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Research Article

Addition of Lentils and Chickpeas to Yogurt: Effects on Physicochemical, Colorimetric, Microbiological, Rheological and Organoleptic Properties During Refrigeration

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Abstract

In this study, yoghurt fortified with lentil and chickpea germinated or not proteins isolate was manufactured. The effects of the addition of lentil and chickpea proteins isolate on the sensory, physico-chemical, colorimetric, microbiological and rheological properties of yoghurt during storage are studied. Physicochemical analyses have shown that for all yogurt samples, a significant decrease in pH and increasing of TA were observed during the first two weeks after which was not significant. Results show that there is not significant difference of DM values during storage period of the different yoghurt samples. Whey separation (Syneresis) was lower in fortified yogurts with lentil and chickpea proteins isolate as compared with control yoghurt. The colorimetric results showed that the factor L*, a* and b* have no significant difference with each other during 21-day storage period for all samples. Microbial evaluation shows that total aerobic mesophilic bacteria, total coliforms, fecal coliforms and S. aureus colonies were not found in any of the yogurt samples until the 21st day of storage. Rheologic analysis shows that the control samples exhibited the lowest viscosity and consistency index values. Sensory results have shown that there is not significant difference between sensory scores for all descriptors of yogurt samples fortified or not with leguminous proteins. Yoghurts YCGPI and YLGPI presented sightly highest sensory scores comparing with the other ones. The results of the present study suggest that leguminous proteins fruit can be effectively used as gelling agent in the manufacture yogurt.

Keywords: Yoghurt; Fortified; Lentil; Chickpea; Proteins

Abbreviations

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate; TA: Titratable acidity; DM: Dry Matter; TAMB: Total Aerobic Mesophile Bacteria Count; TC: Total Coliforms; FC: Fecal Coliforms; STAPH: Staphylococcus Aureus

Introduction

Yogurt or yoghurt is fermented milk obtained by fermenting milk under the action of suitable microorganisms (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), resulting in a decrease in pH, with or without coagulation [1]. Yoghurt is a popular consumer product worldwide. Its consumption has been known for centuries to have health benefits for consumers, and some scientific studies support this. In fact, due to its composition, yogurt is a product with high digestibility and nutrients such as protein, minerals and vitamins [2]. The beneficial effects of yogurt are enhanced by the presence of lactic fermenting bacteria, which make yogurt a functional food and add value to the product [3]. Usually, yoghurt is stabilized and made smoother with the addition of gelatin, mainly from bovine or porcine animal sources. However, there has been a growing interest in alternative protein sources in recent years [4]. In fact, vegetable protein isolates can be effectively used as food stabilizers or gelling agents [4]. Several plant proteins from soybean, sunflower, pea, tomato seeds, wheat germ, chickpeas, green chickpeas, lupins, field beans, and lentils have been successfully tested as stabilizers [4-8]. Various consumer concerns have prompted a desire to replace animal protein in the food system [8,9]. In fact, there is a growing demand for low-processed vegan food options suitable for a variety of diets, and efforts are underway to develop novel foods suitable for consumers who choose not to eat animal protein, whether due to dietary, cultural, traditional or religious factors [6]. Legumes are ideal for making plant-based and meat-free food ingredients because they are rich in protein and have a wide range of technical functions [6,10,11]. The aim of the present study is the development of an innovative dairy product which is a yoghurt supplemented with proteins isolates from chickpea or lentils and the study of their effects on physico-chemical, microbiological rheological and sensory properties.

Materials and Methods

Preparation of yoghurts' formulations

Yoghurt samples were prepared according to Benmeziane., *et al*. [1] method. Fresh semi-skimmed milk was heated up to 40-45°C and the addition of starch (1g/L of milk), sugar (100g/L of milk) and proteins isolate of Lentil and chickpea was realized. The pasteurization was at 93°C for 5 min immediately following with cooling to 45°C. After the incubation with lactic ferments (0,03g/L of milk) and mixing the Packaging and fermentation at 45°C for 4h were realized. Finally, rapid cooling was done and yoghurt samples were stored at 4°C. Each sample of legume protein isolate is incorporated at the concentration of 4% of yoghurt, which is the maximum amount of sample that can be supplemented to yoghurt without disrupting fermentation (Agil., *et al.* 2013). 100 cups of yogurt were prepared and divided into five batches, the first batch: control yoghurt (CY) made without legume proteins isolate, second batch as yoghurt made with the addition of lentil proteins isolate (YLPI), the third one, yogurt made with lentil germs proteins isolate (YLGPI), the fourth one made with the addition of chickpea proteins isolate (YCPI) and the last one made with chickpea germs proteins isolate (YCGPI).

Physicochemical analyzes

The yoghurt samples were mixed and analyzed for pH, titratable acidity (TA), dry matter (DM) and syneresis. Titratable acidity was expressed in terms of % lactic acid [12]. Approximately 10 g of yoghurt with the same volume of distilled water was diluted before titration. The titratable acidity was determined by titrating with 0.1 N NaOH [13]. The pH of the yoghurts was recorded using a digital pH meter (Consort PH C860, Belgium) calibrated using commercial pH 4.00 and 7.00 buffers. Before measurements, yoghurt samples were stirred with a little distilled water [13]. The dry matter of the samples was determined according to the Association of Official Analytical Chemists method AOAC [12]. Yoghurt was mixed and put in a petri dish previously tarred. Samples were placed in an oven at 103 ° C for 24 hours. The dry matter is expressed in percentage [14]. Syneresis (whey quantity released by the yoghurt) was determined according to the method described by Benmeziane., *et al*. [1]. Yoghurt samples are placed in 100 mL cups and stored at 4 ∘ C. The volume of the separated serum on the surface of the yoghurt is determined after 15 days of storage. Results are expressed in (%) (v/v). For all analyses, yoghurt samples were analyzed in triplicate

Color measurements

Surface color measurements of yoghurt were performed using a colorimeter (Minolta CR-300, Japan) according to Rodríguez-Carpena., *et al*. [15]. The instrument was calibrated using the standard white file. Color measurements were made on the surface of each sample in triplicate on three randomly selected locations. Color measurements were made at room temperature (≈25 °C). The yoghurt samples color is expressed as chromatic ordinates a*, b* and L*. Color measurements of all yoghurt samples were analyzed in triplicate.

Microbiological analysis

Total aerobic mesophile bacteria count (TAMB) was determined according to Alexopoulos., *et al.* [16]. Total coliforms (TC) and Fecal coliforms (FC) were counted according to Shahbandari., *et al*. [17]. Staphylococcus aureus (STAPH) count was carried out according to Benmeziane., *et al.* [1]. Plates containing 20-200 colonies were counted, and the results expressed as colony forming units per gram (CFU/g) of sample (Ghasempour., *et al.* 2012; Guler-Akın and Akın, 2007). Three samples were microbiologically examined for each treatment.

Rheological measurements

Apparent viscosity of stirred yoghurt samples was measured using the compact modular rheometer (RM180). The measuring geometry employed was a cup for concentric cylinder CC24 (15,18 mm diameter and 42 mm height) and bob (14.005 mm diameter and 21mm height) system. The yogurts are stored before analysis in a refrigerator at 4°C for 1 day [19]. The samples, vigorously sheared for 1 min in order to suppress the previous rheological history, were equilibrated for 3 min 1 min at 100 rpm before the experiment. All samples were tempered for 10 min at 24˚C ± 1˚C [20]. Easy Match QC Software type (EZMQC-CFLX) was used to collect the values of apparent viscosity.

Sensory evaluation

The sensory characterization was performed by a hedonic test by ranking of appreciation of tested product. Four yoghurt samples were freshly prepared for the hedonic test by 60 untrained subjects. For this test, tasters are asked to rate the coded yogurt samples by indicating their level of appreciation on a nine-point scale ranging from "I don't like it at all" to "I like it a lot". The ratings of each sample are then converted into a numerical rating ranging from 1 for "I don't like it at all" to 9 "I like it a lot". Sensory descriptors of the samples tested were appearance, color, aroma, taste, texture at the spoon and overall acceptability [21,22].

Statistical analysis

An IBM SPSS Statistics, version 23, software was used for ANOVA, and Tukey's test to detect significant differences among all yoghurt samples stored for 28 days at 4°C for different parameters tested. Additionally, correlations between parameters were calculated by person test. A Microsoft Excel 2010 was used for determination of means (± standard deviations) and construction of viscosity and flow curves.

Results and Discussion

Physicochemical characterization

Total acidity (TA) and pH values measured on the 1st, 7th, 14th and 21st day for the different yogurts are presented in figure 1. The storage time significantly affected the level of acidity and pH in the samples. For all yogurt samples, a significant decrease in pH was observed during the first two weeks after which was not significant. Similar results are reported by Benmeziane., *et al.* ca, Senadeera., *et al*. [23] and Haider., *et al*. [24]. A reverse trend was observed by Agil., *et al.* [25], where no significant difference a was found in any of the green lentil-based yogurt, after storage periods of 7th and 14th days at 4 ∘C. Results have shown that TA values increased while the pH values decreased. Indeed, this trend of TA and pH values are reported by many researches about yoghurt [13,18,23,26].

The measured values of dry matter over storage time are shown in figure 2**.** Results show that there is not significant difference of DM values during storage period of the different yoghurt samples. DM values of yoghurt samples are in range of (10.4%- 20.9%). Similar results were reported by Benmeziane., *et al*. [1] for yoghurt with flour of lentil.

Whey separation, which is also called wheying-off, is the appearance of whey on the surface of a curd. It is a common defect during storage of fermented milk products like yoghurts [1]. This phenomenon is related to gels fragility. Whey separation (Syneresis) was lower in fortified yogurts with lentil and chickpea proteins isolate as compared with control yoghurt (Figure 3). The lentil and chickpea proteins isolate incorporation in yoghurt reduces the amount of whey separation from the solid mass from 5,45% (for control) to 0,5% and 0,3% for YCPI and YCGPI, respectively. This shows that legume germ proteins isolate can effectively stabilize yoghurt.

Colorimetric parameters

Results of colorimetric parameters of all yoghurt samples is shown in figure 4. The results showed that the factor L*, a* and b* have no significant difference with each other during 21-day storage period for all samples (P > 0.05). This stability of colori-

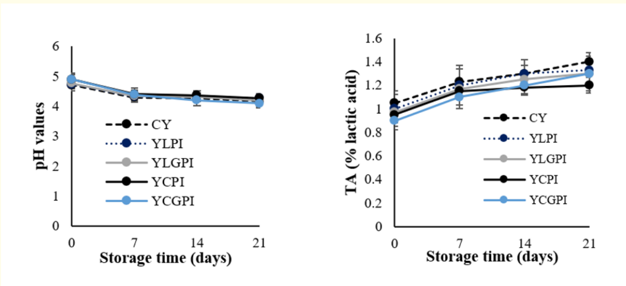

Figure 1: pH and TA values of different yoghurt samples stored at 4°C.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt

Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins

Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

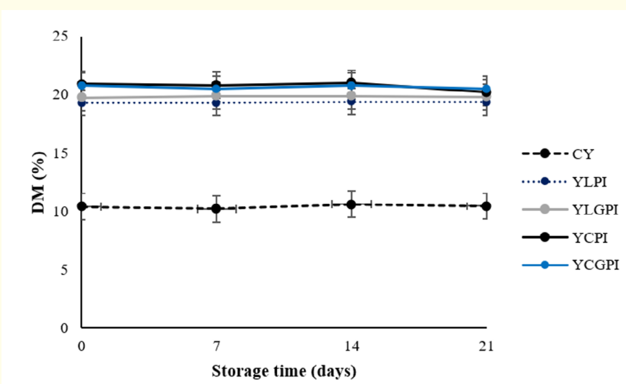

Figure 2: Dry matter (DM) values of different yoghurt samples stored at 4°C.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt

Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins

Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

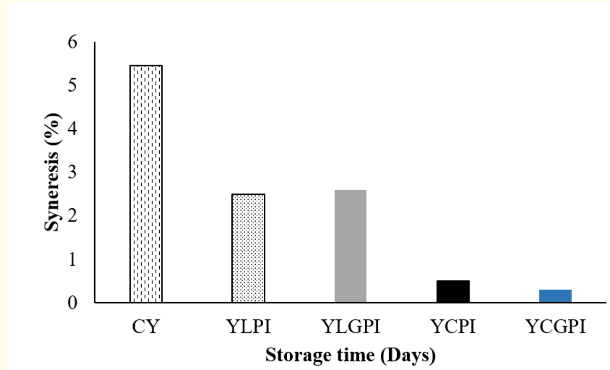

Figure 3: Syneresis values of different yoghurt samples stored at 4°C.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt

Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins

Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

metric parameters values during storage at 4°C were shown by Shahbandari., *et al*. [17]. The brightness of all samples of yoghurt were ranged between 80 and 90. Comparable values were reported by Barakat and Hassan [20]. In this study, the colorimetric parameters a* and b* change slightly according to adding proteins isolate. These finding are in agreement with values reported by Ozcan., *et al*. [27] for control.

Microbial evaluation

The microbial results of yoghurt fortified or not with lentil and chickpea proteins is given in table 1. As can be seen, total aerobic mesophilic bacteria, total coliforms, fecal coliforms and S. aureus colonies were not found in any of the yogurt samples until the 21st day of storage. Therefore, yogurts rich in lentil and chickpea protein are safe to eat. These results are consistent with Çakmakçi and Turgut [28], who did not find coliforms and S. aureus in any strawberry yogurt samples during storage (< 1 log CFU/g). In addition, similar results have also been reported by Benmeziane., *et al*. [1] for yoghurt fortified by flour of lentil.

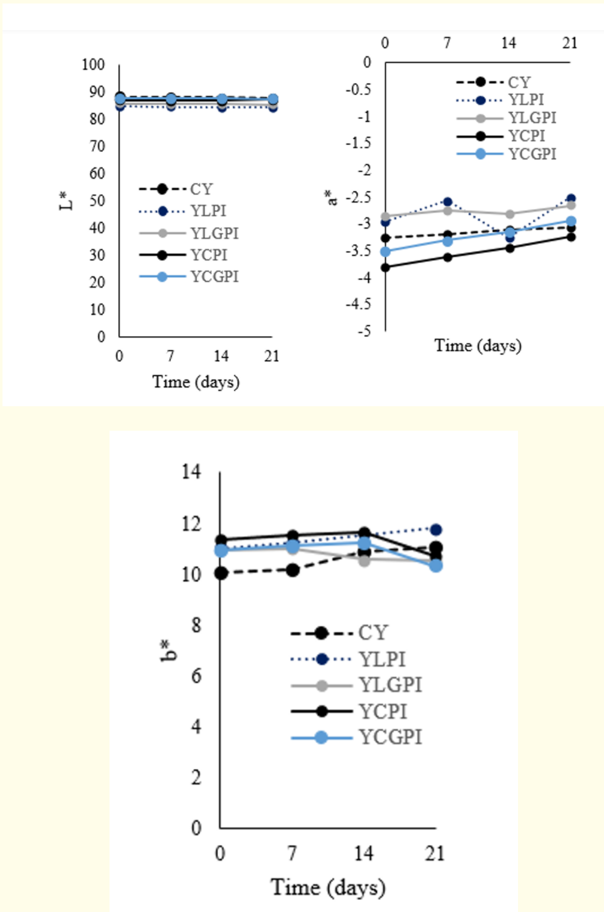

Figure 4: Colorimetric parameters of all yoghurt samples stored at 4°C.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt

Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins

Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

	Storage time (Days)			
Yoghurts	**1**	**7**	**14**	**21**
	Log10 CFU g-1			
TAMB				
CY	Abs	Abs	Abs	Abs
YLPI	Abs	Abs	Abs	Abs
YLGPI	Abs	Abs	Abs	Abs
YCPI	Abs	Abs	Abs	Abs
YCGPI	Abs	Abs	Abs	Abs
FC				
CY	Abs	Abs	Abs	Abs
YLPI	Abs	Abs	Abs	Abs
YLGPI	Abs	Abs	Abs	Abs
YCPI	Abs	Abs	Abs	Abs
YCGPI	Abs	Abs	Abs	Abs
		TC		
CY	Abs	Abs	Abs	Abs
YLPI	Abs	Abs	Abs	Abs
YLGPI	Abs	Abs	Abs	Abs
YCPI	Abs	Abs	Abs	Abs
YCGPI	Abs	Abs	Abs	Abs
STAPH				
CY	Abs	Abs	Abs	Abs
YLPI	Abs	Abs	Abs	Abs
YLGPI	Abs	Abs	Abs	Abs
YCPI	Abs	Abs	Abs	Abs
YCGPI	Abs	Abs	Abs	Abs

Table 1: Microbiological results.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt

Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins

Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

Rheological Measurements

The apparent viscosity, consistency index, and flow index of yogurt with and without lentil and chickpea protein isolates are shown in table 2. All samples are shear thinning ($n < 1$), which means that their viscosity is independent of time. Similar results for low power indices (< 1) were reported by (Najgebauer-Lejko., *et al*. [29]. For stirring milk yogurt controls and other samples with tea infusion. In general, the control samples exhibited the lowest viscosity and consistency index values. The results showed that there was a significant difference ($P < 0.05$) in the storage time of all prepared yogurts. In fact, all prepared yogurts increased in viscosity and consistency up to 21 days after preparation. The highest values of viscosity and consistency were presented for yoghurt fortified with lentil and chickpea germs proteins. Comparable results were also reported by Barakat and Hassan [20]. The results showed that the addition of soy protein resulted in an increase in the viscosity and consistency values of the yoghurt. This may indicate that the soy protein affects the texture of the yoghurt

	Storage time (Days)			
	1	**7**	**14**	**21**
Yoghurts	**Consistency index K (Pa s^n)**			
CY	400,15 ± 11,45	430,19 ± 11,18	413,39 ± 8,01	479,14 ± 22,01
YLPI	527,66 ± 14,03	528,30 ± 10,21	545,93 ± 9,01	555,95 ± 32,01
YLGPI	534,85 ± 12,02	567,76 ± 11,42	593,85 ± 12,00	625,16 ± 11,03
YCPI	517,66 ± 14,03	518,30 ± 10,21	550,93 ± 9,01	545,95 ± 32,01
YCGPI	637,03 ± 12,01	651,05 ± 23,12	712,14 ± 13,41	791,07 ± 10,91
	Flox index (n)			
CY	0,6 ± 0.01	0,4 ± 0.02	0,7 ± 0.01	0,64 ± 0.05
YLPI	0,3 ± 0.01	0,34 ± 0.03	0,8 ± 0.00	0,32 ± 0.01
YLGPI	0,29 ± 0.03	0,12 ± 0.05	0,1 ± 0.00	0,67 ± 0.02
YCPI	0,85 ± 0.01	0,45 ± 0.01	0,93 ± 0.01	0,10 ± 0.05
YCGPI	0,34 ± 0.03	0,32 ± 0.01	0,6 ± 0.01	0,12 ± 0.05
	Apparent viscosity η_o (Pa)			
CY	54,002 ± 4,20	56,23 ± 1,64	62,48 ± 3,62	63,99 ± 3,43
YLPI	60,95 ± 2,20	63,31 ± 2,32	65,47 ± 4,52	65,08 ± 5,11
YLGPI	65,81 ± 3,10	66,06 ± 3,02	63,92 ± 2,32	68,27 ± 2,32
YCPI	61,91 ± 2,30	67,67 ± 5,10	66,91 ± 4,52	66,99 ± 2,52
YCGPI	71,58 ± 3,10	72,86 ± 4,52	84,04 ± 2,32	86,07 ± 2,10

Table 2: Rheologic parameters of yaourt stored 21 days at 4°C.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

Sensorial analysis

The sensory characterization was performed by a hedonic test by 60 untrained subjects and by ranking of appreciation of tested product. The sensory evaluation of the different yoghurt samples aims to determine the tasters' acceptance of yogurts. Results have shown that there is not significant difference between sensory scores for all descriptors of yogurt samples fortified or not with leguminous proteins (Figure 4).

However, yoghurts YCGPI and YLGPI presented sightly highest sensory scores comparing with the other ones. In other words, testers have appreciated these samples more than the other ones.

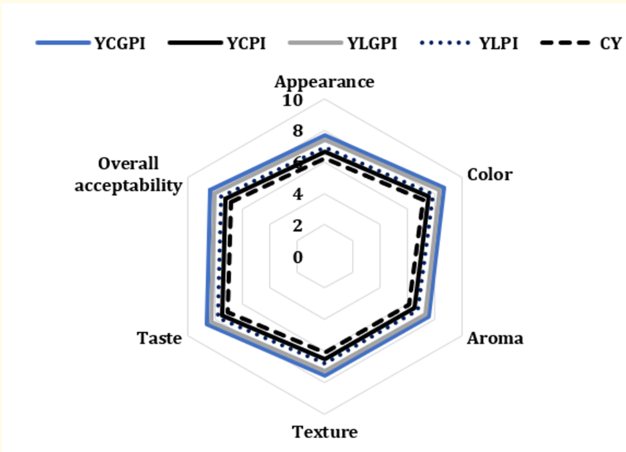

Figure 5: Sensory characteristics of yoghurt samples with or without lentil and chickpea proteins.

CY: Control Yoghurt; YLPI: Yoghurt Made with the Addition of Lentil Proteins Isolate; YLGPI: Yogurt

Made with Lentil Germs Proteins Isolate; YCPI: Yoghurt Made with the Addition of Chickpea Proteins

Isolate; YCGPI: Yoghurt Made with Chickpea Germs Proteins Isolate

Conclusion

In this work, we are interested in the production of a fortified yoghurt with lentil and chickpea proteins isolate. The results showed that the storage time significantly affected the level of acidity and pH in the samples. For all yogurt samples, a significant decrease in pH was observed during the first two weeks after which was not significant. Results have shown that TA values increased while the pH values decreased. In other hand, results show that there is not significant difference of DM values during storage period of the different yoghurt samples. Whey separation (Syneresis) was lower in fortified yogurts with lentil and chickpea proteins isolate as compared with control yoghurt. The colorimetric results showed that the factor L*, a* and b* have no significant difference with each other during 21-day storage period for all samples (P > 0.05). The brightness of all samples of yoghurt were ranged between 80 and 90. Moreover, the colorimetric parameters a* and b* change slightly according to adding proteins isolate. Microbial evaluation shows that total aerobic mesophilic bacteria, total coliforms, fecal coliforms and S. aureus colonies were not found in any of the yogurt samples until the 21st day of storage. Rheologic analysis shows that the control samples exhibited the lowest viscosity and consistency index values. The results showed that there was a significant difference (P < 0.05) in the storage time of all prepared yogurts. Sensory results have shown that there is not significant difference between sensory scores for all descriptors of yogurt samples fortified or not with leguminous proteins. However, yoghurts YCGPI and YLGPI presented sightly highest sensory scores comparing with the other ones. All these findings, clearly shows that lentil and chickpea proteins can be effectively used as stabilizer in the manufacture yoghurt for its quality improvement.

Conflict of Interest

Financial interest or conflict of interest are not existing.

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