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Research Article
Effect of Cold Storage on the Rheological Characteristics of Dry Beans Yogurt

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Abstract

Background and Objective: In addition to being rich sources of several macro and micronutrients, the dry bean is a rich source of dietary fiber and oligosaccharides which could serve as non-dairy based substrates to produce yogurt. The aim of this research was to determine the effects of storage (4°C) on color and the rheological properties of legume yogurts. **Materials and Methods:** Milk was prepared from Kidney Beans (KB), Garbanzo Beans (GB) and Soybeans (SB), which served as control was pasteurized and inoculated with a combination of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies bulgaricus and incubated at 42°C for 4.5 hrs or until the pH reached <4.6. Following fermentation, the yogurt was stored at 4°C for up to 28 days. The pH/acidity, color, viscosity, oscillatory and flow frequency as well as thixotropic measurements were determined weekly during storage of the yogurts. **Results:** Results indicated no significant change in pH during storage. No significant changes in color were noted during storage for both GB and KB yogurts. Results indicated significant ($p\leq 0.05$) changes in the flow behavior index in the control compared to KB and GB yogurt. Even though apparent viscosity (Pa s^{-1}) in all yogurts was increased with storage, viscosity was significantly ($p\leq 0.05$) increased in KB and GB yogurts compared to control (SB) yogurt. The study indicated that storage impacted the viscoelastic and apparent viscosity properties of the legume yogurt. **Conclusion:** Overall, these results indicate KB and GB yogurt demonstrate promise as a potential nondairy-based probiotic product. The utilization of legume crops for such products will offer opportunities for the food industry, in terms of value addition to the development of novel functional foods.

Key words: Yogurt, rheology, dry beans, kidney beans, garbanzo beans

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yogurt is one of the most popular food in the U.S. Yogurt is a dairy-based product that has been fermented with starter cultures or probiotics such as *Streptococcus thermophilus* and *Lactobacilli delbrueckii* subsp. *bulgaricus.* Although, dairy products have been the substrate of choice, probiotics in dairy do not always demonstrate their full growth and survival potential[1]. As such, utilization of non-dairy substrates has become an alternative to dairy-based products. Popular among these are soybeans, however as previously mentioned[2-4], other legume sources can also serve as excellent substrates to support probiotics growth. Moreover, legumes are important sources of relatively inexpensive protein and the introduction of non-dairy products from legumes may contribute to the alleviation of dairy allergen, lactose intolerance as well as meet the needs of individuals concerned with overall well-being.

Legumes, due to the high fiber content are considered as prebiotics and hence are functional food ingredients that target the colon where colonic microflora ferment them, thus, improving the gastrointestinal physiology, immune functions, bioavailability of minerals and generation of novel bioactive compounds (from phenolic compounds) with anti-inflammatory activities. On the basis of this background, the current approach to use dry beans (Kidney and garbanzo beans) appears an important food ingredient that disserves to be explored for its potential as a substrate for yogurt production. The evaluation of this prebiotic takes into account its availability and efficacious response to dietary interventions. Furthermore, there is little information about the use of dry beans as a substrate for yogurt production. While the utilization of dry beans for such products should be promoted, they may impact the physical, textural and rheological properties of yogurt[5,6]. The physical properties are considered important characteristics that define yogurt quality and consumer acceptance[4].

Among the various methods used to access texture properties of yogurt, rheometry is the most useful[7]. Rheological parameters such as viscosity, shear stress and shear rate are important parameters in yogurt[8]. Rheological properties of yogurt can affect its quality, shelf life, product stability and most significantly, consumer acceptance. Furthermore, these quality characterizations are important in non-dairy based yogurts, which often display weak structural disadvantages in comparison to dairy based yogurts[9]. An understanding of these properties can lead to refinement of processing conditions or methods and hence, yogurt quality. Thus, this study aimed to determine the effect of fermentation on the physical properties and rheological characteristics of dry beans yogurt during refrigerated storage.

MATERIALS AND METHODS

Study area: The study was carried out in the Food Science Department at Alabama A and M University, Normal AL from September, 2013 to December, 2015.

Preparation of dry beans milk and inoculation with probiotic bacteria: Milk was prepared by soaking dry beans separately [kidney (KB), garbanzo (GB), soybeans (SB)] in distilled water at ratio of 1:2 (beans: water) for 8-12 hrs. The soaked beans were thoroughly rinsed, then boiled for 30 min. After cooling, the beans were blended with 600 mL of distilled water in a mixing blender until smooth (approximately 5 min). The milk was strained using a miracloth into sterile glass bottles and pasteurized for 5 min at 90°C (High Temperature, Short Time, or HTST pasteurization). Following pasteurization fruit pectin was stirred into the milk at 5% concentration. The bean milk was cooled to room temperature and 100 mL of each milk samples was transferred into sterile cups for inoculation with probiotic bacteria where 2.5% of each probiotic (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) was added and incubated at 42°C for approximately 4-6 hrs or until the pH reach >4.6[10]. Afterwards, yogurt was stored at 4°C for 28 days. The SB was used as the control pH was determined using a pH meter.

Determination of color: The color values, L* (whiteness to blackness), a* (redness to greenness) and b* (Yellowness to blueness) of samples were measured in reflectance mode with Color FlexEZ colorimeter (Hunter Labs, VA, USA) that was previously calibrated with black and white plates. The tests were conducted on samples of 30 mL and the averages of triplicates were recorded on days 1, 7, 14, 21 and 28 of storage.

Determination of apparent viscosity (η): The apparent viscosities of the dry beans yogurt samples were measured on day 1 and at weekly intervals during storage (28-day storage period). All samples were treated at a constant shear rate[11]. Samples were tested using a T-spindle (T-E size 92) coupled to an LVTD digital viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA). The viscometer was set at constant revolutions of 20 rpm. The yogurts were gently stirred for 5, 10, 12, 15, 20 and 30 sec (continuous sweeps) before analysis. All determinations were repeated three times.

Determination of oscillatory and flow frequency sweep: In this study all oscillatory tests were performed using a discovery hybrid rheometer-(DHR2, Delaware and United States). All tests were performed at a frequency range of 0.1-60 Hz, using strain values comprised in the linear viscoelastic region (5%) for each sample. Data were collected and analyzed using the TA instrument software program. All samples calculated data included the shearing component G' (storage modulus), the viscous components G" (loss modulus), complex viscosity as well as a generated temperature profile. In this model, G' and G" were plotted against frequency. Complex viscosity was measured at a range of temperatures (4-60°C) while under constant oscillatory strain.

Statistical analysis: Experiments were repeated three times and each sample was tested in triplicate. All values were expressed as means (±SEM). Data were evaluated with SAS statistical software. Comparisons were done using one-way analysis of variance (ANOVA) followed by Tukey's test. The P value of $\leq$0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effects of pH/acidity in dry beans yogurts during refrigerated storage: Results are displayed in Table 1. The pH of yogurt is an important attribute to its quality, such as taste, mouth-feel and curd formation. It may also be responsible for some of its health benefits, such as maintaining a healthy GI tract. The pH is also an indication of probiotics' survival and viability. With the exception GB yogurt, which saw a significant (p<0.01) drop in pH at day 28, results from the present study showed no significant changes in pH among the legume yogurt over the storage period. The factors, surrounding the survival of probiotics include, the overall food matrix, acidity (pH), presence of oxygen, type and amounts of sugars present, water activity and storage temperature[1,12-14]. However, at the end of fermentation, pH is held in high regards as the most crucial aspect as it relates to the viability of probiotics[13] and a desirable characteristic in the modern yogurt industry[15].

Effects of refrigerated storage on color change in dry beans yogurts: Table 2 showed the effects of fermentation and storage on color change in dry beans yogurt. Results indicated that cold storage had no significant effect on the lightness (L*) of control (SB) and GB yogurts. On the other hand, KB yogurt was significantly (p<0.05) lighter (L*value) during the first 2 weeks of storage compared to the final 2 weeks of storage (days 21 and 28). Control (SB) yogurt presented significantly (p<0.05) higher L* compared to GB and KB yogurts. Redness (a* value) was significantly (p<0.05) higher in KB yogurt (6.00-7.35) compared to GB (3.30-3.31) and control (SB) yogurt, which indicated no redness. The increase in redness in KB yogurt could be due to the presence of colored compounds

Table 1: Effects of pH/Acidity in dry beans yogurts during refrigerated storage

Storage	Control (SB)	KB yogurt	GB yogurt
Day 1	4.83±0.03	4.43±0.13	4.62±0.13^{a}
Day 7	4.43±0.03	4.49±0.19	4.12±0.13^{a}
Day 14	4.57±0.05	4.63±0.06	4.60±0.01^{a}
Day 21	4.64±0.02	4.59±0.01	4.41±0.24^{a}
Day 28	4.63±0.06	4.42±0.16	3.94±0.01^{a}

Values are means±SEM. ^{ab}Values with different letters in a column indicate statistically significant difference among samples at p$\leq$0.05 using Tukey's studentized range test. SB: Soybeans, KB: Kidney Beans, GB: Garbanzo Beans

Table 2: Effect of refrigerated storage on color change in dry beans yogurts

Color values	Storage (days)	Control (SB)	KB-yogurt	GB-yogurt
L*	1	82.40±0.43^{A}	70.99±0.08^{a,B}	76.91±0.44^{B}
	7	81.61±0.92^{A}	70.11±0.03^{a,B}	76.46±0.50^{B}
	14	81.88±0.78^{A}	70.10±0.03^{a,B}	76.22±0.76^{B}
	21	83.79±0.17^{A}	67.98±1.42^{b,C}	75.67±0.74^{B}
	28	84.19±0.21^{A}	67.35±0.91^{b,C}	75.00±0.21^{B}
a*	1	-0.78±0.55^{C}	6.66±0.80^{A}	3.33±0.02^{B}
	7	-0.91±0.41^{C}	6.91±0.67^{A}	3.31±0.02^{B}
	14	-0.68±0.24^{C}	6.00±0.08^{A}	3.30±0.07^{B}
	21	-0.80±0.23^{C}	6.48±0.44^{A}	3.24±0.11^{B}
	28	-0.77±0.35^{C}	7.35±0.66^{A}	3.53±0.10^{B}
b*	1	19.40±0.98^{B}	15.63±0.71^{C}	24.63±0.29^{A}
	7	19.96±0.79^{B}	15.65±0.69^{C}	25.16±6.49^{A}
	14	19.52±0.79^{B}	15.42±0.05^{C}	25.37±0.14^{A}
	21	18.03±0.27^{B}	15.07±0.99^{C}	25.14±0.03^{A}
	28	18.72±0.65^{B}	15.72±0.39^{C}	24.81±0.40^{A}

Values are means±SEM. ^{abc} values with different letters in a column indicate statistically significant difference within samples and ^{ABC} values with different letters in a row indicate statistically significant difference among samples at p$\leq$0.05 using Tukey's Studentized range test, L*: Whiteness to blackness, a*: Redness to greenness and b*: Yellowness to blueness

Table 3: Effect of refrigerated storage on apparent viscosity (η) (Pa s^{-1}) in dry beans yogurts at 20 rpm

Storage	Control (SB)	KB yogurt	GB yogurt
Day 1	$0.16\pm0.01^{c,B}$	$0.39\pm1.85^{d,A}$	$0.37\pm1.17^{d,A}$
Day 7	$0.48\pm0.13^{a,C}$	$0.92\pm1.71^{b,A}$	$0.64\pm1.71^{c,B}$
Day 14	$0.45\pm0.13^{a,C}$	$1.19\pm2.93^{a,A}$	$0.84\pm2.82^{b,B}$
Day 21	$0.16\pm0.01^{c,C}$	$1.03\pm4.58^{ab,A}$	$0.81\pm1.92^{b,B}$
Day 28	$0.22\pm0.03^{b,C}$	$0.68\pm1.97^{c,B}$	$0.97\pm3.03^{a,A}$

Values are means±SEM. ^{abc}Values with different letters in a column indicate statistically significant difference within samples and ^{ABC}Values with different letters in a row indicate statistically significant difference among samples at $p\leq0.05$ using Tukey's studentized range test

such as flavonoids, which are found in larger quantities compared to the other legumes utilized in this study. Overall, there was no significant difference in redness (a* value) with storage among the legume yogurts. Yellowness (b* value) was significantly ($p<0.05$) higher in GB yogurt compared to KB and control (SB) yogurt. The legume yogurts presented no significant change in b* value with storage. The legume yogurts prepared in this study maintained their respective colors from day 1 until day 28, having no significant changes within the groups. As expected, b* values of SB and GB yogurt were statistically similar, this was expected because both soy and garbanzo beans had a yellowish hue prior to processing. Legumes such as GB and KB, although high in other phenolic contents, do not contain those that contribute to bright colors observed with seeds such as navy, pinto and kidney beans (KB). As expected, KB yogurt redness was significantly ($p\leq0.05$) higher than both SB and GB yogurt and maintained its color throughout storage. Though many functional foods obtain a consumer market base due to the exceptional health benefits provided, acceptance through appearance such as color remains the most important marketable aspect. Color is one sensory attribute that should remain consistent throughout the shelf life of the product[7,16,17].

Effect of refrigerated storage on apparent viscosity (η) (Pa s^{-1}) of dry beans yogurt: The apparent viscosity (η) (Pa s^{-1}) of SB, GB and KB yogurts at shear rate of 20 rpm is shown in Table 3. The results in Table 3 show that apparent viscosity (η) (Pa s^{-1}) was increased ($p\leq0.05$) in KB and GB yogurts compared to SB yogurt. In KB and GB yogurts, the apparent viscosity (η) (Pa s^{-1}) increased ($p\leq0.05$) with storage. The highest apparent viscosity (η) (Pa s^{-1}) in KB yogurt was on day 14 and day 21 with increases of more than 60% compared to day 1. In GB yogurt the highest apparent viscosity (η) (Pa s^{-1}) was noted on day 28 (0.97 Pa s^{-1}) with a 61% increase compared to day 1. Results also showed KB yogurt was significantly ($p<0.05$) thicker than GB at least until day 21. These results concur with the findings by Sendra *et al.*[17], in which storage significantly affected the viscoelastic properties of orange fiber enriched yogurt. In the present study, the decrease ($p\leq0.05$) in apparent viscosity (η) (Pa s^{-1}) in SB yogurt compared to KB and GB yogurts could be due to the increased shear rate that was used (20 rpm). In a study by Rinaldoni *et al.*[18], the apparent viscosity of soy yogurt decreased as the shear rate increased, indicating that the yogurt was a shear thinning and a time-dependent product. In another study, Coda *et al.*[19] indicated a 50% decrease in viscosity in yogurt supplemented with cereal, soy and grape must after a 30-day storage period. The rheological analysis performed by Ramirez-Sucre and Velez-Ruiz[20], showed that the flow behavior of caramel flavored yogurt resembled the flow behavior of the dry beans yogurts developed in this study. When the apparent viscosity decreases during shearing with increasing time, the fluid flow behavior is considered thixotropic. Since the apparent viscosity (η) (Pa s^{-1}) of the SB and KB yogurt to some extent GB yogurt remained below 1 during shearing, the yogurts can be considered as being pseudoplastic in nature. Texture, such as smoothness and creaminess are the most important attribute to consumers who purchase yogurt[21]. Although, the texture of yogurt is important, there is no set rheological procedure used to determine the textural parameters of yogurt. Rheological properties of food products are important due to the different processes they may undergo, such as process flow, quality control and storage during shipping or prior to consumption[22]. In the food industry, apparent viscosity is often used to determine mouth-feel or the human perception of thickness[23].

Effect of refrigerated storage on oscillatory and flow frequency sweep in dry beans yogurts: Figure 1 shows the effect of refrigerated storage on oscillatory and flow frequency sweep in dry beans yogurt. The oscillatory and flow frequency sweep are expressed using two parameters, storage (G') and loss (G") modules, which denotes the degree of elastic and viscous behavior, respectively. Figures 1a, b and c show the storage modulus (G') for SB, GB and KB yogurts, respectively. The loss modulus (G") for SB, GB and KB yogurts are depicted in Fig. 1d-f, respectively. The results indicate G' was lowest on day 1 in SB yogurt (Fig. 1a) and lost gel elasticity at >10 Hz. Although G' of SB yogurt increased after day 1 (from 100 Pa on day1 to ~900 Pa on day 21), deformation in the yogurt started to occur at high oscillatory shear (>20 Hz). This observation was evident on day 21 where a dramatic weakening of the yogurt gel occurred at approximately 50 Hz. In GB yogurt (Fig. 1b), G' was noticeably decreased with the concomitant weakening of gel structure at 5 Hz on day 14. However, an increase in G' on day 21 and day 28 resulted in an improvement in gel structure. According to the results, GB

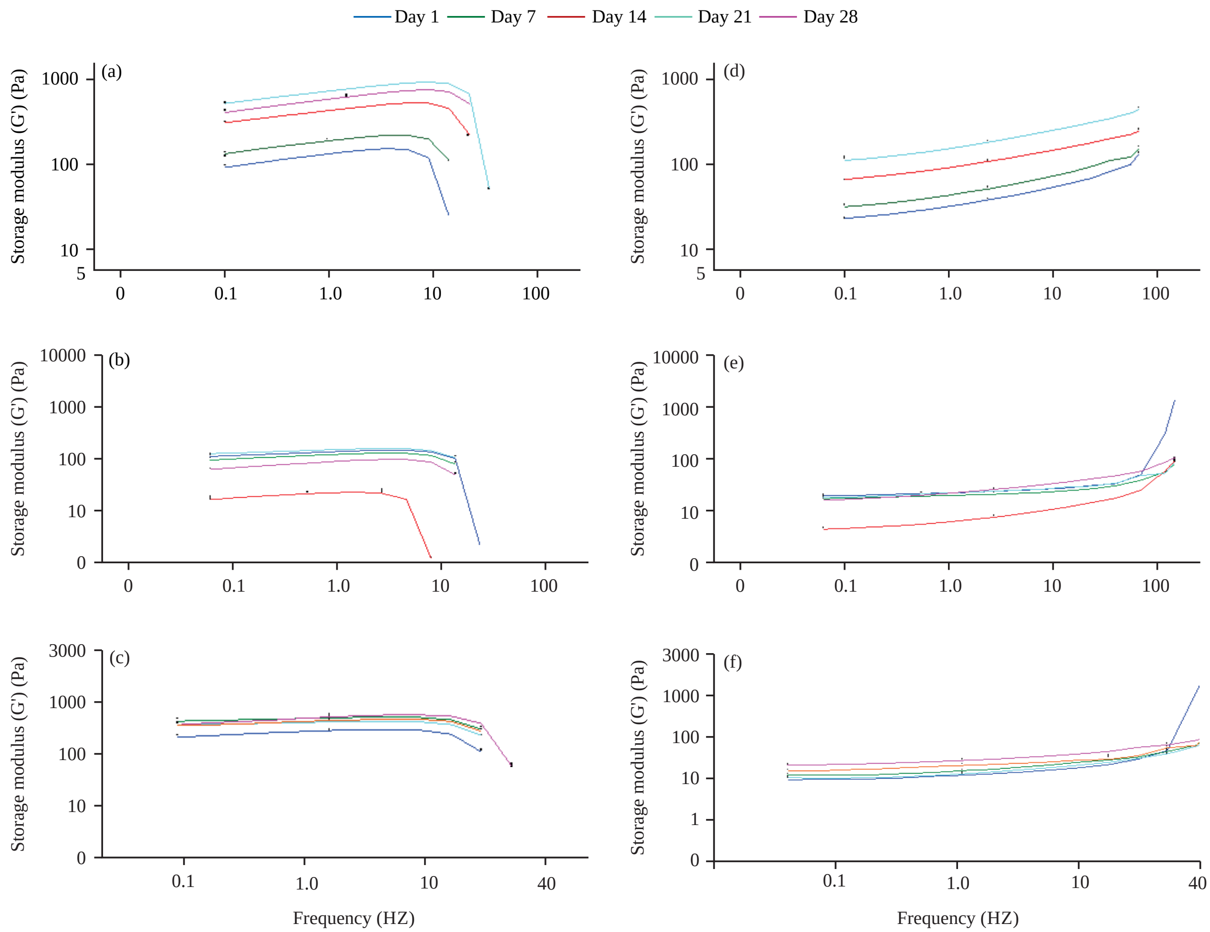

Fig. 1(a-f): Effect of refrigerated storage on oscillatory and flow frequency sweep in dry beans yogurts
Storage modulus(G'): (a) SB yogurt (control), (b) GB yogurt and (c) KB yogurt. Loss Modulus(G"): (d) SB yogurt (control), (e) GB yogurt and (f) KB yogurt

yogurt began to undergo deformation at around10 Hz. Although G′ was high on day 1, the yogurt quickly lost gel strength. While G′ in KB yogurt (Fig. 1c) remained unchanged over the storage time, the yogurt began to lose its gel structure at around 20 Hz on day 28. Similar observations were made with kefir supplemented with faba beans and chickpea mucilage[24]. Compared to SB yogurt (Fig. 1a), G′ was lower in GB (Fig. 1b) and KB (Fig. 1c) yogurts suggesting gel network may have weakened over the storage time in the yogurts. This was most evident in GB yogurt (Fig. 1b) especially on day 14.

The loss modulus (G″) (Pa) was highest in SB yogurt (Fig. 1d) as compared to GB yogurt (Fig. 1e) and KB (Fig. 1f) yogurt. Some studies have suggested this could be due to the aggregation of soy proteins with lipids, thus creating a stronger gel formation[25]. An elevated loss modulus (G″) indicate a more viscous product. In SB yogurt there is a difference of 700, 300, 250, 80 and 60 Pa between the loss modulus (G″) and the storage modulus (G′) for day 21, 28, 14, 7 and 1, respectively, which indicates that as storage time increased the viscosity of the yogurt decreased. This was also noted in GB and KB yogurts. Overall, the results suggested that the dry beans yogurts exhibited viscoelastic properties based on the higher G′ (G>G″). In fact, this is one of the challenges of legume /vegetable-based gels[26]. Saleh *et al.*[27], also noted a decrease in gel strength with increased storage of Turkish beans-based yogurts. Based on their assessments, the study[27] suggested the mechanism of starch gelatinization during the first steps of yogurt making and amylose retrogradation and syneresis could be the reasons. Rheological properties of food products are important due to the different processes they may undergo, such as process flow, quality control and storage during shipping or prior to consumption[22].

Effect of refrigerated storage on temperature-dependent complex viscosity in dry beans yogurts: Figures 2a, b and c show the effect of refrigerated storage on temperature-dependent complex viscosity in SB yogurt, GB yogurt and KB yogurt, respectively. The temperature-dependent complex

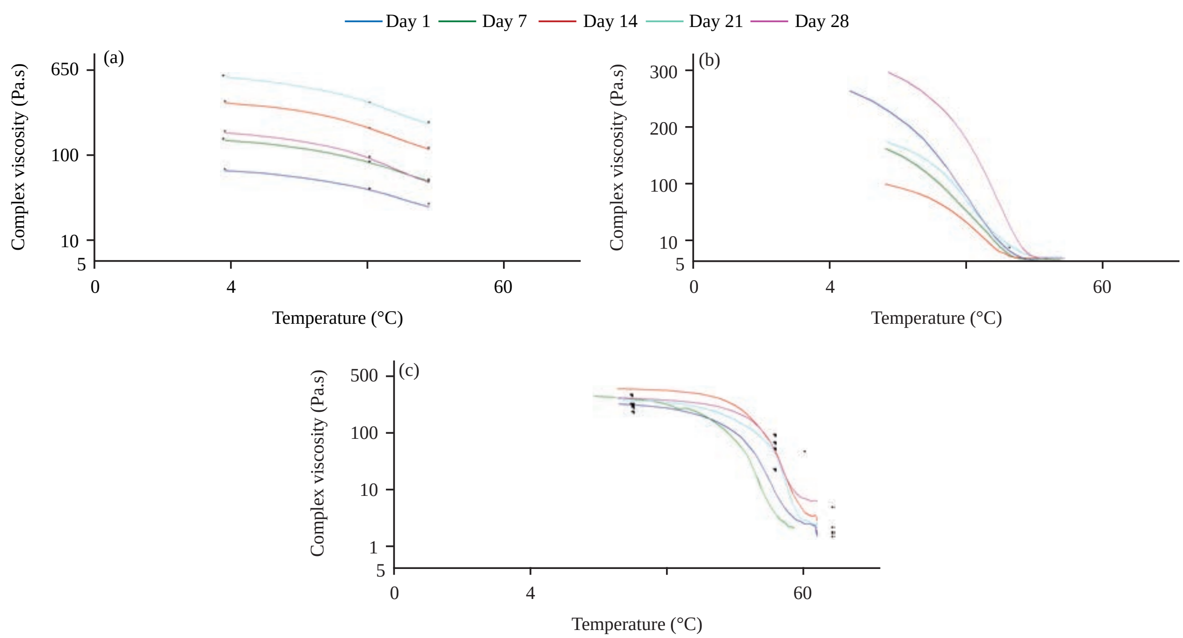

Fig: 2(a-c): Effect of refrigerated storage on temperature- dependent complex viscosity in dry beans yogurt
(a) SB yogurt (control), (b) GB yogurt and (c) KB yogurt

viscosity for the yogurts was measured at a range of temperatures (4-60°C) while under constant oscillatory strain. Complex viscosity is frequency-dependent viscosity determined while under constant shear stress. In this case, the yogurt was previously stored at refrigeration temperatures prior to the temperature ramp. Due to hysteresis in this yogurt product, pectin was added to prevent the separation of the legume solids and liquid portions. Pectin is a polysaccharide that has gelling properties and may be used as a stabilizer as well as contributing to the dietary fiber content. Overall, the results in Fig. 2a-c indicated that complex viscosity (Pa/s) was decreased as the temperature increased in all the yogurts. Based on the results, the decline in complex viscosity of SB yogurt (Fig. 2a) at T_{60} ranged from approximately 30 Pa/s (day 1) to over 100 Pa/s (day 21). The GB yogurt (Fig. 2b) experienced the greatest decline in viscosity from a high of 300 Pa/s (day 28) at T_4 to 0 Pa/s at T_{60}. A decreasing trend in viscosity was also observed for KB yogurt (Fig. 2c) with values ranging from 0.5 Pa/s to 0.8 Pa/s for days 1 and 28 storage, respectively at T_{60}. The slow decline in complex viscosity for SB yogurt could be perhaps attributed to the elevated loss modulus (G”), which is indicative of a more viscous product.

CONCLUSION

Based on rheological properties consistent with yogurt, the dry beans yogurt produced in this study is a thixotropic, shear-thinning fluid due to a decrease in viscosity under constant shear stress. Overall, storage also affected the viscoelastic and apparent viscosity properties of the legume yogurt.

SIGNIFICANCE STATEMENT

Rheological studies indicated probiotic fermented dry beans yogurt is comparable to and can represent a yogurt product according to current U.S. yogurt standards. Therefore, dry beans could be considered as a functional food product to make a non-dairy yogurt product

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