave a viable count less than 0.1% of the original inoculum (1×10^6 cell/ml) were considered as the minimal lethal concentration (MLC). Antibiotics for Gram positive and negative bacteria and fluconazole will serve as standards for comparison in antibacterial and antifungal tests, respectively.

2.2.6. Experimental Procedure

A preliminary experiment was made using different added levels of either Doum or Carob palm fruits powder to find out the acceptable and recommended added levels for making the synbiotic flavored fermented skim milk drinks (SFFSD). In this preliminary experiment the added levels of Doum were, 2%, 4%, 6%, 8% and 10%. While, the added levels of Carob powder were, 1%, 3%, 5%, 10% and 15%. From the initial experiment the sensory evaluation indicated that, the acceptable concentrations of Doum are 2%, 4% and 6%, while, the acceptable concentrations of Carob are 5%, 10% and 15%. The panelists also indicating; the necessity of sifting both of the powder before manufacture due to presence of rough particles in the mouth after tasting the samples of SFFSD.

The steps of making the main experiment of the synbiotic flavored fermented skim milk drinks treatments, were shown in **Figure 1**. The treatments were prepared using either Doum or Carob separately for supplementation and compared by control made of fermented skim milk with probiotic culture and free of flavoring additives. Fresh skim milk was heated to 90°C for 10 - 15 min., subsequently; the sugar (6%) was added to the milk during cooling to 40°C. After that, inoculation with the culture of *Lactobacillus paracasei* (5%) was occur, then mixing and divided to 7 parts. Three of the parts, was supplemented with Doum powder (2%, 4% and 6%), while the other three parts was supplemented with Carob powder (5%, 10% and 15%) and the last part used for making the probiotic control. All synbiotic treatments and control incubated at 37°C till gelation obtained, then cooled to 25°C, stirred, packed and stored for 20 days at 5°C for different analysis.

2.2.7. Analytical Methods

All samples of control and the SFFSD treatments were evaluated for some physical and chemical properties (fresh age). Also, the sensory quality attributes (along 15 days of storage), as well as the living lactic acid bacteria (*L. paracasei*),

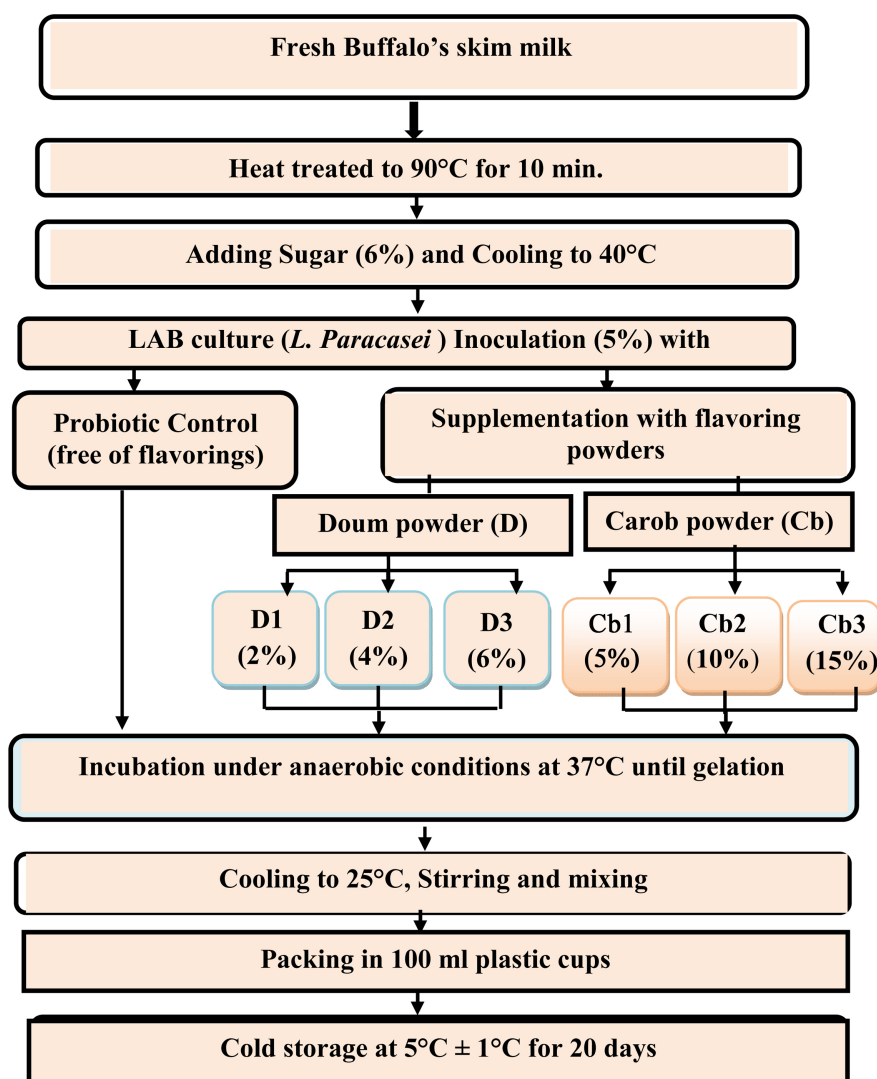


Figure 1. Schematic flow diagram of the basic steps involved in making synbiotic flavored fermented skim milk drinks supplemented with either Doum or Carob fruits powder.

were investigated (along 20 days of storage). The samples of each treatment and control were analyzed in three replicates for each parameter.

1) Chemical analyses and Minerals content

The total solids, total nitrogen, and ash contents of different fresh SFFSD samples were determined using oven drying method, Kjeldahl method, and incineration at 550°C, respectively as described in AOAC [59]. The pH values of the different synbiotic fermented skim milk drink samples were tested during 20 days of storage by laboratory pH meter with a glass electrode Model pH—(ORION 420). Minerals (P, K, Ca, Mg, Zn, and Fe), were determined in all fresh samples of all SFFSD treatments and probiotic control, using Inductively Coupled Plasma (ICP) equipment (Model 6300 Duo UK, England); according to APHA [60].

2) Viscosity measurement

The apparent viscosity (CP) of the fresh SFFSD samples supplemented with

either Doum or Carob fruits powder, were carried out as described by Santillán-Urquiza *et al.* [61]. The viscosity measured using a Brookfield viscometer Model, DV-II + Pro (Brookfield unit, MA, USA) with spindle No. 4 at 25 °C with a rotation speed at 30 rpm. The results were expressed as centipoises (CP) after 30 s of rotation.

3) Viability of LAB (Log cfu/ml)

Investigation of LAB (*L. paracasei*) viability in the probiotic control and the different SFFSD samples was made using MRS agar, which prepared as described in Oxoid [54]. Serial dilutions were made for each sample and 1 ml of different dilution is inoculated into a plate contain MRS agar medium. The plates were incubated at 37 °C for 48 h under microaerophilic conditions and then LAB colony was enumerated.

4) Sensory evaluation

The samples of SFFSD were sensory evaluated during the storage period (15 days) by a regulars' test panels of 10 staff members from Dairy Depart., Fac. Agric., Fayoum Univ., using a scheme of 10 points for color & appearance, 10 points for acidity, 35 points for body & texture and 45 points for flavor, which give a total score of 100 points.

5) Statistical analysis

All results were analyzed using General Linear Models (GLM) procedure of Statistical Package for Social Sciences [62], Version 17.0.0 software. Duncan [63], multiple range tests were used to compare between the means.

3. Results and Discussion

3.1. Extraction Yield, Total Poly Phenolic and Flavonoid Contents of Doum and Carob Fruits Powder

In this study two types of fruit powder; Doum and Carob were extracted with different solvents to determine the phenolic and flavonoid compounds. Results of the extract yield, TPC and TFC contents in Doum and carob fruits powder are presented in **Table 1**. The results revealed that the amount of extract varies according to the type of fruit powder as well as the solvent used in the extraction process. The yield of the carob fruit extract was higher than that of Doum fruit in all the extraction solvents used. Pure water recorded the highest extraction yield in both Doum and Carob fruit. The extract yield was varied from 36.916 to 39.974 for Doum fruit extracts and from 47.905 to 53.063 g/100g DW for Carob fruit. These high extract yields could be attributed to the high content of the total soluble solids, especially soluble carbohydrate in Doum and Carob fruits powder [23] and [44]. These results are in agreement with those reported by Abou-Elalla [20] for Doum fruit and Ydjedd *et al.* [51], for Carob fruit.

The phenolic compounds solubility is dependent on the polymerization degree and the solvent used as well as formation of insoluble complex. The TPC and TFC of the different extracts of Doum and Carob fruits are presented in **Table 1**. The results revealed that there is a considerable difference in TPC and

Table 1. Effect of extraction solvent type on yield, total phenolic and total flavonoids content of Doum and Carob fruits powder.

| Item | Doum fruit powder | | | Carob fruit powder | | |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|---------------------------|
| | yield (g/100g DW) | TPC (mg GAE/g) | TFC (mg QUE/g) | yield (g/100g DW) | TPC (mg GAE/g) | TFC (mg QUE/1g) |
| Methanol: water (80:20) | 36.916 ± 1.03 ^b | 67.540 ± 1.57 ^a | 34.589 ± 1.10 ^a | 49.186 ± 1.21 ^b | 13.840 ± 0.92 ^a | 1.878 ± 0.03 ^a |
| Ethanol: water (50:50) | 36.437 ± 0.97 ^b | 62.356 ± 1.37 ^b | 29.538 ± 0.95 ^b | 47.905 ± 1.67 ^b | 10.189 ± 0.83 ^b | 1.219 ± 0.01 ^b |
| Water (100%) | 39.974 ± 1.17 ^a | 51.436 ± 2.43 ^c | 22.621 ± 1.08 ^c | 53.063 ± 1.08 ^a | 8.885 ± 0.78 ^b | 0.591 ± 0.01 ^c |

Notes: a, b and c: Means in each column with different superscript letters are significantly different ($P \leq 0.05$), TPC: Total phenol content, TFC: Total flavonoid content, DW: Dry weight.

TFC of both Doum and Carob fruit extracts. All Doum extracts show richer source than that in Carob extracts. The total phenolic content of Doum extracts ranged from 51.436 to 67.540 mg/g DW, while total flavonoids varied from 22.621 to 34.589 mg/g DW. These results are in agreement with those reported by Mohamed *et al.* [24] for methanolic extract of Doum fruit and higher than those reported by Aamer [21] for Doum water extract. On the other hand, Abo-shora *et al.* [25], reported about Doum fruit from Sudan that it contains high TPC, ranged from 139.48 to 116.26 mg/g DW and TFC ranged from 47.17 to 24.04 mg/g DW.

The TPC and TFC of Carob fruit powder using three different extraction solvents are presented in **Table 1**. The results showed that the methanol: water (80:20) extract, had the highest total phenolic and flavonoids content with value of 13.84 mg GAE/g DW and 1.878 mg QUE/g DW, respectively; which is higher than those reported by Goulas and Georgion [64] for different extracts of Carob flour ranged between 2.88 to 3.82 mg/g DW for TPC and from 0.08 to 0.8 mg/g DW for TFC. On the other hand, the lowest TPC and TFC contents in this study (8.885 mg GAE/g and 0.591 mg QUE/g DW, respectively), was registered for the water extract of Carob fruit powder. The ethanol: water extract exhibited high TPC and TFC for both Doum and Carob fruits compared by water extract. The results of TPC and TFC are consistent with those reported by Petkova *et al.* [44] for the water extract of Carob and Benković *et al.* [65] for Carob flour with seeds.

3.2. Identification of Phenolic and Flavonoid Compounds in the Methanolic Extract of Doum and Carob Fruits

The HPLC analysis of phenolic compounds in the studied fruits powder extracts is explained in **Table 2**. The results indicated that Chlorogenic acid, catechin, Salicylic, Cinnamic, Gallic, Caffeic and Vanillic acids were the main phenolic acids in Doum fruit extract as it represented, 15.601, 14.89, 8.112, 6.556, 6.467, 6.410 and 4.741, respectively. While, Tannic and Syringic acids were found in low concentrations as the readings were 1.087 and 1.853, respectively. Regarding

Table 2. Concentration of identified phenolic acid and flavonoids in isolated methanolic Doum and Carob fruit extracts as percentage of the total.

| Poly phenolic compounds | Doum extract | Carob extract |
|-------------------------|--------------|---------------|
| Gallic acid | 6.467 | 6.33 |
| Pyrogallol | 1.361 | 2.8830 |
| Tannic acid | 1.087 | 2.3086 |
| Resorcinol | 1.229 | 2.88 |
| Caffeic acid | 6.410 | 4.096 |
| Vanillic acid | 4.741 | 4.049 |
| Chlorogenic acid | 15.601 | 1.696 |
| Catechin | 14.89 | 8.999 |
| E-vanilic | 3.09 | 5.552 |
| p-coumaric acid | 3.507 | 2.78 |
| Syringic acid | 1.853 | 1.403 |
| Salicylic acid | 8.112 | 1.426 |
| Cinnamic acid | 6.556 | 1.243 |
| Quarecetin | 3.105 | 9.66 |
| Naringin | 3.067 | 0.428 |
| Rutin | 1.578 | 1.992 |
| Myricetin | 2.077 | 5.547 |

to the flavonoids, the results revealed that Quercetin, Naringin and Rutin were the main flavonoids in Doum extract sample. Similar results were reported by Aamer [21], furthermore, Habib *et al.* [66] reported that Doum fruit contains more than 14 polyphenolic compounds.

As seen from **Table 2**, six free phenolic acids (Gallic, Syringic, p-coumaric, Cinnamic, Chlorogenic acid and Caffeic acid) and four flavonoids (Naringin, Quercetin Myricetin and Rutin) were identified and semi-quantified in Carob fruit extracts. These results are in agreement with the findings of Owen *et al.* [67] and Benchikh *et al.* [42] for the analysis of phenolic profiles of Carob fiber and pulp, respectively. Indeed, the results showed that Quercetin was the most abundant phenolic compound in Carob extract. Our results are in agreement with that reported by Ydjedd, *et al.* [51], for Algerian Carob.

3.3. Antioxidant Activity of Doum and Carob Methanolic Extract Using DPPH Assay

The antioxidant activity of Carob and Doum isolated methanolic extracts were assessed using the DPPH radical scavenging activity of each extract and compared with the antioxidant potential of pure butylated hydroxy anisole (BHA) as a well-known antioxidant. As shown in **Figure 2**, both Doum and Carob extracts quenched DPPH radical and the scavenging activity of the extract was increased

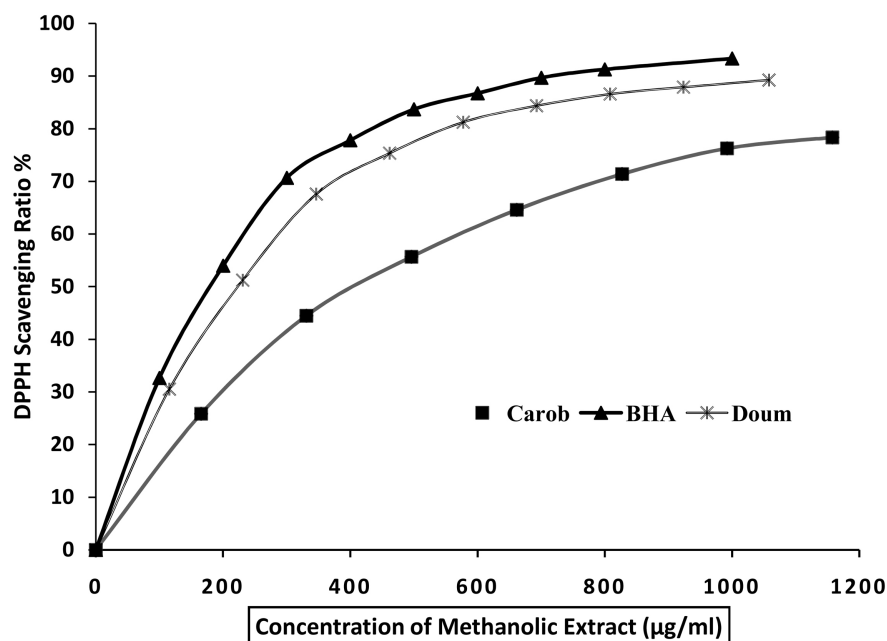


Figure 2. Free radical scavenging activity of different concentrations for Doum and Carob methanolic extracts comparing with butylated hydroxy anisole (BHA).

with increasing the concentration of the phenolic extract. Among the tested extracts, Doum extract shows stronger antioxidant activity; which correlated with more total polyphenol content than that obtained for Carob extract. However as revealed by values of the EC_{50} (extract concentration causing 50% DPPH radical scavenging ability). The BHA had the highest inhibitory effects and this effect was more as its concentration increased for example at concentration of 200 µg/ml the DPPH scavenging ratio was 53.98, followed by Doum extract in DPPH scavenging ability as its reading was 230.9 µg/ml methanolic extract to scavenging 51.25 of DPPH ratio and this was close to the effect of BHA. While, Carob methanolic extract showed the least inhibitory effect for DPPH scavenging as it needed 496.13 µg/ml extract to scavenging 55.66% of DPPH. The difference between both extracts could be attributed to the difference in their poly phenolic and flavonoid contents. Doum extract was reported to have high antioxidant activity [20]. The results for Doum extracts are in compatible with that reported by Aboshora *et al.* [25], El-Kholy [34] and Siddeeg *et al.* [23]. Also results of Carob extract was compatible with that reported by Gharb and Fadhel [33], Petkova *et al.* [44], Goulas and Georgiou [64] and Ydjedd *et al.* [51].

3.4. Antimicrobial Activity of both Doum and Carob Methanolic Extracts

In this study, antimicrobial activity of the isolated methanolic extracts of both Doum and Carob was evaluated against foodborne pathogens including *B. cereus*, and methicillin-resistant *Staphylococcus aureus* as Gram-positive and *Escherichia coli* as Gram-negative, *C. albicans* and *T. mentagrophyte* as food spoilage and dermatophytic molds. The pathogenic foodborne and spoilage

molds tested in this study differed in their sensitivity to the methanolic extract of Doum, wherein *Bacillus cereus* and *C. albicans*, and *S. aureus* were the most sensitive indicators followed by *T. mentagrophyte* and *E. coli*, respectively, while no effect was noticed against *Aspergillus flavus*. Antimicrobial activity (Figure 3 and Table 3) of Doum extract against *B. cereus*, *C. albicans*, *S. aureus*, *T. mentagrophyte*, and *E. coli* were 29, 27, 25 mm 23, and 13 mm, respectively, when compared with the traditional antibiotics, with minimal lethal concentrations (Table 3) ranged of 32, 32, 64, 64 and 128 mg/ml, respectively, these results were different comparing with what obtained by Abdallah [68]. While the methanolic extract of Carob exhibited a weak antibacterial effect (20 mm) only against *B. cereus*, with minimal lethal concentration of 128 mg/ml and no effect observed against the other tested pathogens. Similar results were reported that, methanolic

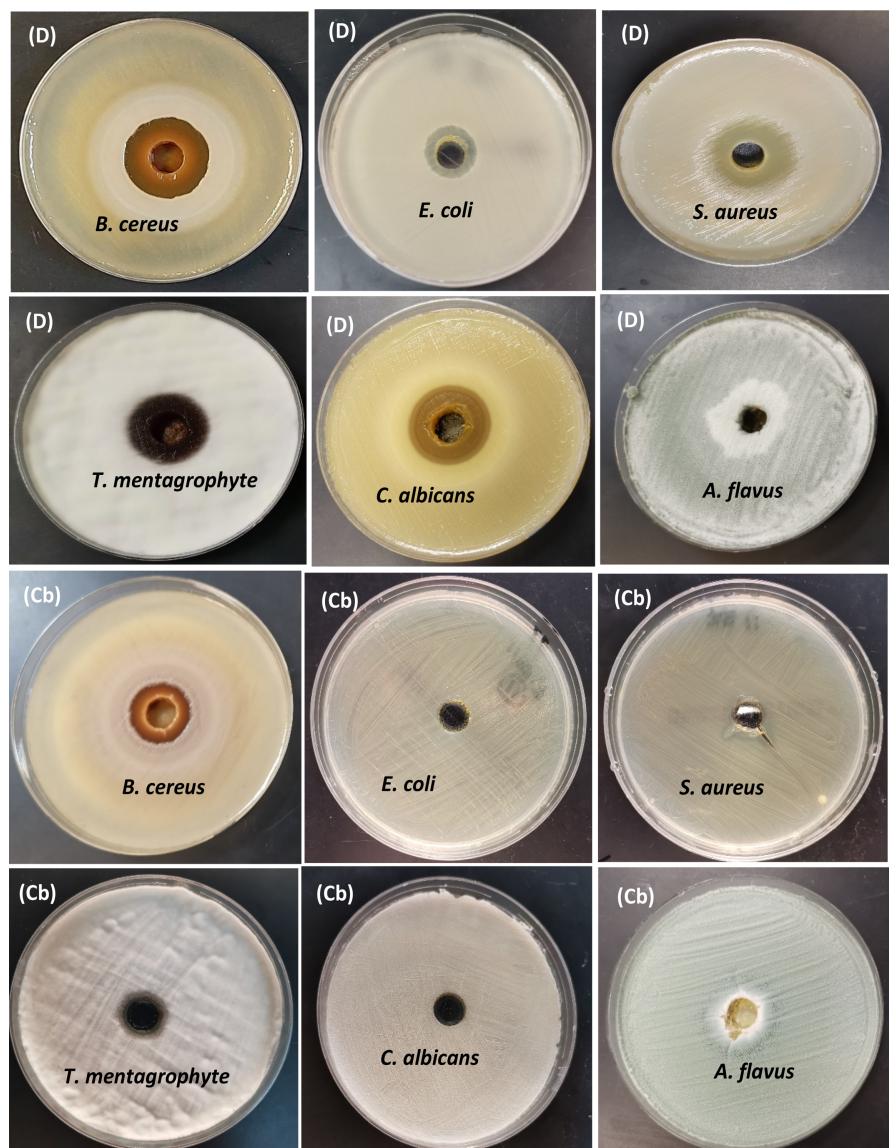


Figure 3. Antimicrobial activity of Doum (D) and Carob (Cb) extracts against the pathogen foodborne bacteria and spoilage fungi as indicated by clear zone diameter (mm).

Table 3. Antimicrobial activity of isolated Doum and Carob fruits extracts against foodborne pathogen bacteria and spoilage fungi as indicated by clear zone diameter (CZD*, mm)^{a,b,c} and minimal lethal concentration (MLC, mg/ml).

| Treatments | Clear zone diameter (mm) and minimal lethal concentration (MLC mg/ml) | | | | | | | | | | | |
|-------------------------|---|-----|------------------|-----|----------------|-----|------------------------------------|-----|--------------------------|-----|------------------|-----|
| | Pathogenic bacteria tested | | | | | | Pathogenic moulds and fungi tested | | | | | |
| | <i>B. cereus</i> | | <i>S. aureus</i> | | <i>E. coli</i> | | <i>C. albicans</i> | | <i>T. mantigrophytes</i> | | <i>A. flavus</i> | |
| | CZD | MLC | CZD | MLC | CZD | MLC | CZD | MLC | CZD | MLC | CZD | MLC |
| Doum extract | 29 | 32 | 24 | 64 | 13 | 128 | 27 | 32 | 23 | 64 | - | - |
| Carob extract | 20 | 128 | - | - | - | - | - | - | - | - | - | - |
| Antibiotics references | | | | | | | | | | | | |
| Fluconazole (100 µg/ml) | Nd | Nd | Nd | Nd | Nd | Nd | 28 | Nd | 36 | Nd | 28 | Nd |
| Chloramphenicol (30 µg) | 24 | Nd | 23 | Nd | 25 | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| Augmentin (30 µg) | 0.0 | Nd | 25 | Nd | 0.0 | Nd | Nd | Nd | Nd | Nd | Nd | Nd |
| Gentamycin (30 µg) | 22 | Nd | 19 | Nd | 13 | Nd | Nd | Nd | Nd | Nd | Nd | Nd |

Note: *CZD = clear zone diameter (mm)^a, MLC: minimum lethal concentrations—no effect. Each value represents the mean of the sample \pm SD (n = 3). ^bThe diameter of the inhibition zone was measured as the clear area centered on the agar well containing the sample. ^cnd = not determined.

extracts of Doum showed higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria [24] [25] [69] [70]. However, the variation in antimicrobial activity of tested Doum extract against different pathogen microorganisms may be due to the different thickness of cell wall between Gram-positive and Gram-negative bacteria.

Gram-positive and gram-negative bacteria have differences in their membrane structure, the most distinctive of which is the thickness of the peptidoglycan layer [71]. The outer membrane of Gram-negative bacteria is often hidden by a slime layer, which in turn hides the antigens of the cell. Thus, it considers as the main reason for resistance to a wide range of antibiotics, any alteration in the outer membrane by Gram-negative bacteria like changing the hydrophobic properties or mutations in porins and other factors can create resistance. Gram-positive bacteria lack this important layer, which makes Gram-negative bacteria more resistant to antibiotics than Gram-positive ones [72] [73] [74]. Worth notably, methanolic Doum extract exhibited strong antimicrobial activity against Gram-positive foodborne pathogenic bacteria as well as food spoilage and dermatophytic molds but exhibited weak effect against Gram-negative *E. coli*. Furthermore, the plates inoculated with pathogens and containing Doum extract continued its antimicrobial activity for more than 10 days as indicated by the clear zone in the agar well diffusion assay. However, antimicrobial activity of Doum extracts may involve complex mechanisms, such as inhibition of cell wall,

cell membrane, nucleic acid, and protein synthesis, as well as the inhibition of nucleic acid metabolism [75] [76] and it possess antibacterial substances such as bacteriocines which retarding growth of pathogenic bacteria [9]. It worth mentioning that, no growth was observed when new agar plates or broth media were inoculated with a loop from the clear zone area, indicating that methanolic Doum extract acted as a lethal effect, not just as an inhibitor against either Gram-positive pathogen bacteria and/or *T. mentagrophyte*. Finally, it could be concluded that methanolic extract of Doum exhibited antimicrobial activity against both foodborne pathogenic bacteria and food spoilage fungi and molds. So, it could potentially serve as an efficient natural antimicrobial agent in food industry against foodborne microorganisms.

3.5. Chemical Composition of Synbiotic Flavored Fermented Skim Milk Drinks

3.5.1. Minerals Content

Minerals content of SFFSD samples were presented in **Table 4**. Several minerals were identified and quantified; iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg) and potassium (K). In general, there is a significant difference ($P \leq 0.001$) in minerals content among SFFSD and control samples. It is apparent from the obtained results that phosphorus is the predominant mineral followed by calcium, sodium, and zinc in control and in SFFSD samples, these results agreement with Aydin and Özdemir [77]. Synbiotic fermented skim milk drinks supplemented with Doum powder, showed the highest content of iron, calcium, potassium, sodium and magnesium, compared with

Table 4. Minerals (mg/100g DM) contents in flavored synbiotic fermented skim milk drink fortified with either Doum or Carob powder.

| Treatments* | Minerals (mg/100g DM) | | | | | | |
|-----------------|-----------------------|--------------------|---------------------|----------------------|--------------------|--------------------|---------------------|
| | Fe | Zn | Ca | P | Na | Mg | K |
| C | 0.210 ^F | 0.42 ^A | 75.28 ^E | 296.81 ^F | 44.13 ^G | 12.11 ^F | 18.28 ^D |
| D ₁ | 0.831 ^C | 0.37 ^E | 91.61 ^{BC} | 316.61 ^E | 54.36 ^D | 20.41 ^C | 51.001 ^B |
| D ₂ | 2.74 ^A | 0.385 ^D | 95.11 ^B | 320.922 ^D | 60.49 ^B | 20.55 ^C | 55.70 ^B |
| D ₃ | 2.45 ^B | 0.409 ^B | 103.26 ^A | 323.84 ^C | 62.23 ^A | 27.87 ^A | 92.94 ^A |
| Cb ₁ | 0.277 ^F | 0.34 ^F | 86.53 ^D | 335.99 ^B | 49.72 ^F | 14.48 ^E | 21.94 ^D |
| Cb ₂ | 0.512 ^E | 0.372 ^E | 87.54 ^{CD} | 336.79 ^B | 50.89 ^E | 18.53 ^D | 37.94 ^C |
| Cb ₃ | 0.622 ^D | 0.395 ^C | 91.67 ^{BC} | 344.99 ^A | 55.83 ^C | 22.47 ^B | 52.27 ^B |
| SE± | 0.027 | 0.001 | 1.47 | 0.86 | 0.007 | 0.456 | 4.26 |

Note: A, B, ..., and G: Means having different superscript within each column are significantly different ($P \leq 0.001$), SE: Standard error, *C: Probiotic skim milk drink (free additives), D₁, D₂ and D₃, are synbiotic flavored fermented skim milk drinks supplemented with Doum at levels of 2%, 4% and 6%, respectively, Cb₁, Cb₂ and Cb₃ are synbiotic flavored fermented skim milk drinks supplemented with Carob at levels of 5%, 10% and 15%, respectively.

samples of SFFSD that supplemented with Carob powder and probiotic fermented control. This increase was more as the level of added Doum fruit powder was increased, hence it can be suggested that Doum powder can served as an excellent source of these elements. The iron content in SFFSD samples supplemented with Doum powder was the highest followed with that supplemented with Carob powder as the readings were, 2.45, 2.74, 0.831, 0.622, 0.512 and 0.277 for D₃, D₂, D₁, Cb₃, Cb₂ and Cb₁ treatments, respectively, while it was 0.210 mg/100 DM in control sample. The content of Zn, Ca, Na, Mg and K take same high trend in SFFSD treatments that supplemented with Doum, followed by that supplemented with Carob powder, comparing with control. It is worth mention that among all treatments and control the samples that supplemented with Carob fruit powder showed the highest phosphorus content; 344.99, 336.79 and 335.99 for Cb₃, Cb₂ and Cb₁ treatments, respectively. Therefore, using Doum fruit powder [29] [30] [31], as well as Carob powder [38] [39], in supplementation of dairy products can be considered as a promising source for essential minerals in human metabolism that are responsible for healthier growth and prevention of diseases. Moreover, all elements in SFFSD were increasing with the added levels of either Doum or Carob powder comparing with control samples. Overall significant ($P \leq 0.001$) differences in individual mineral compositions could also be observed among the different ratio added from Doum and Carob fruits powder. These results are in agreement with El-Deeb, *et al.* [78], Siddeeg, *et al.* [23] and Ibrahim, *et al.* [79].

3.5.2. Changes in pH Values of Synbiotic Flavored Fermented Skim Milk Drinks

Changes in pH values of all treatments and control samples are given in **Table 5**.

Table 5. Changes in pH values of synbiotic flavored fermented skim milk drinks as affected by using different levels of Carob and Doum fruits powder during cold storage.

| Treatments* | Storage period (days) | | | | |
|-------------|-----------------------|----------------------|---------------------|----------------------|----------------------|
| | Fresh (1 day) | 5 | 10 | 15 | 20 |
| C | 5.82 ^a | 5.74 ^{ab} | 5.34 ^{efg} | 5.17 ^{ghij} | 5.12 ^{hijk} |
| D1 | 5.52 ^{cd} | 5.18 ^{ghij} | 5.11 ^{ijk} | 4.84 ^{lm} | 4.7 ^{mn} |
| D2 | 5.43 ^{def} | 5.08 ^{ijk} | 4.85 ^{lm} | 4.67 ^{mn} | 4.56 ^{no} |
| D3 | 5.63 ^{bc} | 5.26 ^{fghi} | 4.84 ^{lm} | 4.57 ^{no} | 4.43 ^o |
| Cb1 | 5.75 ^{ab} | 5.57 ^{bcd} | 5.47 ^{cde} | 5.19 ^{ghij} | 5.04 ^{jk} |
| Cb2 | 5.7 ^{ab} | 5.42 ^{def} | 5.3 ^{efgh} | 4.98 ^{kl} | 4.81 ^{lm} |
| Cb3 | 5.62 ^{bc} | 5.32 ^{efg} | 5.07 ^{jk} | 4.84 ^{lm} | 4.7 ^{mn} |
| SE± | | | 0.057 | | |

Note: a, b, ..., and o: Means in the same column with different superscript letters are significantly different ($P \leq 0.05$), SE±: standard error, *C: Probiotic skim milk drink (free additives), D1, D2 and D3, are synbiotic flavored fermented skim milk drinks supplemented with Doum at levels of 2%, 4% and 6%, respectively, Cb1, Cb2 and Cb3 are synbiotic flavored fermented skim milk drinks supplemented with Carob at levels of 5%, 10% and 15%, respectively.

The results revealed that all pH values of different SFFSD samples affected significantly ($P \leq 0.05$) by the levels of addition from either Carob or Doum fruits powder and also by storage period. The pH was decreased as a result of further fermentation of lactose into lactic acid by the probiotic bacteria. Values of pH for the control samples were 5.82 in fresh age and reached to 5.12 by the end of cold storage period. While, at the first day of storage, SFFSD samples that supplemented with either Doum or Carob fruits powder, were ranged from 5.52 - 5.63 and 5.75 - 5.62, respectively and by the end of storage it reached to 4.7 - 4.43 and 5.04 - 4.70, respectively. These results indicate that, addition of either Doum or Carob fruits powder, had synergistic effect as prebiotics on the probiotic bacteria as confirmed in **Table 5**, therefore more lactic acid produced and decreasing in the pH values comparing to control samples. Similar results were reported by El-Kholy [34] for pH values of frozen yoghurt as decreased by addition of Doum powder. Moreover, Guler-Akin, *et al.* [80], mention that, the addition of Carob in the fermented milk stimulated the metabolic activities of probiotic bacteria and improved development of acidity.

3.5.3. Dry Matter, Ash and Total Nitrogen Contents

Dry matter (DM), total nitrogen (TN) and ash contents of SFFSD and control samples, were shown in **Figure 4** and **Figure 5**, respectively. The results of dry matter (**Figure 4**) and ash (**Figure 5**) contents of the SFFSD samples, was increased by increasing the added level from either Doum or Carob fruits powder compared with that of control samples. Both ash and dry matter content were high in all SFFSD samples comparing to probiotic control sample and increased with the added level from either Doum or Carob; the ash were 0.731, 0.835, 0.926, 0.989, 0.90, 1.21, 1.23 for control, Cb₁, Cb₂, Cb₃, D₁, D₂ and D₃, respectively. Regarding, DM content the lowest reading was for probiotic control sample

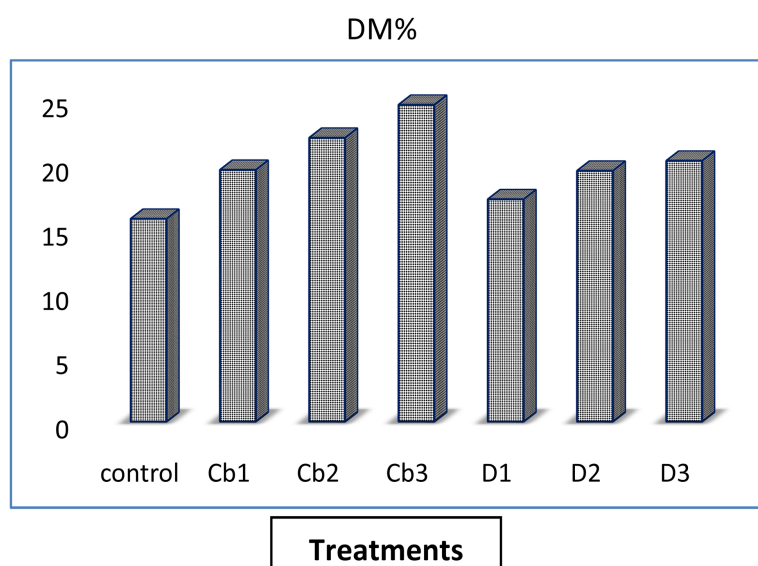


Figure 4. Effect of adding either Doum (D) or Carob (Cb) fruits powder on dry matter (DM) content of the synbiotic flavored fermented skim milk drinks.

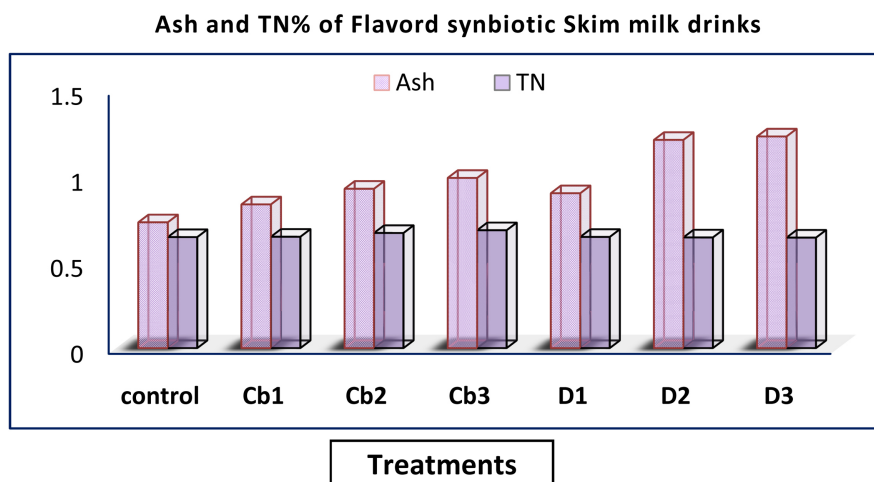


Figure 5. Effect of adding either Doum (D) or Carob (Cb) fruits powder on ash and total nitrogen (TN) content of the synbiotic flavored fermented skim milk drinks.

(15.79%) and the highest one (24.67%), was for SFFSD that supplemented with 15% Carob powder. The present results were in agreement with that reported by Aumara and Hassan [81], Aboshora *et al.*, [28]. Total nitrogen content of probiotic control and SFFSD samples are given in **Figure 5**, there is a little increase in the TN content of SFFSD supplemented with Carob fruit powder comparing with control. On the other hand, no significant effect in the TN content of the samples supplemented with Doum fruit powder. The highest TN content recorded in Cb₃ treatment and the lowest one was recorded in D₃ treatment. Similar results reported that Carob contain low amounts of nitrogen [45].

3.6. Viability of Lactic Acid Bacteria (*L. paracasei*)

The number of lactic acid bacteria (LAB) in the all samples of SFFSD and control along 20 days of cold storage, is presented in **Table 6**. The probiotic control and SFFSD samples showed a significant difference ($P \leq 0.05$) in the counts of *L. paracasei*, the lowest count was registered in control samples as it recorded 8.36, 8.71, 8.75, 8.99 and 8.44 Log cfu/ml, at 1, 5, 10, 15 and 20 days of cold storage, respectively. On the other hand, the counts of LAB in SFFSD samples were increased with increasing the added levels of either Doum or Carob fruit powder. It was noticed that the increasing rate of LAB counts (Log cfu/ml) was more in the samples supplemented with Doum fruit powder than that made with Carob fruit powder or that for control; this could be due to the stimulating effect of some components in Doum like polysaccharides, which the probiotic bacteria use it as source for energy [8]. The highest reading (9.24 Log cfu/ml), was recorded in synbiotic D₃ treatment at day 15 of storage. So it is concluded that Doum fruit exhibited more synergistic effect than that for Carob towards LAB growth and both were more effective for the growth of the probiotic bacteria than in control during the cold storage period (20 days). These results are in accordance with that reported by El-Kholy [35]. Also, Carob fruit act as a good

Table 6. Effect of adding Doum and Carob fruits powder on viability (Log cfu/ml) of *L. paracasei* in probiotic control and synbiotic flavored fermented skim milk drinks during storage period (20 days).

| Treatments* | Viability (Log cfu/ml) of <i>L. paracasei</i> | | | | |
|-----------------|---|--------------------|--------------------|--------------------|----------------------|
| | Age (days) | | | | |
| | Fresh (1 day) | 5 | 10 | 15 | 20 |
| C | 8.63 ^t | 8.71 ^q | 8.75 ^p | 8.99 ^m | 8.44 ^u |
| D ₁ | 8.71 ^q | 8.85 ⁿ | 9.08 ^e | 9.14 ^c | 8.65 st |
| D ₂ | 8.81 ^o | 9.07 ^{ef} | 9.12 ^d | 9.16 ^b | 8.96 ^j |
| D ₃ | 8.81 ^o | 9.04 ^{gh} | 9.08 ^e | 9.24 ^a | 8.99 ⁱ |
| Cb ₁ | 8.66 ^s | 8.77 ^p | 8.95 ^{jk} | 9.05 ^{fg} | 8.89 ^m |
| Cb ₂ | 8.68 ^r | 8.89 ^m | 8.96 ⁱ | 9.08 ^e | 8.92 ^l |
| Cb ₃ | 8.79 ^o | 8.94 ^{kl} | 9.03 ^h | 9.11 ^d | 8.94 ^{ijkl} |
| SE± | | | 0.007 | | |

Note: a, b and u: Means in the same column with different superscript letters are significantly different ($P \leq 0.05$), SE±: standard error, C: Probiotic skim milk drink (free additives), D₁, D₂ and D₃, are synbiotic flavored fermented skim milk drinks supplemented with Doum at levels of 2%, 4% and 6%, respectively, Cb₁, Cb₂ and Cb₃ are synbiotic flavored fermented skim milk drinks supplemented with Carob at levels of 5%, 10% and 15%, respectively.

prebiotic, because its addition was stimulating the growth of *L. paracasei* comparing to probiotic control, as it may have prebiotic activity similar to inulin [39]. It worth mention that, the counts of LAB were high in day 15 in all samples of control and the SFFSD, moreover, the counts of LAB, were above the therapeutic levels.

3.7. Sensory Evaluation

Sensory evaluations of SFFSD were evaluated during storage period of 15 days to check the maximum shelf life and when the taste will be acidic if stored for longer time. The organoleptic properties of SFFSD supplemented with either Doum or Carob fruit powder during 15 days of storage is presented in **Table 7**. There are highly significant variations ($P \leq 0.05$) in all sensory attributes among all samples of the SFFSD and probiotic control. Addition of either Carob or Doum fruits powder leads to an improvement for flavor, color, texture and the total score points of the SFFSD samples comparing with that of probiotic control samples. Generally, all sensory parameters and the total score points of all treatments and probiotic control samples were decreased as the storage period progressed. Comparing to the probiotic control, the SFFSD samples that supplemented with Doum fruit powder gives high scores in all parameters during storage and the best added concentration was 2% and 4%, these results were close to what reported by Abd EL-Rashid and Hassan [82], El-Kholy [34] [35], Tawfeuk and Khalil [36] and Ismail, *et al.* [37]. Moreover, it is noticed that, the total

Table 7. Sensory evaluation of synbiotic fermented skim milk drinks flavored with either Doum or Carob fruits powder during storage period (15 days) at 5 °C.

| Parameters | Age (day) | Treatments* | | | | | | | SE± |
|---------------------------|-----------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|------|
| | | C | D ₁ | D ₂ | D ₃ | Cb ₁ | Cb ₂ | Cb ₃ | |
| Flavor (45) | Fresh | 41.40 ^{a,f} | 43.50 ^{a,d} | 41.80 ^{a,e} | 40.60 ^{b,f} | 44.10 ^{abc} | 44.50 ^a | 44.10 ^{abc} | 1.05 |
| | 5 | 40.50 ^{c,f} | 41.80 ^{a,e} | 41.00 ^{a,f} | 38.00 ^{fg} | 43.80 ^{abc} | 44.40 ^a | 43.80 ^{abc} | |
| | 10 | 39.10 ^{ef} | 41.00 ^{a,f} | 41.00 ^{a,f} | 38.00 ^{fg} | 43.20 ^{a,d} | 44.20 ^{ab} | 43.40 ^{a,d} | |
| | 15 | 35.50 ^g | 40.00 ^{def} | 38.60 ^{efg} | 35.40 ^g | 41.80 ^{a,e} | 43.60 ^{abc} | 41.60 ^{a,e} | |
| Body & Texture (35) | Fresh | 31.70 ^{c,f} | 33.30 ^{a,d} | 33.10 ^{a,d} | 32.70 ^{a,e} | 33.60 ^{abc} | 34.70 ^a | 33.40 ^{a,d} | 0.60 |
| | 5 | 31.50 ^{def} | 33.60 ^{abc} | 33.10 ^{a,d} | 33.20 ^{a,d} | 34.10 ^{ab} | 34.60 ^{ab} | 33.60 ^{abc} | |
| | 10 | 30.90 ^{ef} | 33.80 ^{ab} | 33.50 ^{a,d} | 33.30 ^{a,d} | 34.10 ^{ab} | 34.50 ^{ab} | 32.80 ^{a,e} | |
| | 15 | 30.10 ^f | 33.90 ^{ab} | 33.30 ^{a,d} | 32.70 ^{a,e} | 34.20 ^{ab} | 33.60 ^{abc} | 32.60 ^{b,e} | |
| Color and Appearance (10) | Fresh | 9.20 ^{abcd} | 8.70 ^{bcd} | 8.80 ^{abcd} | 9.10 ^{abcd} | 9.60 ^{ab} | 9.20 ^{abcd} | 9.30 ^{abc} | 0.29 |
| | 5 | 9.10 ^{abcd} | 9.00 ^{abcd} | 8.90 ^{abcd} | 8.70 ^{bcd} | 9.60 ^{ab} | 9.80 ^a | 9.00 ^{abcd} | |
| | 10 | 9.00 ^{abcd} | 9.20 ^{abcd} | 9.10 ^{abcd} | 8.70 ^{bcd} | 9.50 ^{abc} | 9.80 ^a | 8.50 ^{c,d} | |
| | 15 | 8.50 ^{cd} | 9.50 ^{abc} | 9.50 ^{abc} | 8.30 ^d | 9.50 ^{abc} | 9.70 ^{ab} | 8.50 ^{c,d} | |
| Acidity (10) | Fresh | 7.10 ^b | 8.60 ^a | 8.80 ^a | 8.40 ^a | 8.90 ^a | 9.30 ^a | 8.80 ^a | 0.36 |
| | 5 | 7.30 ^b | 8.80 ^a | 8.80 ^a | 8.80 ^a | 9.40 ^a | 9.30 ^a | 8.80 ^a | |
| | 10 | 8.60 ^a | 9.30 ^a | 9.00 ^a | 9.20 ^a | 9.50 ^a | 9.30 ^a | 9.10 ^a | |
| | 15 | 8.90 ^a | 9.40 ^a | 9.30 ^a | 9.30 ^a | 9.50 ^a | 9.50 ^a | 9.20 ^a | |
| Total (100) | Fresh | 89.40 ^{f,i} | 94.10 ^{a,g} | 92.50 ^{b,h} | 90.80 ^{e,i} | 96.20 ^{a,d} | 97.70 ^{ab} | 95.60 ^{a,e} | 1.69 |
| | 5 | 88.40 ^{ghi} | 93.20 ^{a,h} | 91.80 ^{c,h} | 88.70 ^{ghi} | 96.90 ^{a,d} | 98.20 ^a | 95.20 ^{a,e} | |
| | 10 | 87.60 ^{hi} | 93.30 ^{b,g} | 92.60 ^{b,h} | 89.20 ^{g,i} | 96.30 ^{a,d} | 97.80 ^{ab} | 93.80 ^{a,g} | |
| | 15 | 83.00 ^{ij} | 92.80 ^{b,h} | 90.70 ^{e,i} | 85.70 ^{hij} | 95.00 ^{a,f} | 96.40 ^{a,d} | 91.9 ^{c,h} | |

Notes: a, b and j: Means in the same row with different superscript letters are significantly different ($P \leq 0.05$), SE±: Standard error, C: Probiotic skim milk drink (free additives), D₁, D₂ and D₃, are synbiotic flavored fermented skim milk drinks supplemented with Doum at levels of 2%, 4% and 6%, respectively, Cb₁, Cb₂ and Cb₃ are synbiotic flavored fermented skim milk drinks supplemented with Carob at levels of 5%, 10% and 15%, respectively.

score points of the synbiotic fermented drinks that supplemented with Carob fruit powder, registered the highest total score points during storage period, at all added levels especially Cb₂ treatment that contain 10% concentration (98.2, 97.8, 97.7 and 96.4 at 5, 10, fresh and 15 days, respectively), comparing with samples of control and that supplemented with Doum fruit powder, this may relate to the sweet taste for Carob fruit powder which make it used as natural sweetener [44]. The sensory evaluation characterized all SFFSD samples that supplemented with either Doum or Carob fruits powder by cocoa like color, acceptable flavor and described with soft body & texture.

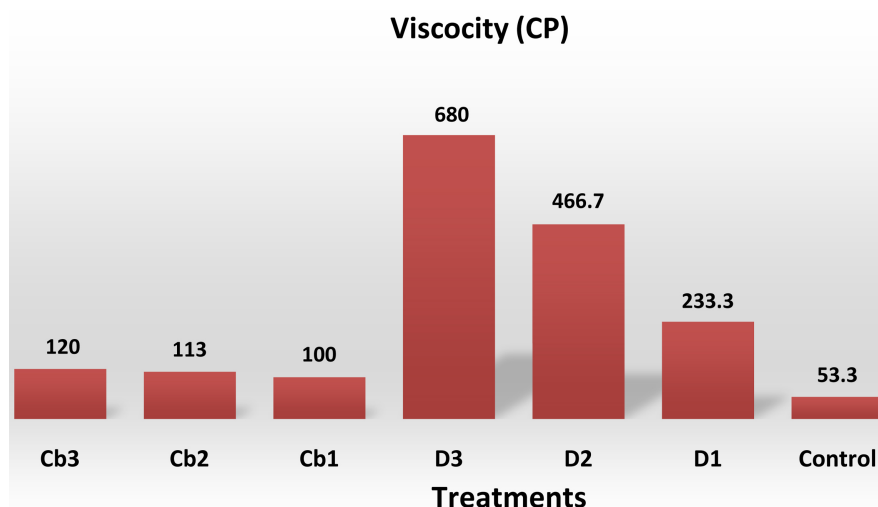


Figure 6. Viscosity of synbiotic flavored fermented skim milk drinks supplemented with either Doum (D) or Carob (Cb) fruits powder.

3.8. Viscosity of the Synbiotic Flavored Fermented Skim Milk Drinks

The viscosity of food is affected by many factors including, the presence and concentration of some components; e.g., fat, polysaccharides and proteins. Also it is affected by the hydration phenomena that related to protein aggregation, etc. [83]. The viscosity of the SFFSD is shown in **Figure 6**, it is noticed that adding either Doum or Carob fruits powder improved the viscosity of the flavored drinks as they contain polysaccharide. There is a proportional relation between the added ratios of Doum and Carob fruits powder and the apparent viscosity of the produced SFFSD drinks. Samples made with Doum fruit powder shows more viscosity than that of control (53.3 CP) and that made with Carob fruit powder. The viscosity in different SFFSD samples were, 100, 113.3, 120, 233.3, 466.7 and 680 CP, for Cb1, Cb2, Cb3, D1, D2 and D3 synbiotic treatments, respectively. These results shown that the content of dry matter (**Figure 4**) directly affected the apparent viscosity, whereas, it increased with increasing the added levels of either Doum or Carob fruits powder. Similar results were reported by Penna *et al.* [84] and El-Kholy [34], as they mentioned that, increasing the dry matter subsequently increase the apparent viscosity.

4. Conclusion

The purpose of the current study was to produce a novel healthy dairy product with antioxidant, antimicrobial and functional properties. This study shows that Doum fruit has more antioxidant and more antimicrobial effect than that for Carob fruit powder, which may relate to more TPC and TFC in the former than the latter. Also, both Doum and Carob exhibit high content of minerals which increases the nutritional value. Moreover, fermented milk drinks can be improved to produce high-quality synbiotic products with good nutrient sources, antimicrobial, antioxidant properties, moderate viscosity and good taste, by

adding natural flavorings and coloring agents like Doum and Carob fruit powder, which emphasized flavor, color and texture. Also, acting as a good prebiotic source, the counts of *L. paracasei* were stimulated in the synbiotic skim milk drinks during the cold storage compared to the control. This proves that there are opportunities to develop some new functional foods with health benefits and also good sensory properties. It also concluded that adding either Doum or Carob stimulates the growth and increases the number of the probiotic bacteria in the synbiotic-flavored fermented skim milk drinks that remain high (8 Log cfu/ml) during the storage period (20 days). The above-mentioned results emphasize the utilization of Doum and Carob fruit powder, to produce good flavored fermented milk drinks and to ensure the health and nutrition of human-kind, especially in the areas of its availability.

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Conflicts of Interest

The authors declare no conflict of interest.

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