

QUALITY EVALUATION OF DEVELOPED IRON AND RETINOL ACETATE FORTIFIED YOGURT

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ABSTRACT

Yogurt is one of the most nutritious probiotic food and serves as a medium for nutrients supplementation. The prevalent deficiency of iron and vitamin A within the population prompted the creation of retinol acetate and iron-fortified yogurt. In the yogurt-making process, temperatures typically remain below 100°C for 5 to 10 minutes, and the fortified milk exhibited remarkable heat stability, surpassing even the effects of sterilization treatment (140°C for over 20 minutes). Sensory evaluations of the fortified yogurts yielded scores comparable to the control yogurt. The fortified variety set in a similar time frame, and the quantity of microorganisms used in the inoculation mirrored that of the control yogurt. Acetaldehyde, a key flavoring compound, was produced in a similar manner to the control yogurt ($p > 0.05$). Physico-chemical properties of the fortified yogurt closely resembled those of the control, with improvements seen in viscosity and textural attributes, though these values were statistically similar ($p > 0.05$). The fortified yogurt demonstrated stability along with consistent quality, texture, and sensory appeal, suggesting its potential for commercialization to address nutrient deficiencies.

Keywords: Iron; vitamin A; retinol acetate; yogurt; sensory analysis; texture

INTRODUCTION

Yogurt is the most popular fermented milk product all over the globe. Yogurt has thousands of variants like fruit yogurt (Mittal *et al.*, 2020), micronutrient fortified yogurt (Kaushik and Arora, 2017), prebiotic yogurt (Hussein *et al.*, 2020), probiotic yogurt, omega-3 fortified yoghurt (Goyal *et al.*, 2016), bio yogurt (Santivarangkna, 2016). Yogurt starter cultures metabolize lactose into lactic acid reduce pH and lead to milk protein coagulation (Ye *et al.*, 2022). Yogurt culture produces exopolysaccharides, vitamins, bioactive peptides, flavouring compounds, etc. (Popovic *et al.*, 2020).

The yogurt itself has several health benefits and the functional properties of yogurt are enhanced by micronutrient fortification (Kaushik *et al.*, 2017). Milk and products prepared from milk are deficient in iron and the best option to overcome is its fortification (Ziena and Nasser, 2019). Le-port *et al.* (2017) carried out a cluster randomized control trial on children aged between 2 to 5 years in which iron-fortified yogurt was fed to children for 1 year and the result showed that Anaemia prevalence decreased to 20% and haemoglobin increased by 0.55g/dl in one year.

Anaemia is an iron deficiency condition in which red blood cell count or haemoglobin concentration is less than normal. The associated factors responsible for anaemia are deficiencies of cyanocobalamin, vitamin B9, and vitamin A. Global anaemia prevalence in women is 30% and in children between 0.5 to 5 years is 40% (WHO, 2021).

The main ingredient of yogurt is milk which is inoculated with two types of lactic acid bacteria (*Lactobacillus delbrueckii subsp. Bulgaricus* and *S. thermophiles*). It is the source of live lactic acid bacteria that modulates the digestion system and performs as a functional food (Le-Roy *et al.*, 2022). Yogurt can be prepared from cow milk, goat milk (Papaioannou *et al.*, 2022), buffalo milk (Swelam *et al.*, 2021), Sheep milk (Mendoza-Taco *et al.*, 2022), and cow and buffalo mixed milk (Kaushik *et al.*, 2017). The vitamin A content of mixed cow and buffalo-toned milk has been reported 325IU/L (Sachdeva *et al.*, 2021). Herrero *et al.* (2002) reported vitamin A content of full fat, reduced fat, and skimmed yoghurt as 103 to 123, 36 to 53, and 1.5 IU/100 ml, respectively. The iron content of mixed cow and buffalo-toned milk was ± 1 ppm (Sachdeva, 2012). Similarly, Mandecka *et al.* (2022) reported 1.3 ppm of iron in yoghurt. All dairy products are deficient in iron, whereas low-fat dairy products are deficient in vitamin A (Chawla *et al.*, 2021). Vitamin A deficiency in children was highest in Sub-Saharan Africa (48%) and South Asia stands second (44%) (Stevens *et al.*, 2015). Vitamin A is fortified in two forms 1) Carotenoids or retinyl esters *viz.* retinol acetate and 2) Retinol palmitate (Hooper *et al.*, 2022). As per FSSAI (2018) regulations milk fortification

with vitamin A and D is compulsory. The level of addition of vitamin A is 270 to 450 $\mu\text{g RE}$.

Fortification of yogurt with iron has been performed by several researchers (El-Kholy *et al.*, 2011; Ziena and Nasser, 2019), however, there are limited reports on developing vitamin A and iron-fortified yogurt. It is reported that vitamin A has a proven effect on the increased bioavailability of iron and its utilization (Sachdeva *et al.*, 2015). Therefore, the fortified (iron+retinol acetate) yogurt was developed and quality, sensory, microbial, and textural characteristics were determined.

MATERIALS AND METHODS

Materials

Milk was obtained from the experimental dairy plant, ICAR-NDRI (Karnal, India). Yogurt was prepared using NCDC 074 (*S. thermophilus*) and NCDC 009 (*Lactobacillus delbrueckii subsp. Bulgaricus*) bacterial cultures obtained from National Collection of Dairy Cultures (NCDC), Karnal, India. Retinol acetate encapsulated with a water-soluble layer (3,25,000 IU/g) was used (DSM Nutritional Products, Singapore). Ferrous gluconate hydrate and Ferric pyrophosphate were procured from Sigma Aldrich, St. Louis, MO, USA and Shanpur Industries Pvt., Vadodara, India, respectively.

Fortification of milk

The addition of retinol acetate and iron to milk was carried out as per the method of Sachdeva *et al.* (2015). Retinol acetate was fortified at the level of 2500 IU/L and iron at the levels of 15, 20 and 25 mg/L using soluble ferric pyrophosphate and ferrous gluconate, respectively, in toned (3.0% fat and 8.5%) mixed milk. The milk was preheated at 45 °C and the fortificants were added and mixed thoroughly for 10 min. using an electric stirrer.

Heat coagulation time (HCT)

When the temperature of milk surpasses the milk stability limit may lead to grittiness, phase separation, separation of milk fat, and sediment formation is considered HCT (Dumpler *et al.* 2020). Therefore, the heat stability (HCT) of fortified and unfortified raw milk samples was analysed (Kaushik *et al.* 2015). Heat coagulation time was noted down at 140°C in minutes and it represents the stability of milk during heating treatments. The experiment was conducted in a paraffin oil bath (Elmech Pneumatic Industries Pvt. Ltd., Okhla, Delhi, India).

Yogurt Preparation

Yogurt was prepared from unfortified and fortified milk (Nevestani et al., 2015). Milk was preheated, filtered, homogenized (2500 psi) and heated to 90 °C for 60 min, and cooled to 45°C and *Streptococcus thermophilus* (1.25%) and *Lactobacillus bulgaricus* (1.25%) cultures were added for fermentation, mixed thoroughly, and incubated at 42 °C till the coagulum was set.

Setting time

Setting time is the time taken by a milk sample to reach a set coagulum. After mixing the culture in the milk, started the stopwatch and noted down the time till the coagulum was set. The setting time of yoghurt was noted down in hours.

Syneresis and moisture binding capacity

Syneresis of set yogurt were estimated using the syphon method. Yogurt was placed over a 45-degree slide and the whey separated was calculated as percentage syneresis. The moisture binding capacity was determined by centrifugation of yogurt (Hammad et al., 2022).

pH and titratable acidity (TA)

Lactic acid bacteria (LAB) metabolize milk carbohydrates to lactic acid that shifts the pH to casein isoelectric point. Therefore, pH and acidity of yogurt were analysed. The yogurt pH was analysed using an electrochemical pH meter (Mettler-Toledo, India). The electrode of pH meter was placed in yogurt and the pH was recorded. The TA of the yogurt was determined by the derivatization with 0.1 N NaOH (Kaushik et al., 2017b).

Viscosity

When milk is converted into yogurt, the viscosity increases due to gel formation. The effect of the addition of fortificants on yogurt viscosity was recorded with a viscometer (Viscosimetro DV-E, Brookfield, Barcelona). Spindle TL-7 was used for viscosity measurement (centipoise) at 10, 20, and 30 rpm, respectively.

Textural properties of yogurt

Yogurt was prepared in 100 ml glass beakers with equal dimensions (5 cm internal diameter). The stickiness, firmness, work of Adhesion, and work of shear of yogurt were analysed using a texture analyser (Malvern Panalytical Ltd., England). The texture analyser was equipped with a 5 kilogram load cell. Yogurt was compressed mono-axial 25 mm with a crosshead speed of 1 mm/s at a constant temperature of 25 °C. The force of compression versus time was recorded in the form of a graph. Three replications with 5 samples in each replication of the yoghurt sample were analysed for textural properties.

Lactic acid bacteria count

The yogurt sample was serially diluted to 10⁻⁷ dilution factor and spread over M17 agar plates for enumeration of *S. thermophilus* (ST) and incubated at 37°C for 48 h. The *L. bulgaricus* ssp. *delbrueckii* (LB) was inoculated over Acidified MRS media plates and incubated at 37°C for 72 h (Nikbakht et al., 2019). The enumeration of bacterial colonies was carried out using a colony counter (Bio Techno Lab, Mumbai, India).

Acetaldehyde content in yoghurt

Acetaldehyde is a prominent flavouring compound metabolized by yogurt cultures. Acetaldehyde content was analysed in yoghurt using Acetaldehyde assay kit (Wicklow, Ireland).

Sensory analysis

Descriptive sensory analysis is the most sophisticated method for assessing the eating quality and acceptability of dairy Products (Kaushik et al., 2017b). Milk is a sensitive food and the addition of a low amount of foreign material can change in taste, flavour, aroma, mouthfeel, and colour. Thirty trained sensory panellists from the same institute evaluate the yogurt samples. The samples were analysed on a 100-rating scale bifurcated to 10, 20, 40 and 30 for colour and appearance, odour, taste, and mouthfeel, respectively. The scores were reduced from the maximum scores if any defect was observed.

Statistical analysis

The experimental data was noted down in Microsoft 365 (Microsoft Corp., New Mexico, US) and data was interpreted with the help of Microsoft excel. A single-way ANOVA was used to determine the critical value (P < 0.05) (Sharma et al., 2017).

RESULTS AND DISCUSSION

Heat stability of milk samples

The time of resistance to coagulation at temperature above boiling point of milk is termed as heat stability (Dumpler et al., 2020). The highest heat treatments used to process milk are sterilization (121°C for 0.25 h at 1.055kg/cm² pressure) and ultrahigh treatment (135 °C for 2-3 s). The result presented in Figure 1 showed that all control unfortified milk samples and pH adjusted fortified milk samples (pH 6.4 to 6.7) showed higher heat coagulation time than standard sterilization and UHT processes. Therefore, it can be inferred that retinol acetate and iron-fortified milk samples can withstand all heat treatments used in milk processing and all type of milk products will be stably prepared from it. The natural pH of control milk was 6.62 and the maximum heat coagulation time was observed at acidic side (pH 6.5) of control milk.

The milk or milk products are processed using heat viz. pasteurisation, sterilization or UHT. Before preparing any product from milk, checking heat stability is a must. Heat stability graph is of two types: Type A and Type B. Milk having HCT/pH maxima at natural pH or acidic side is termed as Type A, however in Type B milk HCT/pH maxima at the alkaline side of natural pH (Dumpler et al., 2020). In present heat stability results, all milk samples were Type-A.

A similar HCT/pH curve was reported for iron-fortified milk and reported that HCT–pH maxima was shifted from natural pH to slightly acidic side (Sachdeva et al., 2015). Heat stability studies were carried out for calcium-fortified milk, and they also reported that HCT–pH maxima was shifted from natural pH to slightly acidic side (Kaushik et al., 2015). Sweetsur and Muir (1980) and Sindhu and Singh (1987) determined the heat stability of cow milk and reported that all milk samples were Type-A. However, Holt et al. (1978); Singh et al. (2007) reported maximum heat stability was slightly alkaline side than the milk natural pH. The milk takes 52.8 min for coagulation (natural pH 6.65) and HCT/pH maxima was shifted to pH 6.7 with 54.26 min (Singh et al., 2007).

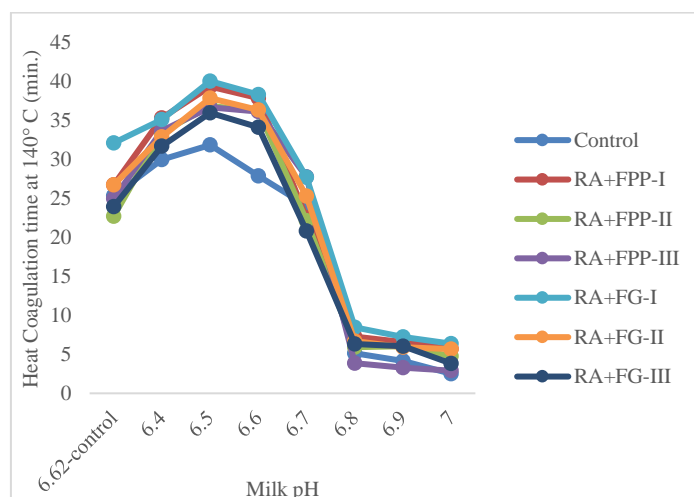


Figure 1 Heat coagulation time of milk samples

The value represented in the figure are the mean of three concordant readings and the value after ± is the standard error mean.

Sensory acceptability

Iron and retinol acetate fortified milk was analysed for its yogurt-making quality. Yogurt was prepared and its sensory evaluation was carried out. It is inferred from Figure 2 that all fortified samples were at par (p>0.05) with unfortified yogurt. The overall scores obtained were lowest for 25 ppm iron-fortified yogurt samples and highest for control yogurt, respectively in both RA+FPP and RA+FG iron salts.

Elbehairy and Mohamed (2010) developed ferric chloride and ferrous sulphate fortified yogurt and reported sensory acceptability level at 20 and 40 mg iron per kg yogurt, respectively. Ferrous lactate fortified yogurt scored similar sensory scores as control (Simova et al., 2008). Hekmat and McMahon (1997) reported that the Thiobarbituric Acid value of unfortified yogurt and 40 ppm iron fortified yogurt were at par (p>0.05), however no other type of off flavour, and the defect were observed. Chelated iron addition in soy yogurt showed similar sensory scores as control (Cavallini and Rossi, 2009).

Previously, Schiffman and Dackis (1975) determined the taste of vitamin A and they reported that it has no perception as bitter, sweet, salty, or sour and judged as tasteless. In, the present study, yogurt was fortified with 2500 IU of vitamin A per liter of milk which is equal to 750 µg, it is a very less amount, therefore, its addition to complex food like milk and yogurt does not induce any sensory change (Sachdeva et al., 2021). Ilic and Ashnoor (1988) fortified yogurt with vitamin A at 10,000 IU/227g and reported no significant change in sensory characteristics, pH, and acidity of yogurt.

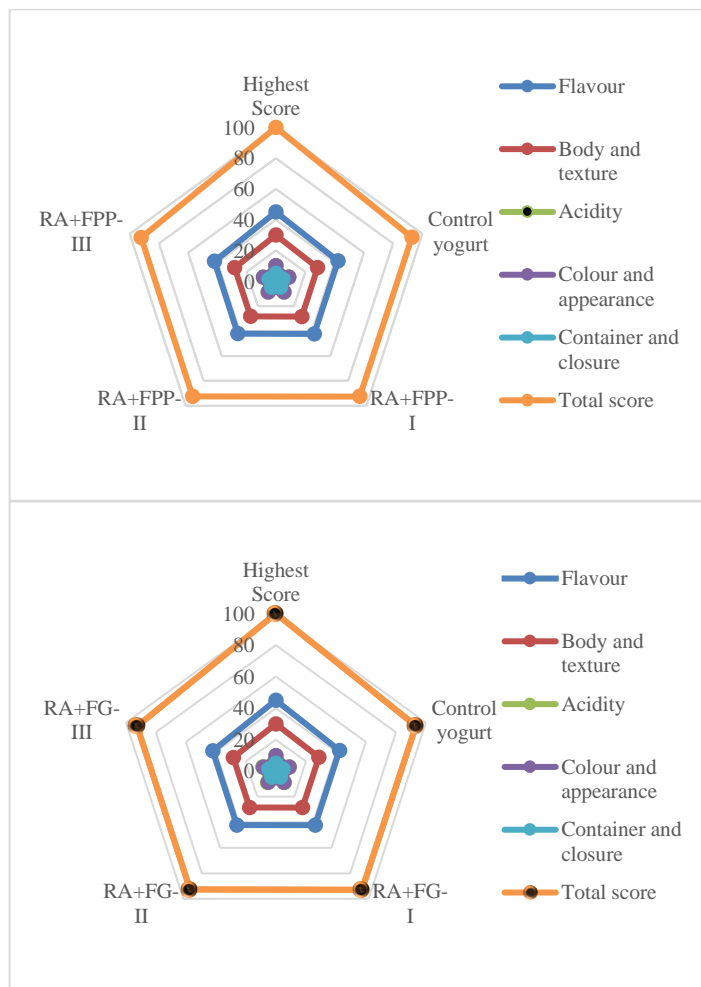


Figure 2 Comparative Sensory analysis of control and fortified yogurt a) retinyl acetate and Ferric pyrophosphate fortified yogurt b) retinyl acetate and Ferrous gluconate fortified yogurt
The value represented in the figure are the mean of thirty concordant readings and the value after ± is the Standard error mean

Yogurt Quality indices

Yogurt cultures metabolize lactose into lactic acid that is responsible for production of free H⁺ ions that lowers pH and raise the acidity. The acidification rate was reported between 0.0022 to 0.0042pH/min (Sanusi et al., 2022). The pH 4.6 is the isoelectric point of milk, therefore, the pH of a set yoghurt should be below 4.6 (Medeiros et al., 2015). Yogurt samples reported pH in range of 4.38 to 4.40 and pH values of all samples were at par (p>0.05) to each other (Tab 1). Therefore, it can be inferred that addition of iron does not accelerate the H⁺ production in yogurt significantly. The control sample has 0.772% LA and fortified yogurt samples have acidity ranging from 0.886 to 0.916% LA. All yogurt samples acidity were at par (p>0.05) to each other. The results showed that acid production (lactose to lactic acid) was higher in iron fortified yogurt, which might be because of iron fortification on the higher metabolism of lactic acid bacteria (Ziena and Nasser, 2019). Water holding capacity of yogurt samples ranged between 77.99 to 78.81% and all the samples were at par to each other (p>0.05). An imperceptible and insignificant increase (p>0.05) was observed in WHC of retinyl acetate and iron-fortified yogurt, samples. Reason behind it might be due to improving the gelling ability of casein by ferrous ions (Ziena and Nasser, 2019). The milk gel system is made up of casein micelles with entrapped water (Lucey, 2002). Ziena and Nasser (2019) reported that iron fortification may underpin gel structure of yogurt, which strengthened the gel structure due to higher bonding density per unit volume. A very low syneresis (3.07 to 3.33%) was observed for yogurt samples. All fortified yogurt samples were at par (p>0.05) to control. It is observed that iron fortification reduces the syneresis which might be due to an increase in the gelling ability of casein upon fortification with iron (Ziena and Nasser, 2019). Previous articles also reported that iron fortification reduced pH slightly (ElBehairy and Mohamed, 2010; El-Kholy et al., 2011) and increased acidity of yogurt (Cavallini and Rossi, 2009; El-Kholy et al., 2011; Ziena and Nasser, 2019). The increase or decrease in acidity and pH is dependent on iron salt. Chelated and complexed iron salt showed less change in pH and acidity in comparison to iron salts (El-Kholy et al., 2011). Achanta et al. (2007) reported that iron fortification reduced the syneresis in yogurt and increased the water holding properties. They also reported that WHC also increased in iron fortified yogurt. Fortification of dairy products with iron would help in fighting nutritional deficiencies as iron-fortified yogurt has a relatively higher iron bioavailability (Ziena and Nasser, 2019). Moreover, it has also been reported that iron fortification increases the gumminess of yogurt with a strong gel formation due to the interaction of iron to free amino acids produced during fermentation (Ziena and Nasser, 2019). Gaucheron et al. (1997) reported that iron interacts with casein and leads to specific change in its structure, and it is considered as the factor responsible for higher WHC/lower syneresis in iron fortified yogurts.

Table 1 Yogurt Quality characteristics

Sample	Acidity (% Lactic acid)	Syneresis (%)	WHC (%)	pH
RA+FPP-I	0.879±0.012 ^a	3.30±0.27 ^a	78.21±0.51 ^a	4.39±0.01 ^a
RA+FPP-II	0.892±0.020 ^a	3.27±0.24 ^a	78.30±0.56 ^a	4.39±0.02 ^a
RA+FPP-III	0.902±0.021 ^a	3.17±0.30 ^a	78.63±0.68 ^a	4.38±0.01 ^a
RA+FG-I	0.886±0.016 ^a	3.23±0.27 ^a	78.27±0.54 ^a	4.39±0.02 ^a
RA+FG-II	0.899±0.023 ^a	3.13±0.27 ^a	78.54±0.67 ^a	4.38±0.02 ^a
RA+FG-III	0.916±0.024 ^a	3.07±0.30 ^a	78.81±0.77 ^a	4.38±0.02 ^a
Control	0.879±0.012 ^a	3.33±0.27 ^a	77.99±0.54 ^a	4.40±0.02 ^a

The value represented in the table are the mean of three concordant readings and the value after ± is the Standard error mean
The different superscript alphabets ^{a-b} inferred a significant (P<0.05) difference in column.

Abbreviations: (Same for all tables and figures)

- RA+FPP-I = Retinyl acetate (2500 IU/L) and Ferric pyrophosphate (15 ppm)
- RA+FPP-II = Retinyl acetate (2500 IU/L) and Ferric pyrophosphate (20 ppm)
- RA+FPP-III = Retinyl acetate (2500 IU/L) and Ferric pyrophosphate (25 ppm)
- RA+FG-I = Retinyl acetate (2500 IU/L) and Ferrous gluconate (15 ppm)
- RA+FG-II = Retinyl acetate (2500 IU/L) and Ferrous gluconate (20 ppm)
- RA+FG-III = Retinyl acetate (2500 IU/L) and Ferrous gluconate (25 ppm)

Yogurt Microbiological indices

Fan et al. (2023) reported 46 volatile flavour compounds in yoghurt, and they belong to ketones, aldehydes, acids, aromatic compounds, alcohols, and carbonyl compounds. These flavouring compounds were generated by hydrolysis of fat and microbial bioconversion of citrate and lactose in yoghurt (Wang et al., 2022). To check the intensity of flavouring compound in yoghurt analysis of acetaldehyde is carried out. (Fan et al., 2023; Tian et al., 2020). The obtained results were at par (p<0.05) in setting time, acetaldehyde content, LB and ST count of all yogurt samples (Tab 2). However, it was observed that with increase in iron content and addition of vitamin A, increase in acetaldehyde content and LB and ST count, and decrease in gel setting duration. All three parameters are associated with microbial

growth, and as per observations, it can be seeming that vitamin A and iron fortification increased the growth rate of yogurt culture (LB and ST). Ziena and Nasser (2019) reported that count of both yogurt cultures *Streptococcus* and *Lactobacillus* was increased in iron fortified yogurt. Acetaldehyde is a flavouring metabolic product produced by yogurt culture (Gezginc et al., 2015). Iron fortification increased diacetyl content of yogurt (El-Kholy et al., 2011). Iron fortification also increased the count of yogurt culture bacteria (ElBehairy and Mohohamed, 2010), whereas non-significant difference was observed in lactic acid bacterial count in iron fortified and control yogurt (Simova et al., 2008). The gel setting time for yogurt samples also depends on the same factors as discussed in syneresis and water holding capacities. Ocak and Kose (2010) reported similar gel setting time for iron fortified yogurt and its control. It is also reported that

bacteria are living organism, and they require iron as an essential micronutrient, and with increase in iron increased viability, metabolic activity, and fermentation power was observed (Gaensly et al., 2011; Novin et al., 2020).

Table 2 Yogurt Microbiological indices

Sample	Acetaldehyde (mg/g)	Setting time (h)	ST (10 ⁶ cfu/ml)	LB (10 ⁶ cfu/ml)
RA+FPP-I	1.369±0.029 ^a	4.80±0.15 ^a	591.00±13.20 ^a	529.33±8.95 ^a
RA+FPP-II	1.370±0.028 ^a	4.73±0.15 ^a	595.33±13.91 ^a	533.67±9.82 ^a
RA+FPP-III	1.375±0.028 ^a	4.67±0.12 ^a	603.00±16.17 ^a	538.00±9.82 ^a
RA+FG-I	1.369±0.029 ^a	4.73±0.15 ^a	593.30±13.91 ^a	532.30±9.26 ^a
RA+FG-II	1.370±0.029 ^a	4.67±0.18 ^a	599.00±14.05 ^a	538.00±10.12 ^a
RA+FG-III	1.375±0.029 ^a	4.57±0.15 ^a	606.00±16.17 ^a	541.67±10.60 ^a
Control	1.368±0.028 ^a	4.83±0.15 ^a	586.33±12.81 ^a	527.67±8.41 ^a

The value represented in the table are the mean of three concordant readings and the value after ± is the Standard error mean. The different superscript alphabets ^{a-b} inferred a significant (P<0.05) difference in column.

Viscosity

The viscosity was measured at three different speeds of spindle of viscometer and, due to the increase in speed of the spindle, significantly (p<0.05) lower down viscosity of yogurt (Figure 3). Viscosity decreased with an increase in spindle speed. At each speed, the yogurt viscosity values of all samples were at par (p>0.05) with each other, respectively. However, it was observed that addition of iron and retinol acetate increased the viscosity in fortified samples as compared to control. Achanta et al. (2007) also observed similar results for the viscosity of iron fortified and control yogurt. However, Cavallini et al. (2009) observed no alteration in the viscosity of iron-fortified soy yogurt.

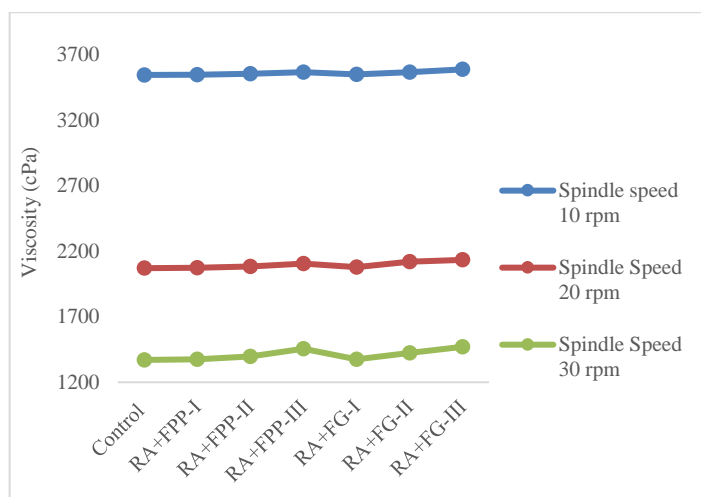


Figure 3 Viscosity trend of developed yogurt. The value represented in the figure are the mean of three concordant readings and the value after ± is the Standard error mean

Texture analysis

The texture analyser recorded four properties of yogurt, viz. stickiness, firmness, work of adhesion, and work of shear. Textural analysis revealed that firmness and work of adhesion were at par (p>0.05) with each other, respectively, except RA-FG-II and RA-FG-III yogurt. It was observed that Firmness increased, whereas work of adhesion decreased with fortification. The work of shear values for all yogurt samples were at par (p>0.05). However, in the case of stickiness, nutrients added yogurt reported a significant difference (p<0.05) from the unfortified yogurt. It was observed that stickiness increased with the addition of iron and retinol acetate (Tab 3). Textural results were in accordance with other quality characteristics of yogurt. Yogurt was firmer and cohesive, and the same trend was reported by Ocak and Kose (2010) in yogurt. The mechanism behind the increase in firmness is aggregation of protein matrix due to iron fortification (Sandoval-Castilla et al. 2004). Udenigwe et al. (2017) reported that the surface properties of peptides can affect their metal binding capacity. The hydrolysed casein and hydrolysates casein and hydrolysates have varying Fe²⁺ chelating capacities (0.049 to 0.134 mg.ml⁻¹). The negatively charged surface of the particles facilitated peptide-Fe²⁺ chelate complex formation via electrostatic interaction. When milk is converted to yoghurt, acidity increases which increases the negative charge and more negative charge increase the Fe²⁺-casein complex, which may be responsible for the increase in the hardness of yoghurt.

Table 3 Texture profile of developed yogurt

Sample	Stickiness (N)	Work of adhesion (N.s)	Firmness (N)	Work of shear (N.s)
RA+FPP-I	-0.268±0.010 ^a	-1.754±0.038 ^a	1.946±0.029 ^a	15.571±0.337 ^a
RA+FPP-II	-0.255±0.012 ^{ab}	-1.714±0.039 ^a	1.956±0.032 ^a	15.644±0.338 ^a
RA+FPP-III	-0.244±0.014 ^{bc}	-1.567±0.046 ^c	2.003±0.044 ^a	15.813±0.338 ^a
RA+FG-I	-0.258±0.013 ^{ab}	-1.734±0.039 ^a	1.952±0.029 ^a	15.582±0.334 ^a
RA+FG-II	-0.242±0.014 ^{bc}	-1.676±0.037 ^b	1.975±0.029 ^{ab}	15.663±0.334 ^a
RA+FG-III	-0.230±0.015 ^c	-1.497±0.049 ^c	2.026±0.038 ^b	15.856±0.337 ^a
Control	-0.280±0.012 ^a	-1.761±0.036 ^a	1.942±0.029 ^a	15.565±0.337 ^a

The value represented in the table are the mean of three concordant readings and the value after ± is the Standard error mean. The superscript alphabets ^{a-b} if different inferred a significant (P<0.05) difference in column.

CONCLUSION

Overall, iron and vitamin A fortified yogurt was developed. The milk was stable to heat after fortification and found suitable for yoghurt fortification. The eating quality of fortified yoghurt was acceptable and physicochemical properties were comparable to control yogurt. Fortification improves gel strength, water holding capacity, firm texture, and viscosity. All fortified samples exhibited comparable physico-chemical properties to control; therefore, the highest level of iron (25 ppm iron) was selected with 2500 IU of vitamin A as retinol acetate as fortificants. Multiple fortification has proven symbiotic effect; therefore, the fortified yogurt may be helpful in reducing deficiency of fortified nutrients.

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