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Chemical, Nutritional, Rheological, and Organoleptical Characterizations of Stirred Pumpkin-Yoghurt

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Abstract

Pumpkin contains considerable amounts of bioactive compounds which hardly valorized in dairy products. This study aims to investigate the chemical, nutritional, rheological, and organoleptical properties of prepared stirred pumpkin-yoghurt. Stirred yoghurt with three different pumpkin varieties pulp [American (AP), Domestic (DP), and Indian (IP) pumpkin] at 15% was prepared and stored at 5˚C ± 1˚C up to 14 d. The chemical properties of yoghurts include protein, fat, crude fiber, and available carbohydrates were significantly affected by adding pumpkin without remarkable change in caloric value. Addition of pumpkin pulps decreased the pH and increased the acidity of stirred pumpkin-yoghurt significantly ($P < 0.05$). WHC of pumpkin-yoghurts was found to be statistically improved compared to control yoghurt (CY). L*, a*, b* means were differed significantly by adding pumpkin to yoghurt. Apparent viscosity was increased after manufacturing up to 14 d. Lactic acid bacteria (LAB) were counted from 8.59 to 8.73 CFU mL^{-1} in all stirred yoghurts after 1 d. After that, it was increased without notable effect of adding pumpkin pulps, and with slight simulation in LAB, growth was noticed in DP and IP yoghurts. Total phenolic compounds (TPC), total flavonoid (TF), total flavonols (TFL), carotenoids contents, and antioxidant capacity of pumpkin-yoghurts were significantly increased. Fortifying yoghurt with pumpkin pulp was associated with a statistically significant effect on sensory parameters such as flavor, color, and overall acceptability. Scores of AP yoghurt showed the highest score which significantly differed from CY. It is obviously showed that panelists were favored the prepared stirred pumpkin-yoghurt. Thus, it is recommended that adding pumpkin pulps increases yoghurt health benefits and could be scaled up further.

Keywords

Pumpkin, Stirred Yoghurt, Fortification, Bioactive Compounds, Quality, Consumer Acceptability

1. Introduction

Yoghurt is one of the most popular fermented milk products consumed worldwide not only for its perfect sensory properties but also for its high nutritive as well as its therapeutic values. It is offered in a variety of types of concerning fat and total solids content, the form of the body (stirred, set-style, frozen, concentrated), with or without additives, probiotic microflora and in different flavors [1]. It is produced by milk fermentation with a mixture of LAB strains. Quality and sensory acceptance of manufactured yoghurt ensured its physical properties like perceived viscosity and lack of visible wheying-off [2]. Rheologically, yoghurt is a viscoelastic, pseudoplastic fluid which exhibits highly time-dependent shear thinning in flow. Yoghurts may also be fortified with bioactive rich-extract like: caffeine, guarana, green tea extract, coenzyme Q10, ginseng, aloe vera, cranberry, fiber, and olive vegetable water [3] [4]. Lutchmedial *et al.* [5] found that most panelists preferred yoghurt with 10% and 15% soursop fruits and confirmed that provided high percentage daily values of zinc, phosphorus, calcium, and much of protein. Fayed [6] investigated ingredients namely, isoflavones and γ-Aminobutyric acid, with emphasis on their fitness for fortification of yoghurt to be consumed as a functional food. Among non-traditional additives also vegetable powders, pulps, and their extracts have been used in the production of fermented milk products [7]. The manufacturing process of food formulation containing milk or soy yoghurt and 30% - 70% pureed cooked vegetables was described by Shereshevsky [8]. The therapeutic and prophylactic effect of cultured dairy products containing vegetable and fruit powders (beetroot, carrot, pumpkin, marrow and apple) for patients with gastrointestinal disorders was reported by Arkhipova *et al.* [9]. Vegetables present a valuable source of nutrients and are also low in calories. They are rich in dietary fiber, minerals as well as many bioactive compounds, such as antioxidants, e.g. carotenoids, ascorbic acid, tocopherols, phenolic substances [10]. Increased fruit and vegetable consumption is an effective strategy to increase antioxidant intake and may help to prevent developing chronic diseases, especially cancer and cardiovascular disease [11]. Supplementation of yoghurt with some selected vegetables will provide additional health properties, especially concerning antioxidant properties, and manufacturing novel functional dairy products. Mataragas *et al.* [12] developed model to establish shelf-life predictions of yoghurt with fruits stored under constant temperature conditions. However, addition of fruits to the yoghurt significantly improved the quality. The yoghurt containing papaya pulp had the highest overall acceptability scores as compare to other fruit yogurt samples and also plain yogurt [13]. Incorporation of flaxseed oil, flaxseed flour and fruits 20%

improved sensory attributes, physicochemical and fatty acid profile [14]. The quality characteristics and antioxidant activities of supplemented yogurt with 1%, 2%, and 3% aronia juice were improved. Aronia juice may be a useful additive for improving the taste and antioxidant potential of yogurt [15].

TPC and antioxidant activity of hot pepper-yoghurt significantly increased, however yoghurt contains fermented red pepper by *Bacillus licheniformis* at a concentration of 0.05% received higher scores for taste, flavor, and overall acceptability than yoghurt prepared with non-fermented pepper [16]. The highest ability to scavenge DPPH radicals was stated for yoghurts with broccoli and red sweet pepper. The red sweet pepper additive was the most beneficial regarding antioxidant properties and organoleptic acceptance of the studied yoghurts [17].

Therefore, the objective of the current investigation was to study the effect of adding pumpkin to stirred yoghurt on the physicochemical properties, polyphenolics content, antioxidant capacity, rheological properties, microbiological and organoleptic attributes of stirred pumpkin-yoghurt.

2. Materials and Methods

2.1. Materials

Milk was purchased from El Marai Company, Al-Qassim, KSA. Yoghurt DVS culture (YC-X11) were obtained from Chr. Hansens, Denmark. Instant skim milk powder (SMP-97% MSNF) was obtained from local market (El Marai Company, KSA) was used for milk standardization. The fresh pumpkin fruits (different size and maturity, season 2017) of three different common consumed varieties [AP: American pumpkin (*Cucurbita moschata* L.) butternut type, DP: Demotic pumpkin (*Cucurbita maxima* L.), and IP: Indian pumpkins (*Cucurbita pepo* Mill.) were purchased from the local market at Buraydah city, Al-Qassim region, KSA. The scientific names were nominated by Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, KSA.

2.2. Pumpkin Pulp Preparation

Fresh pumpkin was washed, manually peeled and cut into cubes. Pumpkin cubes were blanched under live steam for 10 min then vigorously mixed to obtain homogenies puree using (Santos, VITA-MAX CORP-Light Industrial Food Preparing Machine Model, VM0122E, USA). Pumpkin puree was filled in glass jars then pasteurized at 85˚C - 90˚C for 10 min, cooled in an ice-water bath for 20 min and stored at 4˚C for further use in pumpkin-yoghurt manufacturing.

2.3. Yoghurt Manufacturing

Milk with pumpkin was pasteurized at 90˚C for 10 min, cooled down to 42˚C, (subsequently subjected to pumpkin-yoghurt formulation according to Table 1, inoculated with yoghurt DVS culture at a level of 0.05%, incubated at 42˚C until coagulation pH 4.7 up to 3 - 4 h, and transferred to the cold store at 5˚C ± 1˚C. On the second day, the obtained yoghurt was mixed for 1 min using a sterile

Table 1. Different formulas of prepared stirred pumpkin-yoghurt.

Ingredients (g)	Stirred pumpkin-yoghurt			
	CY	APY	DPY	IPY
Fresh full milk	975	817.5	817.5	817.5
Dried full milk powder	25	33.5	33.5	33.5
Sucralose*	0.1	0.1	0.1	0.1
Starter %	0.05	0.05	0.05	0.05
American Pumpkin (AP)	–	150	–	–
Domestic Pumpkin (DP)	–	–	150	–
Indian Pumpkin (IP)	–	–	–	150

*: Sucralose had been added as safe sweetener calculated based on 3% sucrose and sweetness strength 600 times of sucrose.

stainless steel blender to obtain the stirred yoghurt. The yoghurts were kept at 5 ± 1˚C and the three replicates of the stirred yoghurt were analyzed after 1, 7 and 14 d of manufacturing.

2.4. Chemical and Physiochemical Analysis

Moisture, total solids, total protein, fat, ash, crude fiber, available carbohydrates, energy value, pH and titratable acidity of samples were determined in accordance with methods of AOAC [18]. Water holding capacity (WHC) was determined according to Arslan and Ozel [19] with minor modification. Stirred pumpkin-yoghurt was centrifuged for 10 min at 1520 *xg* (Universal 320R, D-78532 Hettich, Germany), supernatant was removed and weighted then WHC % was calculated according to given equation.

Apparent viscosity of stirred pumpkin-yoghurt samples was measured using the (Brookfield Programmable Rheometer, Model RVDV-III Ultra; Brookfield Engineering Laboratories, Stoughtm, MA, USA). All samples were tempered for 10 min at 24˚C ± 1˚C. The RV spindle number 3 was used for apparent viscosity measurement after 1 min at 100 rpm. Rheocalc software (ver. 2.5, Brookfield Engineering Laboratories, Inc.) was used to collect the values of apparent viscosity.

2.5. Microbiological Examination

The LAB count was determined by the plate technique at 37˚C using MRS media under anaerobic conditions in all prepared stirred pumpkin-yoghurt samples after 1, 7 and 14 d of preparation according to Dave and Shah [20].

2.6. Phytochemicals and Antioxidant Activity

2.6.1. Determination of TPC

The TPC of pumpkin-yoghurts were determined using the Folin-Ciocalteu reagent according to modified method by Bettaieb *et al.* [21]. The absorbance at 760 nm was measured and measurements were compared to a prepared standard curve of gallic acid (GA) solution then TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (mg of GAE g^{-1} dw).

2.6.2. Determination TF and TFL

The TF content of pumpkin-yoghurt samples was determined according to the method of Mohdaly *et al.* [22]. The TF content were expressed as mg quercetin equivalent (QE) per 100 g^{-1} dw. The TFL content was determined according to Kumaran and Karunakaran [23]. The absorbance at 440 nm was measured. TFL content was expressed as mg quercetin equivalent (QE) per 100 g^{-1} dw.

2.6.3. DPPH radical Scavenging Assay

Radical scavenging activity by donation capacity of samples extracts was determined spectrophotometrically based on bleaching of the purple-colored solution of the DPPH radicals according to modified method by Lu *et al.* [24]. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The antiradical activity was expressed as micromoles of Trolox equivalents (TE) per gram of dry weight (μmol TE g^{-1} dw).

2.7. Organoleptical Properties

Organoleptical attributes of the different formulas was carried out. Twelve panelists of the staff members from the Food Science and Human Nutrition Department, Faculty of Agriculture and veterinary medicine, Qassim University were asked to evaluate the prepared pumpkin-yoghurt. The following parameters such as flavor (30), color (20), consistency (20), mouth feel (20), acidity (10), and overall acceptability (100) were judged. Results were statistically analyzed and average of the mean values of the aforementioned attributes and their standard error were calculated.

2.8. Statistical Analysis

The statistical analysis was carried out using SPSS program (ver. 19) with multi-function utility regarding to the experimental design and multiple comparisons were carried out applying LSD according to comparisons were carried out applying LSD according to Steel *et al.* [25].

3. Results and Discussion

3.1. Approximate Chemical Composition of Different Prepared Stirred Pumpkin-Yoghurt

The chemical composition of prepared stirred pumpkin-yoghurt and their caloric value were illustrated in **Table 2**. General mean of treatments indicated significant differences between CY and pumpkin-yoghurt (APY, DPY, and IPY) in total protein, fat, crude fiber, and available carbohydrates. Yoghurts supplemented with pumpkin were characterized by a lower concentration of TS, fat and protein compared to CY which confirmed by Najgebauer-Lejko *et al.* [17]. However, a little of fiber have been detected as a function of adding pumpkin pulp in range of 0.09% - 0.1% on fresh weight which had similarly confirmed by Matter *et al.* [13]. On the other side, there is no significant differences between CY and pumpkin-yoghurt (APY, DPY and IPY) were found in moisture, TS, and

Table 2. Approximate chemical composition of different prepared stirred pumpkin-yoghurt after 1, 7 and 14 days at 5˚C ± 1˚C (Mean ± SE), $n = 3$.

Component (%)	Treatment	Storage period (day)			Mean
		1	7	14	
Moisture	CY	86.08 ± 0.06^{aA}	85.98 ± 0.01^{aA}	85.87 ± 0.06^{aA}	85.98 ± 0.04^{ab}
	APY	85.31 ± 0.06cA	85.23 ± 0.03^{bAB}	85.01 ± 0.07^{cB}	85.18 ± 0.05^{c}
	DPY	86.20 ± 0.03^{aA}	85.94 ± 0.04^{aB}	85.92 ± 0.06^{aB}	86.02 ± 0.06^{a}
	IPY	85.93 ± 0.07^{aA}	85.81 ± 0.06^{bA}	85.44 ± 0.52^{bB}	85.72 ± 0.17^{b}
TS	CY	13.92 ± 0.06^{cA}	14.02 ± 0.01^{bA}	14.13 ± 0.06^{cA}	14.02 ± 0.04^{bc}
	APY	14.69 ± 0.06^{aB}	14.77 ± 0.03^{aAB}	14.99 ± 0.07^{aA}	14.82 ± 0.05^{a}
	DPY	13.80 ± 0.03^{cB}	14.06 ± 0.04^{cA}	14.08 ± 0.06^{bA}	13.98 ± 0.07^{c}
	IPY	14.07 ± 0.07^{bB}	14.19 ± 0.06^{bB}	14.56 ± 0.52^{bA}	14.28 ± 0.17^{b}
Total protein	CY	4.35 ± 0.11^{aA}	4.29 ± 0.00^{aA}	4.27 ± 0.08^{aA}	4.30 ± 0.04^{a}
	APY	3.73 ± 0.08^{bA}	3.72 ± 0.13^{bA}	3.71 ± 0.10^{bA}	3.72 ± 0.05^{b}
	DPY	3.68 ± 0.11^{bA}	3.61 ± 0.07^{bA}	3.55 ± 0.05^{cA}	3.61 ± 0.06^{b}
	IPY	3.69 ± 0.14^{bA}	3.62 ± 0.02^{bA}	3.58 ± 0.08^{bcA}	3.63 ± 0.05^{b}
Fat	CY	4.30 ± 0.06^{aA}	4.27 ± 0.03^{aA}	4.23 ± 0.03^{aA}	4.27 ± 0.02^{a}
	APY	4.13 ± 0.03^{bA}	4.00 ± 0.06^{bB}	3.97 ± 0.03^{bB}	4.03 ± 0.03^{b}
	DPY	3.93 ± 0.03^{cA}	3.87 ± 0.07^{cAB}	3.83 ± 0.09^{cB}	3.88 ± 0.04^{c}
	IPY	3.97 ± 0.03^{cA}	3.97 ± 0.03^{bA}	3.87 ± 0.03^{cB}	3.93 ± 0.02^{c}
Ash	CY	0.79 ± 0.00^{bA}	0.80 ± 0.01^{bA}	0.79 ± 0.01^{bA}	0.79 ± 0.00^{b}
	APY	0.81 ± 0.00^{aA}	0.81 ± 0.01^{abA}	0.81 ± 0.00^{aA}	0.81 ± 0.00^{a}
	DPY	0.82 ± 0.00^{aA}	0.82 ± 0.00^{aA}	0.79 ± 0.00^{bB}	0.81 ± 0.01^{a}
	IPY	0.78 ± 0.00^{bB}	0.78 ± 0.01^{cB}	0.80 ± 0.01^{aA}	0.80 ± 0.01^{ab}
Crude fiber	CY	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{bA}	0.00 ± 0.00^{b}
	APY	0.10 ± 0.02^{aA}	0.10 ± 0.02^{aA}	0.10 ± 0.02^{aA}	0.10 ± 0.01^{a}
	DPY	0.09 ± 0.01^{aA}	0.09 ± 0.01^{aA}	0.09 ± 0.01^{aA}	0.09 ± 0.00^{a}
	IPY	0.10 ± 0.01^{aA}	0.09 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.09 ± 0.01^{a}
Available carbohydrates	CY	4.51 ± 0.06^{cA}	4.70 ± 0.05^{cA}	4.66 ± 0.05^{cA}	4.62 ± 0.04^{c}
	APY	5.92 ± 0.10^{aB}	6.19 ± 0.10^{aA}	6.38 ± 0.14^{aA}	6.16 ± 0.09^{a}
	DPY	5.34 ± 0.08^{bB}	5.63 ± 0.10^{bA}	5.79 ± 0.15^{bA}	5.59 ± 0.09^{b}
	IPY	5.66 ± 0.1^{5aB}	5.76 ± 0.11^{bB}	6.15 ± 0.52^{aA}	5.85 ± 0.17^{b}
Energy value (kcal 100 g^{-1} fw)	C	73.36 ± 0.20^{bA}	73.50 ± 0.10^{aA}	73.92 ± 0.30^{bA}	73.59 ± 0.14^{b}
	APY	74.58 ± 0.21^{aB}	75.10 ± 0.40^{aAB}	75.63 ± 0.44^{aA}	75.10 ± 0.24^{a}
	DPY	70.59 ± 0.27^{cA}	71.17 ± 0.47^{bA}	71.37 ± 0.24^{cA}	71.05 ± 0.21^{c}
	IPY	71.47 ± 0.22^{bB}	72.42 ± 0.38^{bB}	74.05 ± 2.14^{bA}	72.65 ± 0.73^{b}

$^{a,b\&c}$: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter, $^{A\&B}$: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

ash. Practically, the obtained results of proximate chemical composition concluded that prepared pumpkin-yoghurt by adding different pumpkin pulps had no remarkable effect of caloric values. Interestingly, similar findings were found [6] [13] [16] [17].

3.2. Physicochemical Parameters of Different Prepared Stirred Pumpkin-Yoghurt

Physicochemical compositions of freshly prepared pumpkin-yoghurt are shown in **Table 3**. The addition of pumpkin pulps decreased the pH of pumpkin-yoghurt significantly ($P < 0.05$). The pH of different pumpkin-yoghurt limited from 4.57 - 4.63 when compared to CY (4.7). The lowest acidity for the sample was 0.71% that related to CY and the maximum was 0.80% that belongs to IPY. Initially, increase in acidity of pumpkin-yoghurt might be due to the acidity of pumpkin pulp which not be significantly differed [6] [13] [16] [17]. During storage period, acidity increased in all yoghurt treatments as a result of LAB growth [3]. As shown in **Table 3**, there was a significant difference between the water holding capacity (WHC) of pumpkin-yoghurt and CY. The higher WHC was obtained for all pumpkin-yoghurt (15%). After one day, the WHC ranged from 58.10% - 63.11% then decreased with increasing the shelf-life period to be 55.86% - 60.60%. The mean value of WHC of pumpkin-yoghurt was found to be statistically higher than that of CY. Low WHC and/or high whey separation is related to a weakness of curd network [26]. The WHC of pumpkin-yoghurt was found to be higher than CY. This may be due to increasing the available carbohydrates, fiber and pectic substances with have water binding force in acidic medium as a result of adding pumpkin as mentioned [13] [26].

Color properties as (L*, a*, b*, chroma and intensity) of stirred pumpkin-yoghurt are shown in **Table 3**. L* values, indicating brightness, ranged from 85.42 to 88.26 pumpkin-yoghurts. The control sample showed the highest L* values 91.57. During storage period the L* values were not obviously changed as time increased. The a* values, indicating redness, ranged from −0.33 to 2.94 in all yogurt samples. The b* values, indicating yellowness, ranged from 23.39 in DPY to 28.90 in APY in pumpkin-yoghurts while lowest b* was recorded for CY. As calculated from L*, a* and b* results, chroma and color intensity means were also differed significantly by adding pumpkin to yoghurt. These results are in harmony with those obtained by adding different fruits to yoghurt, whereas change in yoghurt instrumental color was fruit-dependable color [5] [15] [16].

3.3. Apparent Viscosity of Different Prepared Stirred Pumpkin-Yoghurt

Apparent viscosity of pumpkin-yoghurt is shown in **Table 4**. Generally, CY showed the lowest values of viscosity among all yoghurts. The viscosity of all prepared yoghurts increased after manufacturing up to two weeks. The mean of all treatment indicated a significant difference ($P < 0.05$) among all prepared yoghurts. The results revealed that addition of pumpkin demonstrated increases

Table 3. Physicochemical parameters of different prepared stirred pumpkin-yoghurt after 1, 7 and 14 days at 5˚C ± 1˚C (Mean ± SE), n = 3.

Items	Treatment	Storage period (day)			Mean
		1	7	14	
pH	CY	4.83 ± 0.01^{aA}	4.71 ± 0.01^{aB}	4.56 ± 0.02^{aC}	4.70 ± 0.04^{a}
	APY	4.77 ± 0.01^{bA}	4.63 ± 0.01^{bB}	4.48 ± 0.02^{cC}	4.63 ± 0.04^{b}
	DPY	4.63 ± 0.01^{bA}	4.61 ± 0.01^{cA}	4.53 ± 0.02^{bB}	4.59 ± 0.02^{c}
	IPY	4.78 ± 0.02^{bA}	4.51 ± 0.02^{cB}	4.42 ± 0.01^{dC}	4.57 ± 0.05^{c}
Acidity	CY	0.60 ± 0.01^{aC}	0.75 ± 0.01^{bB}	0.78 ± 0.01^{bA}	0.71 ± 0.03^{c}
	APY	0.62 ± 0.01^{aC}	0.76 ± 0.01^{cB}	0.83 ± 0.01^{bA}	0.74 ± 0.03^{b}
	DPY	0.63 ± 0.02^{aC}	0.76 ± 0.01^{cB}	0.82 ± 0.01^{bA}	0.74 ± 0.03^{b}
	IPY	0.64 ± 0.01^{aC}	0.86 ± 0.01^{aB}	0.89 ± 0.01^{aA}	0.80 ± 0.04^{a}
WHC	CY	58.10 ± 0.83^{cA}	55.29 ± 0.23^{cB}	54.20 ± 0.14^{cC}	55.86 ± 0.63^{c}
	APY	61.00 ± 0.01^{bA}	58.67 ± 0.22^{bB}	57.05 ± 0.03^{bC}	58.91 ± 0.58^{ab}
	DPY	63.11 ± 0.54^{aA}	59.92 ± 0.21^{aB}	58.77 ± 0.09^{aC}	60.60 ± 0.67^{a}
	IPY	60.47 ± 0.6^{bA}	57.98 ± 0.83^{bB}	56.36 ± 0.49^{bC}	58.27 ± 0.68^{b}
L*	CY	91.30 ± 0.28^{aB}	91.40 ± 0.53^{aB}	92.01 ± 0.15^{aA}	91.57 ± 0.21^{a}
	APY	85.58 ± 0.07^{dA}	84.57 ± 0.99^{cB}	86.12 ± 0.12^{cA}	85.42 ± 0.37^{c}
	DPY	87.68 ± 0.44^{cA}	87.38 ± 0.51^{bA}	87.99 ± 0.08^{bA}	87.68 ± 0.21^{b}
	IPY	88.67 ± 0.20^{bA}	87.70 ± 0.51^{bB}	88.41 ± 0.13^{bA}	88.26 ± 0.22^{b}
a*	CY	-2.88 ± 0.06^{cA}	-2.94 ± 0.02^{dA}	-3.01 ± 0.06^{cA}	-2.94 ± 0.03^{d}
	APY	-1.00 ± 0.02^{aC}	0.32 ± 0.00^{aA}	-0.33 ± 0.04^{aB}	-0.33 ± 0.19^{a}
	DPY	-0.79 ± 0.45^{aC}	-0.46 ± 0.00^{bB}	-0.18 ± 0.04^{aA}	-0.48 ± 0.16^{b}
	IPY	-1.92 ± 0.19^{bA}	-1.88 ± 0.01^{cA}	-2.00 ± 0.06^{bA}	-1.93 ± 0.06^{c}
b*	CY	6.73 ± 0.40^{dB}	7.76 ± 0.05^{dA}	7.88 ± 0.16^{dA}	7.46 ± 0.22^{d}
	APY	28.25 ± 0.05^{aB}	29.06 ± 0.34^{aA}	29.37 ± 0.13^{aA}	28.90 ± 0.20^{a}
	DPY	23.47 ± 0.80^{cA}	23.16 ± 0.14^{cA}	23.53 ± 0.15^{cA}	23.39 ± 0.25^{c}
	IPY	25.25 ± 0.34^{bA}	24.24 ± 0.14^{bB}	24.33 ± 0.10^{bB}	24.61 ± 0.20^{b}
Chroma	CY	7.16 ± 0.38^{dC}	14.98 ± 0.84^{cB}	15.95 ± 0.78^{cA}	12.70 ± 1.43^{c}
	APY	23.54 ± 0.04^{aB}	24.56 ± 0.07^{aA}	24.58 ± 0.07^{aA}	24.22 ± 0.17^{a}
	DPY	20.38 ± 0.66^{cA}	20.99 ± 0.23^{bA}	20.71 ± 0.15^{bA}	20.69 ± 0.22^{b}
	IPY	21.91 ± 0.26^{bB}	25.03 ± 0.21^{aA}	25.06 ± 0.20^{aA}	24.00 ± 0.53^{a}
Intensity	C	89.25 ± 0.38^{aB}	89.45 ± 0.58^{aB}	90.05 ± 0.54^{aA}	89.58 ± 0.28^{a}
	APY	85.26 ± 0.08^{dB}	85.60 ± 0.40^{dB}	86.02 ± 0.37^{dA}	85.63 ± 0.19^{d}
	DPY	86.92 ± 0.68^{cB}	87.58 ± 0.21^{cA}	87.38 ± 0.12^{cAB}	87.29 ± 0.23^{c}
	IPY	88.47 ± 0.30^{bA}	88.57 ± 0.20^{bA}	88.30 ± 0.14^{bA}	88.45 ± 0.12^{b}

$^{a,b\&c}$: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter, $^{A\&B}$: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

Table 4. Apparent viscosity (cP) of different prepared stirred pumpkin-yoghurt after 1, 7 and 14 days at 5˚C ± 1˚C (Mean ± SE), $n = 6$.

Items	Treatment	Storage period (day)			Mean
		1	7	14	
Apparent viscosity (cP)	CY	413.17 ± 13.37^{cC}	460.70 ± 5.14^{cB}	517.97 ± 0.44^{cA}	463.94 ± 15.70^{d}
	APY	471.67 ± 0.84^{bC}	489.23 ± 4.81^{bB}	545.90 ± 1.01^{aA}	502.27 ± 11.29^{b}
	DPY	477.20 ± 0.61^{bB}	486.00 ± 3.84^{bA}	498.80 ± 0.40^{dB}	486.67 ± 1.76^{c}
	IPY	524.10 ± 3.20^{aB}	544.80 ± 4.59^{aA}	559.67 ± 6.01^{bB}	542.86 ± 3.90^{a}

$^{a,b\&c}$: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter, $^{A\&B}$: There is no significant difference ($P > 0.05$) between any two means for the same attribute, within the same row have the same superscript letter.

in viscosity values of stirred yoghurt.

This might indicated that pumpkin positively affected the texture of the stirred yoghurt. It is expected that available carbohydrates, fiber and pectic substances improved the structure of the curd network of stirred yoghurt and slightly increase the viscosity [16] [26].

3.4. LAB count of Different Prepared Stirred Pumpkin-Yoghurt

Figure 1 illustrated the LAB counts in yogurts fortified with various pumpkin pulps with 15% as substitution level.

The LAB counts were 8.59 to 8.73 CFU mL^{-1} after 1 d of preparation in all stirred pumpkin-yoghurts. During storage up to 7 d, LAB count increased rapidly in both control and pumpkin-yoghurts to be 8.90 - 9.19 CFU mL^{-1}. Obviously, the number of LAB in yogurt was not affected and increased with adding pumpkin pulp which simulated the LAB growth initially. A slight decrease was remarked in number of LAB after 14 days in some treatments. However, longer incubation times resulted in higher numbers of LAB and the number of LAB in stirred pumpkin-yoghurts met the required number of LAB in semi-liquid yogurt as mentioned [15]. Interestingly, pumpkin pulp does not inhibit the growth of LAB and actually improves the growth of LAB during the incubation from 7 days, possibly by providing some sugars for LAB growth [12] [15]. Previous studies showed that adding flowering cherry into yogurt improves LAB growth [25], but adding acanthopanax powder to yogurt prevents LAB growth which may contain antimicrobial compounds that may affect LAB growth [28].

3.5. Phytochemicals and Its Antioxidant Capacity of Different Prepared Stirred Pumpkin-Yoghurt

Table 5 shows the TPC, total flavonoid (FT), total flavonols (TFL) and carotenoids contents as well as antioxidant capacity applying DPPH radicals of yogurt fortified by 15% pumpkin pulp for 14 days of storage. The highest TPC content (256.56 mg GAE g^{-1} dw) was found in the yogurt fortified with 15% AP pulp and the lowest TPC content (228.56 mg GAE g^{-1} dw) was found in IPY yogurt. However, control yoghurt exhibited 167.05 mg GAE g^{-1} dw. TPC content in

Table 5. Phytochemicals composition of different prepared stirred pumpkin-yoghurt after 1, 7 and 14 days at 5˚C ± 1˚C (Mean ± SE), $n = 3$.

Items	Treatment	Storage period (day)			Mean
		1	7	14	
Total Phenolic compounds [mg GAE 100 g^{-1}]	CY	172.68 ± 7.81^{cA}	165.24 ± 6.45^{cA}	163.24 ± 4.25^{dA}	167.05 ± 5.25^{d}
	APY	233.68 ± 9.91^{aC}	254.41 ± 5.73^{aC}	281.58 ± 6.9^{aB}	256.56 ± 7.94^{a}
	DPY	229.41 ± 7.38^{aC}	238.61 ± 4.72^{bC}	265.61 ± 2.18^{bB}	244.54 ± 6.02^{b}
	IPY	208.65 ± 8.75^{bB}	231.00 ± 5.52^{bB}	248.04 ± 2.09^{cB}	228.56 ± 4.64^{c}
Total flavonoid [mg QE 100 g^{-1}]	CY	-	-	-	-
	APY	1.74 ± 0.04^{bC}	1.89 ± 0.04^{bB}	3.00 ± 0.19^{aA}	2.21 ± 0.21^{a}
	DPY	1.92 ± 0.03^{aC}	2.23 ± 0.04^{aB}	2.78 ± 0.17^{bA}	2.31 ± 0.14^{a}
	IPY	1.91 ± 0.06^{aB}	2.16 ± 0.02^{aA}	2.24 ± 0.13^{cB}	2.10 ± 0.06^{b}
Total flavonols [mg QE 100 g^{-1}]	CY	-	-	-	-
	APY	0.70 ± 0.02^{bB}	0.72 ± 0.02^{bB}	1.20 ± 0.08^{aA}	0.87 ± 0.09^{a}
	DPY	0.77 ± 0.01^{aC}	0.85 ± 0.02^{aB}	1.11 ± 0.07^{bA}	0.91 ± 0.06^{a}
	IPY	0.76 ± 0.03^{abB}	0.82 ± 0.01^{abA}	0.94 ± 0.05^{cB}	0.84 ± 0.02^{b}
Carotenoids [mg 100 g^{-1}]	CY	-	-	-	-
	APY	0.80 ± 0.01^{dC}	0.75 ± 0.01^{dB}	0.72 ± 0.01^{cA}	0.75 ± 0.01^{d}
	DPY	0.60 ± 0.01^{cC}	0.57 ± 0.02^{cB}	0.54 ± 0.03^{bA}	0.57 ± 0.01^{c}
	IPY	0.52 ± 0.02^{bC}	0.49 ± 0.01^{bB}	0.46 ± 0.02^{bA}	0.49 ± 0.01^{b}
DPPH$^{\bullet}$ [μmol TE 100 g^{-1}]	CY	17.85 ± 1.27^{c}	14.24 ± 0.47^{b}	13.25 ± 1.05^{b}	15.11 ± 1.74^{b}
	APY	46.76 ± 0.96^{a}	49.62 ± 3.38^{ab}	57.80 ± 1.29^{a}	51.39 ± 1.98^{a}
	DPY	48.24 ± 0.68^{a}	50.47 ± 3.72^{a}	59.08 ± 2.32^{a}	52.60 ± 2.09^{a}
	IPY	46.73 ± 2.72^{b}	46.85 ± 2.00^{a}	58.98 ± 0.66^{a}	50.85 ± 2.26^{a}

$^{a,b\&c}$: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter, $^{A\&B}$: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter, -: not detected.

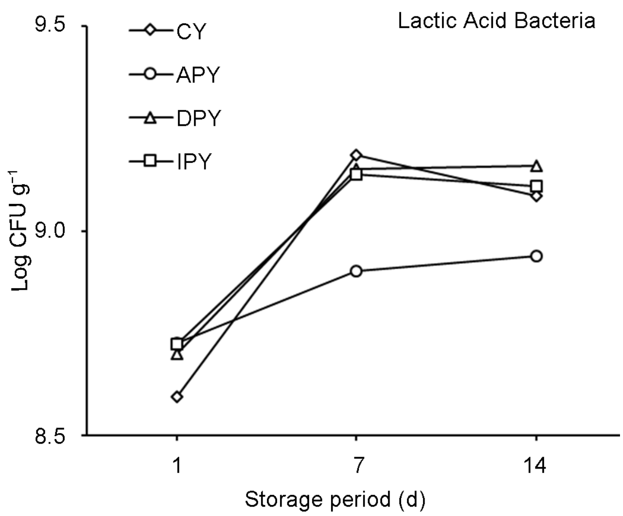

Figure 1. Changes in LAB number of yogurt containing different pumpkin varieties at 15% pulp during Storage at 5˚C ± 1˚C up to 14 days, values expressed as log CFU g^{-1}. CY: Control yoghurt, APY: Stirred yoghurt with American pumpkin pulp, ADY: Stirred yoghurt with Domestic pumpkin pulp and IPY: Stirred yoghurt with Indian pumpkin pulp (see materials and methods).

creased 33% - 54% in yogurt containing 15% pumpkin pulp. The TF content was ranged from 2.10 to 2.31 mg QE 100 g^{-1} dw in pumpkin-yoghurt while no TF and TFL were detected in CY. In the same context, TFL content was ranged from 0.49 to 0.75 mg QE 100 g^{-1} dw in pumpkin-yoghurt.. Compared to CY, incorporation of pumpkin pulp into yoghurt resulted in higher TPC, TF and TFL contents as similarly indicated by adding different pumpkin pulps to yoghurt [6] [15] [16]. The antioxidant activities of yogurt samples containing various pumpkin pulps are shown in the same table. Pumpkin-containing yogurts had higher antioxidant activity than CY. The DPPH radical scavenging activity of CY was 15.11 μ·mol TE 100 g^{-1} dw, and DPPH radical scavenging activity of yogurt increased in pumpkin-yoghurt significantly ($P < 0.05$) not only in mean score but also during storage period. The higher antioxidant activity in pumpkin- yoghurt might result from the phytochemicals content of pumpkin and microbial metabolic activity releasing some bounded bioactive materials. Similar findings had been previously mentioned by Nguyen and Hwang [15].

3.6. Organoleptical Parameters of Different Prepared Stirred Pumpkin-Yoghurt

Table 6 shows the sensory scores of pumpkin-yoghurt. Fortifying yogurt with pumpkin pulp was associated with a statistically significant effect on sensory parameters such as flavor, color, and overall acceptability while no significant different was remarked in consistency and acidity by panelists. The flavor mean score of control showed the lowest score while the highest scores were given to APY. Adding pumpkin to yoghurt was slightly improved the flavor. Color score of plain yoghurt showed the highest score while pumpkin-yoghurts were the lowest significantly. Pumpkin contains a yellow color from carotenoids contributes to yogurt color which may not favored for panelists. Improving the flavor and color of yoghurt fortified with fruits and vegetables were confirmed [5] [16] [27]. Mouth feel of pumpkin-yoghurt were slightly improved as pumpkin was added when compared to CY. Scores of APY showed the highest score which differed significantly from control yoghurt. Opposite finding was found by Nguyen and Hwang [15] who confirmed that flavor, mouth feel, thickness, or overall acceptance were not statistically differed by adding 3% aronia juice to plain yoghurt.

For overall acceptability, APY showed the highest score followed by IPY and DPY while control recorded the lowest score. It is obviously showed that panelists were favored the prepared stirred pumpkin-yoghurt. Moreover, pumpkin contained several bioactive compounds that may contribute to the antioxidative effects and recommended for health benefits (**Table 4**). Thus, scaling up stirred pumpkin-yoghurt as described in this investigation could be concerned as also recommended for many trails [1] [5] [6] [13] [15] [16] [17].

4. Conclusion

Production of more healthy fermented milk product by adding pumpkin pulp to yoghurt was aimed in this study. Adding 15% pumpkin pulps improved some

Table 6. Organoleptical parameters of different prepared stirred pumpkin-yoghurt after 1, 7 and 14 days at 5˚C ± 1˚C (Mean ± SE), $n = 12$.

Items	Treatment	Storage period (day)			Mean
		1	7	14	
Flavor	C	23.75 ± 1.26^{aA}	22.92 ± 1.23^{bA}	25.25 ± 1.14^{aA}	23.97 ± 0.70^{b}
	APY	25.08 ± 1.26^{aA}	27.42 ± 0.92^{aA}	27.83 ± 0.90^{aA}	26.78 ± 0.62^{a}
	DPY	24.25 ± 1.41^{aAB}	22.83 ± 1.04^{bB}	26.50 ± 0.58^{aA}	24.53 ± 0.65^{b}
	IPY	24.83 ± 1.41^{aA}	24.83 ± 1.30^{abA}	25.42 ± 0.91^{aA}	25.03 ± 0.69^{ab}
Color	C	17.83 ± 0.94^{aA}	19.00 ± 0.37^{aA}	19.00 ± 0.30^{aA}	18.61 ± 0.35^{a}
	APY	15.33 ± 1.20^{aA}	15.67 ± 0.77^{bA}	17.08 ± 0.75^{aA}	16.03 ± 0.54^{b}
	DPY	16.42 ± 1.05^{aA}	17.17 ± 0.55^{abA}	17.75 ± 0.65^{aA}	17.11 ± 0.45^{b}
	IPY	17.42 ± 0.95^{aA}	17.00 ± 0.43^{abA}	16.83 ± 0.80^{aA}	17.08 ± 0.43^{b}
Consistency	C	18.67 ± 0.50^{aA}	18.08 ± 0.51^{aA}	17.75 ± 0.48^{aA}	18.17 ± 0.29^{a}
	APY	18.17 ± 0.51^{aA}	17.33 ± 0.54^{aA}	17.67 ± 0.61^{aA}	17.72 ± 0.32^{a}
	DPY	18.00 ± 0.49^{aA}	17.17 ± 0.76^{aA}	18.75 ± 0.35^{aA}	17.97 ± 0.33^{a}
	IPY	18.42 ± 0.47^{aA}	18.17 ± 0.44^{aA}	17.92 ± 0.48^{aA}	18.17 ± 0.26^{a}
Mouth feel	C	17.08 ± 0.53^{aA}	17.58 ± 0.48^{aA}	17.08 ± 0.91^{aA}	17.25 ± 0.38^{b}
	APY	18.25 ± 0.51^{aA}	18.42 ± 0.53^{aA}	18.58 ± 0.45^{aA}	18.42 ± 0.28^{a}
	DPY	17.67 ± 0.54^{aA}	16.67 ± 0.71^{aA}	18.00 ± 0.49^{aA}	17.44 ± 0.34^{ab}
	IPY	18.00 ± 0.49^{aA}	18.17 ± 0.44^{aA}	16.83 ± 0.90^{aA}	17.67 ± 0.38^{ab}
Acidity	C	9.17 ± 0.27^{aA}	8.75 ± 0.33^{aA}	9.08 ± 0.26^{aA}	9.00 ± 0.16^{a}
	APY	8.83 ± 0.34^{aAB}	8.00 ± 0.37^{aB}	9.08 ± 0.19^{aA}	8.64 ± 0.19^{ab}
	DPY	8.25 ± 0.45^{aA}	8.08 ± 0.31^{aA}	8.83 ± 0.21^{aA}	8.39 ± 0.20^{b}
	IPY	9.08 ± 0.23^{aA}	8.17 ± 0.30^{aB}	8.25 ± 0.30^{aAB}	8.50 ± 0.17^{ab}
Overall acceptability	C	85.58 ± 2.64^{aA}	81.42 ± 2.90^{bA}	88.17 ± 2.02^{aA}	85.06 ± 1.50^{b}
	APY	90.67 ± 1.96^{aA}	93.08 ± 1.92^{aA}	92.58 ± 1.42^{aA}	92.11 ± 1.01^{a}
	DPY	85.25 ± 2.77^{aA}	83.50 ± 2.68^{bA}	87.33 ± 2.99^{aA}	85.36 ± 1.60^{b}
	IPY	90.42 ± 2.30^{aA}	87.83 ± 2.22^{abA}	86.67 ± 2.67^{aA}	88.31 ± 1.37^{ab}

$^{a,b\&c}$: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter, $^{A\&B}$: There is no significant difference ($P > 0.05$) between any two means, within the same row have the same superscript letter.

chemical, physicochemical and sensory properties of stirred yoghurt. Moreover, bioactive compounds and their antioxidant capacity were noticed for stirred pumpkin-yoghurts when compared to the natural yoghurt. Pumpkin additions may have an increasing effect on yoghurt consumption and may give more choices to consumers in the market. Thus, it is recommended that adding pumpkin pulps increases yoghurt health benefits and could be scaled up further.

Conflict of Interest

The authors declare that no conflict of interest.

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