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Rheological and physico-chemical attributes of lactose hydrolyzed micronutrients rich low lactose yoghurt

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Abstract
The study focuses on the development of low lactose yoghurt by lactose hydrolysis and fermentation process and fortification with micronutrients rich plant sources such as avocado, pumpkin seeds and lemon. The product was optimised for lactose hydrolysis and addition of avocado pulp powder (0.50%), pumpkin seeds powder (0.75%) and fresh lemon juice (0.75%) based on sensory quality. The developed product was analysed for rheology and physico-chemical characteristics; and nutritional quality. Degree of lactose hydrolysis was 54.80% at 0.30% lactase enzyme concentration and fermentation by yoghurt cultures brought down the lactose content to 1.00% (20 - 30% lowered). The lactose hydrolysed micronutrient rich yoghurt showed a significant improvement in the rheological properties such as firmness, consistency, cohesiveness, index of viscosity and apparent viscosity and physico-chemical properties enhanced by reduction in syneresis and setting time. The nutritional quality was improved with respect to fat, protein, minerals and vitamin C. The developed micronutrient rich (minerals and vitamins) low lactose yoghurt possesses superior sensory quality (sweetness and consistency) along with improved nutritional value which can attract lactose intolerant population.

Keywords: Low lactose yoghurt, lactose hydrolysed milk, rheological attributes, physico-chemical properties, micronutrients, nutritional value

Introduction
Milk is the liquid food, secreted by the mammary gland for the nourishment of the newly born containing water, lactose, fat, protein and the minor constituents. It has long been recognised as the most complete single food available in nature for the maintenance of health and promotion of growth' of the mammal. India stands 1st in the milk production, which contribute to 24% of the global milk production in the year 2021-22. India produced 230.58 MT (2022-23); 239.30 MT in 2023-24 which is higher by 6% as compared to the previous year's 221.06 million MT (Economic Survey 2023-24; GOI).

Lactose is a unique disaccharide in milk which is hydrolyzed by enzyme lactase present in the epithelial cells of brush board of small intestine. It is estimated that approximately 68% of world and 65% of Indian population are suffering lactose intolerance due to deficiency of lactase enzyme (Dominici *et al.,* 2022) [1]. β -galactosidase (EC 3.2.1.23) is the scientific name of lactase enzyme that catalyses the hydrolysis of lactose to glucose and galactose. In dairy industry, various applications of β-galactosidase enzyme have been reported like prevention of lactose crystallization, to increase the sweetness of the milk products, to produce low lactose food products and for cheese whey utilization by which water pollution can be controlled Kaur *et al*., 2017; Joon *et al*., 2018) [2,3]. The hydrolysis of lactose alters the carbohydrate composition, leading to the generation of prebiotic galacto-oligosaccharides (GOS), potentially influencing the viability and function of starter and probiotic cultures as well as various parameters determining yoghurt quality (Venica *et al*., 2016) [4]. Martins *et al*. (2014) [5] documented a tenfold rise in GOS levels and a fourfold reduction in lactose content in lactose hydrolyzed yoghurt compared to fermentation without enzyme supplementation. Lactase enzyme also used for the preparation of low lactose and lactose free dairy products. As per FSSAI standards Low Lactose or Lactose free milk means the product prepared from any type of milk (Species identified milk, Mixed Milk, Full Cream Milk) in which, lactose content has been reduced significantly through hydrolysis by enzymatic or any other appropriate process.

Low lactose milk" shall have less than 1% lactose and Lactose free milk" shall have less than 0.1% lactose. Lactose-free dairy can provide the essential nutrients present in regular dairy products, like calcium and vitamins, to those that are not able to digest lactose. This product category currently has a wide and growing health appeal to consumers. In recent years, the quality and product variety in the lactose-free dairy segment has been increasing significantly, giving consumers more tempting products to decide. As a result, lactose-free dairy is now the fastest growing market in the dairy industry.

The fermented food products enhance the metabolic activity and the composition of gut microflora. This helps maintain a healthy microbial balance in the gut, which is usually altered during gastrointestinal disorders, stress, use of antibiotics, and from other diseases. Yoghurt is an ancient fermented dairy product and has been consumed worldwide since the domestication of mammals (Tamime and Robisons, 2007) [6]. According to Food Safety Standards Authority of India, (FSSAI, 2021) yoghurt means a coagulated product obtained from pasteurised or boiled milk or concentrated milk, pasteurised skimmed milk and /or pasteurised cream or a mixture of two or more of these products by lactic acid fermentation through the action of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Yoghurt and other fermented milk products are rich in vitamins $\mathrm{A}, \mathrm{B}_1, \mathrm{B}_2, \mathrm{B}_6$, and B_{12} (Chandan, 2016) [7]. Lactic acid bacteria (LAB) have also been shown to possess the ability to synthesize B vitamins, such as folic acid during fermentation (Le Blanc *et al*., 2013) [8]. In general, the content of vitamins in yoghurt is higher than in milk.

A healthy and balanced diet is one that helps to maintain or improve overall health. Moreover, it was established that the ingredients of food, including nutritive and non-nutritive ingredients, can be used to modify the risk of developing or aggravating human illness conditions (Udenigwe and Aluko, 2012) [9]. The consumers' increasing awareness of the influence of diet on health is reflected in their selection of natural products, abundant in vitamins, minerals, and other bioactive compounds like carotenoids, anthocyanins, polyphenols or peptides especially after Covid 19 (Ahmed *et al*., 2023) [10]. Therefore, in order to attract the health-conscious customers, food producers must incorporate functional ingredients into their products.

Persea americana is commonly known as avocado, avocado pear, or butter fruit, which is native to Mexico and Central America. The term "superfood" refers to foods that are beneficial to human health due to their high levels of nutrients and/or bioactive phytochemicals such as antioxidants. In particular, avocado has recently gained dramatic popularity and is often referred to as a "superfood" because of its unique nutritional and phytochemical composition compared to other fruits. It is one of the few fruits in the human diet with a high content of both water-soluble and fat-soluble vitamins, health-related compounds (Duarte *et al*., 2016) [11]. Pumpkin seeds may be tiny, but they are densely packed with useful nutrients and nutraceuticals such as amino acids, phytosterols, unsaturated fatty acids, phenolic compounds, tocopherols, cucurbitacins and valuable minerals. All these bioactive compounds are important to a healthy life and well-being. They are high in vitamin E (tocopherols), carotenoids magnesium, potassium, phosphorus, as well as other minor minerals such as zinc, manganese, iron, calcium, sodium, and copper (Amin *et al*., 2019) [12]. Lemon is a citrus fruit belong to the species of small evergreen tree in the flowering plant family *Rutaceae*, which is native to Asia. Lemons contain approximately 10% carbs, which are mostly soluble fibers and simple sugars. Lemon extract contains 3 calories per 1 tablespoon and according to "World's Healthiest Foods a quarter cup of it contains 31% of recommended vitamin C intake and 3% of Folate and 2 percent of potassium which make up total 13 calories". With this background the study is designed to hydrolyse lactose in milk and prepare yoghurt with fortification of micronutrients through plant sources such as avocado, pumpkin seeds and lemon. The developed product was analysed for sensory, rheology, physico chemical and nutritional quality.

Materials and Methods

Materials

Yoghurt culture: Mixed culture of *Streptococcus thremophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* in the ratio 1:1 as brand name YO-MIX procured from Danisco Company was used as the yoghurt culture.

Avocado fruit powder: Avocado freeze-dried powder was purchased from Naturzo, Sigulda, Latvia, Europe.

Pumpkin seeds powder: Pumpkin seeds powder was purchased from Nutriwish, Cherlapally, Hyderabad, Thelangana- 500051.

Lactase enzyme: Lactase enzyme procured from DSM food specialities, Heelen, Netherland.

Lemon juice: The lemon is purchased from nearby local market. It is washed and cut into two pieces and squeezed and filtered to obtain juice.

Methodology

Preparation of lactose hydrolysed Milk

Homogenised cow milk (3.5% fat and 8.5% SNF) was heated to 90 ± 1ºC for 10 min. followed by cooling to 40 ± 1°C. Addition of lactase enzyme (DSM food specialities, Heelen, Netherland) at 3 different concentrations i.e 0.20, 0.30, 0.40% and mixed it for 40ºC for 1 hr for the preparation of lactose hydrolysed milk. After the incubation period, lactose hydrolysed milk was analyzed for degree of hydrolysis (Nickerson *et al*., 1976) [13].

Preparation of lactose hydrolysed yoghurt

The starter culture of *Streptococcus ssp thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* (YO-MIX procured from Danisco Company) were added to lactose hydrolysed milk in the ratio of 1:1 inoculated at the rate of 1.50%. The mixture was packed in poly propylene cups and incubated at 42±1°C for 4-5 hrs followed by storage at 5 ± 1°C.

Preparation of micronutrients rich yoghurt using lactose hydrolysed milk

Value added lactose hydrolysed yoghurt was prepared by incorporation of avocado fruit powder, pumpkin seed powder and lemon extract is presented in Fig 1 (flow chart). The ingredients were homogenized at 1000 rpm and then pasteurized at 90 ± 1ºC /10 min. The mixture was cooled rapidly to 42°C, inoculated with yoghurt cultures, packed in poly propylene cups and stored at 5 ±1°C.

Sensory evaluation

Optimisation of different levels of lactase enzyme, avocado fruit powder, pumpkin seeds powder and lemon extract by semi trained judges on a 9-point hedonic scale. The samples for evaluation were coded appropriately before serving the samples to the judges for sensory evaluation. The panellists were requested to grade the sample based on sensory attributes: colour and appearance, flavour and overall acceptability.

Texture profile analysis

Texture profile analysis parameters were determined by using the Micro System TA-XT2-Texture analyzer (UK) fitted with suitable aluminum probe (P/25) to analyze force. A stainless-steel probe 25 mm diameter with flat end was inserted to a depth of 1 cm. Various textural parameters were determined which includes firmness, consistency, cohesiveness and index of viscosity. The firmness is the maximum peak force (g) required during the penetration and the unit is Newton (N). The consistency is the area of the positive curve and is given by force x time (g. sec). The cohesiveness is the maximum negative peak force (g) and index of viscosity is the negative curve area and is given by force x time (g sec).

Apparent viscosity

Viscosity is the property of fluid having resistance to a change in shape, or movement of neighboring portions relative to one another. Viscosity of the developed yoghurt will be evaluated by Lamy viscometer (B-one plus LR module). In this viscometer low viscosity (LV), medium and high viscosity (RV) using different spindles. Spindle can be varied based on the product characteristics with ideal temperature for measurement (25 °C).

Physico-chemical analysis

The effect of addition of avocado fruit powder, pumpkin seeds powder, fresh lemon juice and final developed product were studied in lactose hydrolysed yoghurt. The physico-chemical parameters, *viz*. titratable acidity (IS: SP 18 (Part XI) 1981), syneresis (Nagaraj *et al*., 2009) [14] and setting time were analysed in the samples.

Statistical analysis

Experimental data obtained in the study was analysed by Randomized Column Block design as per the method described by Snedecor and Cochran (1991) [15] to test for 'F' values to know the statistical significance. Critical Difference (CD) value was calculated to determine whether the treatment means were similar or not. The results are the average of three replications and were statistically analysed by subjecting to statistical analysis using R Programme, R - Version 4.0.3 for accurate interpretation that helped in discussion.

Results and Discussion

Lactose hydrolysed micronutrients rich low lactose yoghurt

Lactase enzyme was incorporated into cow milk at concentrations of 0.30% showed lactose hydrolysis of 54.80% after incubation for one h at 40ºC. Upon fermentation using yoghurt cultures, the lactose content in the yoghurt further reduced by breakdown propose and resulted in 1.00% lactose. The sensory quality of low lactose yoghurt obtained optimum scores as against other levels of lactose concentration (0.20 and 0.40%) as observed by Anjana (2024) [16].

These findings align with previous research by Nagaraj *et al*. (2009) [14], who investigated lactase enzyme concentrations ranging from 0.12% to 0.56% at 0.04% intervals, achieving a degree of lactose hydrolysis ranging from 46.3% to 90.06%. Additionally, results from Sharanagouda *et al*. (2022) [17] indicated that lactase enzyme concentrations of 0.16 ml/l, 0.24 ml/l,.32 ml/l, and 0.40 ml/l of milk resulted in lactose hydrolysis rates of 60.48%, 65.69%, 70.85%, and 78.21%, respectively.

The lactose hydrolysed yoghurt enriched with avocado fruit powder (0.25, 0.50 and 0.75%), pumpkin seeds powder (0.50, 0.75 and 1.00%) and fresh lemon juice (0.50, 0.75, and 1.00%) were analysed for sensory, rheology and physico chemical attributes. The lactose hydrolysed developed micronutrient rich low lactose yoghurt with 0.50% avocado fruit powder, 0.75% pumpkin seeds powder, 0.75% lemon juice were analysed for the same attributes. The results obtained are presented and discussed below.

Sensory quality

The lactose hydrolysed yoghurt enriched with avocado fruit powder at levels of 0.25%, 0.50%, and 0.75% was evaluated for its sensory attributes, as depicted in Fig. 2a. The sensory analysis revealed that the mean scores for sensory attributes, including colour and appearance, body and texture, flavour, and overall acceptability (8.80, 8.76, 8.56 and 8.56, respectively), were significantly higher for yoghurt fortified with 0.50% avocado fruit powder compared to both unhydrolyzed (7.43, 7.55, 7.07 and 7.18, respectively) and hydrolysed control samples (7.71, 8.21, 8.31 and 8.10, respectively). This enhancement in sensory attributes could be attributed to the higher solid content, which imparts a glossier and creamier appearance to the product, as well as the increased fat content from avocado, contributing to its creamy texture and flavour. The elevated scores for colour and appearance; and flavour in avocado yoghurt are consistent with the findings of Atmanaji *et al*. (2019) [18], who demonstrated that the addition of avocado fruit pulp at a concentration of 7.5% resulted in higher scores (6.73 and 6.63, respectively) as compared to control (5.80 and 5.93, respectively).

Lactose-hydrolyzed yoghurt fortified with pumpkin seeds powder at concentrations of 0.50, 0.75 and 1.00% was assessed for its sensory attributes, as illustrated in Fig. 2b. The sensory evaluation revealed that the mean scores for all sensory attributes of lactose-hydrolyzed yoghurt, including colour and appearance, body and texture, flavor, and overall acceptability (8.53, 8.80, 8.51 and 8.36, respectively)), were significantly higher for the sample fortified with 0.75% pumpkin seed powder compared to both unhydrolyzed and hydrolyzed control samples. This improved sensory profile associated with the addition of 0.75% pumpkin seeds powder may be attributed to the enhanced shiny and firm gel appearance it imparts to the product, along with an increase in total solids that contributed to a pleasant nutty taste. These findings agree with the results reported by Robert (2018) [19], who observed that yoghurt fortified with 10%

pumpkin seeds achieved higher scores (4.06) as compared to the control (3.93).

Lactose-hydrolysed yoghurt fortified with lemon extract at concentrations of 0.50, 0.75 and 1.00% was evaluated for its sensory attributes, as depicted in Fig. 2c. The sensory analysis revealed that the mean scores for all sensory attributes (8.61, 8.63, 8.40 and 8.47, respectively)) were significantly higher for yoghurt fortified with 0.75% lemon extract compared to both unhydrolyzed and hydrolysed control samples. This elevated score for colour and appearance in the presence of 0.75% lemon extract is attributed to its ability to impart a shiny and firm gel appearance to the product. Additionally, the improved body and texture may be attributed to the accelerated growth of starter microbes compared to the control. Furthermore, the addition of 0.75% lemon extract imparted a pleasant tangy taste to the product due to the presence of citric acid. These findings are consistent with the results reported by Bhat *et al.* (2016) [20], who incorporated lemon extract into curd at concentrations of 2.50%, 5.00%, and 7.50%. They observed that the 5% lemon extract concentration scored significantly higher in colour and appearance (7.83), flavour (7.32), body and texture (7.96), and overall acceptability (7.84) as compared to the control (7.67, 6.58, 7.89 and 7.49, respectively).

Hence, the lactose-hydrolysed yoghurt fortified with 0.50% avocado fruit powder, 0.75% pumpkin seeds powder, and 0.75% lemon extract levels were optimised and evaluated for its sensory attributes, as illustrated in Fig. 2d. The sensory analysis indicated significantly higher mean scores for all sensory attributes (8.53, 8.53, 8.50 and 8.45, respectively) compared to both unhydrolyzed and hydrolysed control samples. The resultant product exhibited a glossy, creamy appearance, suggesting an enhancement in visual appeal. The improved body and texture of the yoghurt can be attributed to the higher content of total solids, fat, soluble fiber, protein, and ascorbic acid present in avocado fruit powder, pumpkin seeds powder, and lemon extract. The flavour profile of the final product, which encompasses the sweetness from the lactase enzyme, creamy richness from avocado, nutty undertones from pumpkin seeds, and tanginess from lemon extract, contributed to its superiority over the control. These findings are consistent with the results of Guo (2021) [21], who investigated the incorporation of 10% avocado pulp with 2% ascorbic acid into yoghurt. The author observed that 2% ascorbic acid effectively preserved the colour of avocado in yoghurt, mitigating enzymatic browning, and reported higher sensory scores for flavour and colour appearance.

Rheological characteristics: Textural properties and Apparent viscosity

Rheological properties influence sensory property and ultimately acceptance of product by final consumer**.** Rheological properties of yoghurt are influenced by solid content, fats and proteins content, temperature, and milk composition (Prajapati *et al*., 2016) [22]. The rheological properties of value-added yoghurt (avocado, pumpkin seeds, lemon and final developed combination product) were studied using Texture profile analyser for parameters *viz.* firmness, consistency, cohesiveness and index of viscosity and Brookfield Viscometer for apparent viscosity which is depicted in Fig. 3 (a to d).

Firmness and Consistency

Firmness, a critical parameter in evaluating yoghurt quality, refers to the maximum force required to induce a specific deformation or the peak force during the initial compression cycle. It is closely linked to the total solid content of yoghurt and significantly influences its texture. In this study, all treatments (avocado, pumpkin seeds, lemon and final developed combination product) exhibited a noteworthy increase in firmness values (N) of 1.30, 1.22, 1.18 and 1.67, respectively as compared to the hydrolysed control (1.17) and hydrolysed control (0.94), which might be likely attributed to the rise in total solids resulting from fortification. Notably, among all treatments, developed final product demonstrated superior firmness in yoghurt and yoghurt fortified with avocado fruit powder, owing to its elevated total solids and fat content.

Furthermore, consistency, reflecting the internal bond strength of food, holds substantial importance in consumer preference. In comparison to the unhydrolyzed control (84.66) and hydrolysed control (91.66), all treatments *viz.* avocado, pumpkin seeds, lemon and final developed combination product demonstrated a significant increase in consistency (g.sec) of 111.0, 109.71, 106.33 and 122.33, respectively. This enhancement in consistency is consistent with the findings of Ahmed *et al.* [10], who highlighted that the addition of pumpkin seeds (20%) contributed to higher consistency due to its rich dietary fiber content and high water-holding capacity. For instance, the consistency of yoghurt fortified with pumpkin seeds was reported as 1056.62±1.05 g.sec, whereas the control exhibited a consistency of 1022.45±1.08 g.sec.

Cohesiveness and Index of viscosity

Cohesiveness, initially defined as the ratio of the positive force area during the second compression cycle to that during the first compression cycle (area 2/area 1), serves as an indicator of the internal strength of a product and is crucial in evaluating yoghurt quality. In this study, a significant increase in cohesiveness (N) was observed for all treatments avocado, pumpkin seeds, lemon and final developed combination product (-38.16, -29.33, -25.76 and -38.66, respectively) as compared to the control (hydrolysed, -24.33; unhydrolyzed, -15.70). This finding aligns with the observations of Al-Zahraa (2020) [23], who noted higher cohesiveness values in yoghurts containing pumpkin powder as compared to the control. This increase in cohesiveness can be attributed to the high viscosity contributed by pumpkin powder. Additionally, the higher fiber content of avocado fruit powder and pumpkin seeds powder may enhance the bonding between sugar and casein molecules, thereby strengthening the compact structure of yoghurt, resulting in a cohesive structure.

The index of viscosity (g.sec), a measure of the temperature influence on the product's viscosity, exhibited a significant increase for avocado, pumpkin seeds, lemon and final developed combination product (-16.96, -12.63, -13.55 and -17.93, respectively) as compared to the control (unhydrolysed, -13.10; hydrolysed, -12.43) This finding is in agreement with the results reported by Ahmed *et al.* (2023), who observed that the index of viscosity of yoghurts increased with the addition of pumpkin powder (123.34±0.61 g.sec) compared to the control (106.32±0.34 g.sec).

Apparent viscosity

Yoghurt, characterized as a gel comprising water-entrapped caseinate micelles, exhibited a significant increase in apparent viscosity (cP) for all treatments is given in Fig. 4 such as avocado, pumpkin seeds, lemon and final developed combination product (73.20. 74.80, 74.80. 70.00 and 96, respectively) as compared to the unhydrolyzed control (40.66) and hydrolysed control (64.30). This finding is consistent with the results reported by Yamamoto *et al.* (2021) [24], where lactose-hydrolyzed yoghurt demonstrated higher viscosity (1400 mPa.s) compared to control yoghurt (1000 mPa.s). The augmentation in apparent viscosity can be attributed to the increased cell count of *L. bulgaricus*, leading to higher concentrations of EPS (Exopolysaccharides), which consequently enhances the apparent viscosity.

Among the treatments, the addition of pumpkin seeds exhibited a notable influence on increasing apparent viscosity compared to others. This observation is corroborated by the findings of Barakat and Hassan (2017) [25], who demonstrated the positive impact of pumpkin on stirred yoghurt texture. It is postulated that the presence of available carbohydrates, fiber, and pectic substances in pumpkin enhances the structural integrity of the curd network in stirred yoghurt, thus augmenting viscosity. Furthermore, Atmanaji *et al.* (2019) [18] illustrated that the addition of avocado pulp to cow milk yoghurt at varying concentrations (2.5%, 5%, 7.5%) led to viscosity improvements (control: 173.5 cP, 2.5%: 205.37 cP, 5%: 228.23 cP, 7.5%: 230.87 cP). This viscosity enhancement is attributed to the higher concentration of total solids added, contributing to increased viscosity in cow's milk yoghurt. Consequently, the optimized final product exhibited a higher apparent viscosity than the control yoghurt.

Physico-chemical characteristics

The lactose hydrolysed yoghurt with 0.50% avocado fruit powder, 0.75% pumpkin seeds powder, 0.75% lemon juice and combination in developed product were subjected for analysis of physical characteristics, comparing with unhydrolyzed and hydrolysed control samples. The results obtained are presented in Table 1 for titratable acidity, syneresis and setting time of developed yoghurt along with the control.

Acidity

The titratable acidity for unhydrolyzed and hydrolysed control yoghurt were 0.62 and 0.67% LA. The avocado, pumpkin seeds, lemon incorporated and final developed combination yoghurt showed significant increased acidity of 0.79, 0.78, 0.82 and 0.72% LA, respectively. Addition of lemon juice increased acidity due to citric acid but the developed product had lower acidity among the treated samples. Statistical analysis shows that there was a significant difference ($P \leq 0.05$) observed for the lemon added yoghurt as compared to control.

Syneresis

The unhydrolyzed and hydrolysed control yoghurt showed average syneresis value of 27.46 and 29.50 ml/100 g and that of avocado, pumpkin seeds, lemon incorporated yoghurt and final developed combination yoghurt showed decreased syneresis value of 25.90, 26.06, 26.23 and 21.43 ml/100 g, respectively. The developed yoghurt showed statistically significant decrease ($P \leq 0.05$) in the syneresis as compared to other treatments and control.

Setting time: The setting time of yoghurt for unhydrolyzed and hydrolysed control was 224.67 and 191.00 min. The avocado, pumpkin seeds, lemon incorporated yoghurt and final developed combination yoghurt showed decreased setting time value of 178.33, 175.00, 126.67 and 154.33 min., respectively. The addition lemon and developed yoghurt had a significant effect ($P \leq 0.05$) on decreasing the time required to set yoghurt as compared to yoghurt and other treatments.

Gross composition of developed yoghurt

The gross composition of optimized lactose hydrolysed yoghurt with 0.50% avocado fruit powder, 0.75% pumpkin seeds powder, 0.75% lemon juice and its comparison with both controls were analysed for total solids, fat, protein, ash, vitamin C are delineated in Table 2 are discussed below.

An augmentation in total solid content was observed with the incorporation of avocado fruit powder, pumpkin seed powder and developed product. In a study conducted by Dangal *et al.* (2021) [26], investigating the addition of avocado pulp to cow milk yogurt at various concentrations, the total solid content was recorded as 16.88% for the control sample and 17.92% for the optimized sample. Similarly, findings from Hettige *et al.* (2022) [27] revealed that the total solids content of control yogurt was 22.29±0.13%, whereas the addition of 10% avocado fruit powder increased the total solids to 24.76±0.41%. In a study by Robert (2018) [19], it was found that the total solids in pumpkin seed yogurt (10%) were higher (24.54±1.32%) compared to plain yogurt (18.16±0.68%).

An increase in fat content was observed with the fortification of avocado fruit powder and pumpkin seeds powder as compared to the control. This can be attributed to the high-fat content of avocado, which significantly contributes to the fat content of yogurt. This finding is consistent with the report by Dangal *et al.* (2021) [26], who noted fat contents of 2.89% for the control sample and 3.68% for avocado fruit-added yogurt. Similarly, Hettige *et al.* (2022) [27] reported an increase in fat content with the addition of 10% avocado fruit to yogurt (4.08±0.14%) compared to the control (2.91±0.14%). Robert [19] documented fat contents of 3.06±0.45% for plain yoghurt and 4.04±0.26% for pumpkin seed yogurt.

A notable difference in protein content was observed in yogurt with the inclusion of avocado fruit powder and pumpkin seeds powder. pumpkin seeds, being rich in protein, significantly contributed to the protein content of yogurt. This finding aligns with the research by Robert (2018) [19], who reported protein contents of 5.01±0.33% for plain yoghurt and 5.71±0.52% for pumpkin seed yoghurt. In the present study, the optimized yoghurt with added avocado fruit powder exhibited a protein content of 4.11%. Similarly, Hettige *et al.*2022) [27] reported protein contents of 4.11±0.05% for avocado yoghurt and 3.78±0.04% for the control samples. Therefore, the developed product demonstrated a higher protein content.

An augmentation in ash content was observed with the fortification of avocado fruit powder, pumpkin seeds powder, and lemon juice as compared to control. Dangal *et al.* (2021) [26] investigated the addition of avocado pulp to cow milk yoghurt at various concentrations and reported ash contents of 0.71±0.14% for the control sample and 0.78±0.12% for the optimized sample. Similarly, Hettige *et al*. (2022) [27] also reported an increase in ash content with the addition of avocado fruit to yoghurt. Robert (2018) [19] reported ash contents of 1.46±0.50% for pumpkin seed yoghurt and 0.93±0.11% for plain yogurt. Therefore, the developed product in the present study exhibited a higher ash content as compared to both control samples.

An elevation in vitamin C content was observed with the fortification of avocado fruit powder, lemon juice and developed yoghurt (0.04. 0.05 and 0.07 g/L, respectively) compared to the control (0.02 g/L). Both lemon and avocado fruit are rich sources of vitamin C, which contributed significantly to the fortified yoghurt.

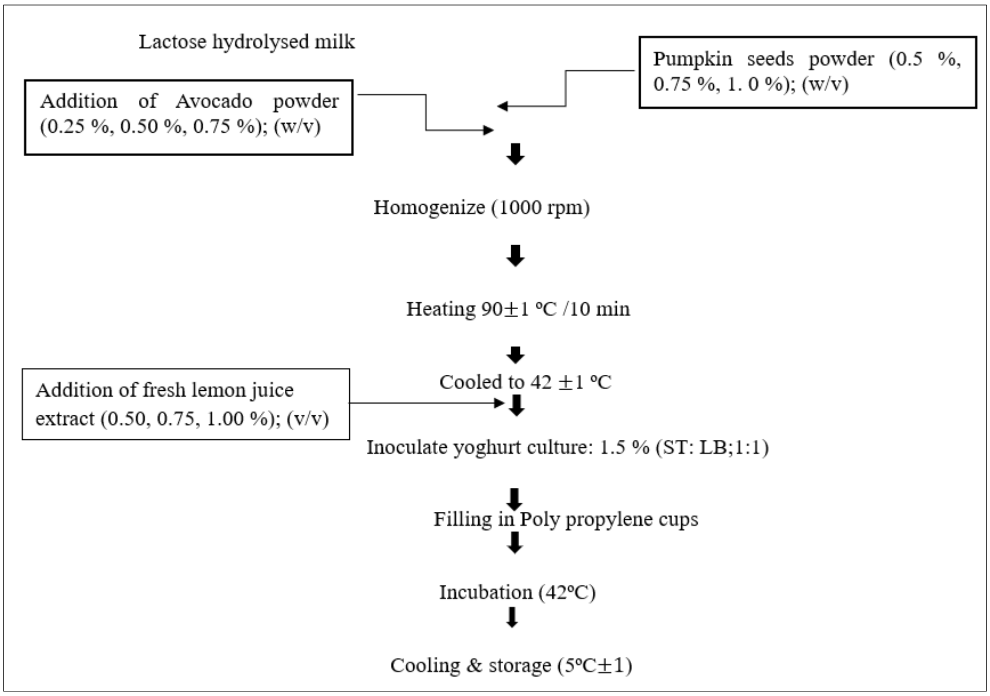

Fig 1: Preparation of micronutrient rich yoghurt using lactose hydrolysed milk

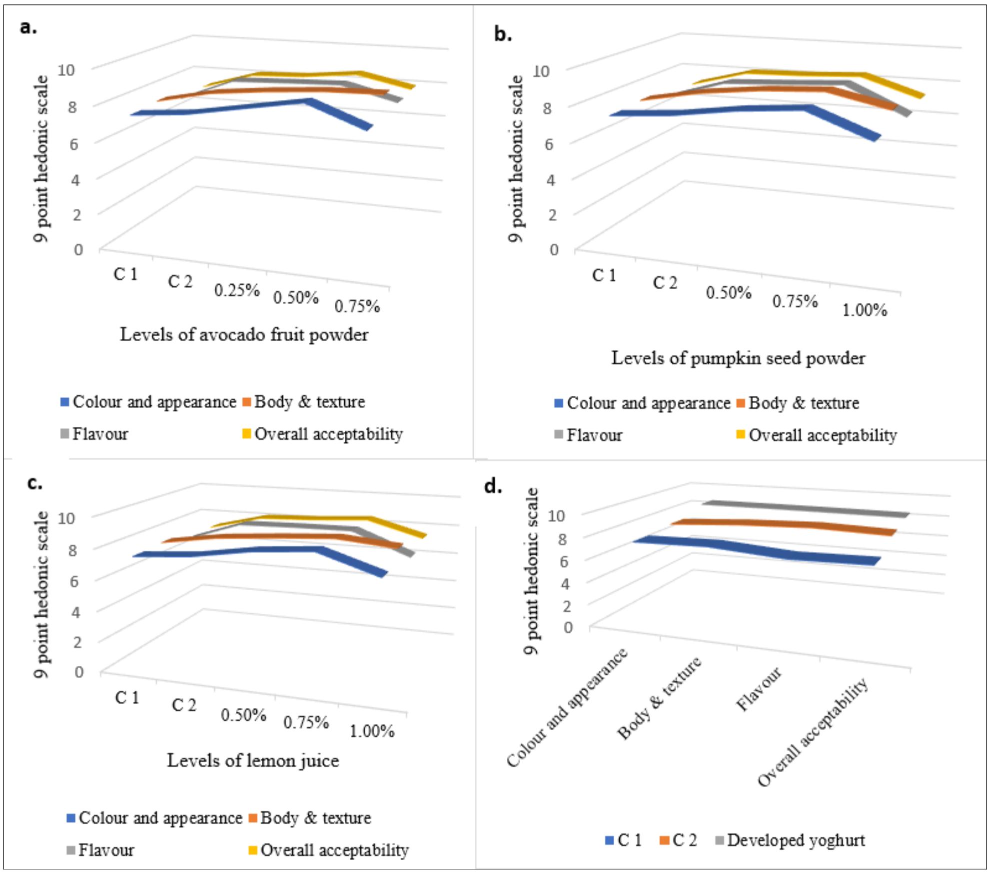

Fig 2: Sensory attributes of lactose hydrolysed micronutrient rich yoghurt with, a. Avocado fruit powder b. Pumpkin seed powder c. Lemon juice d. Developed yoghurt, C1: Unhydrolyzed control. C2: Hydrolysed control

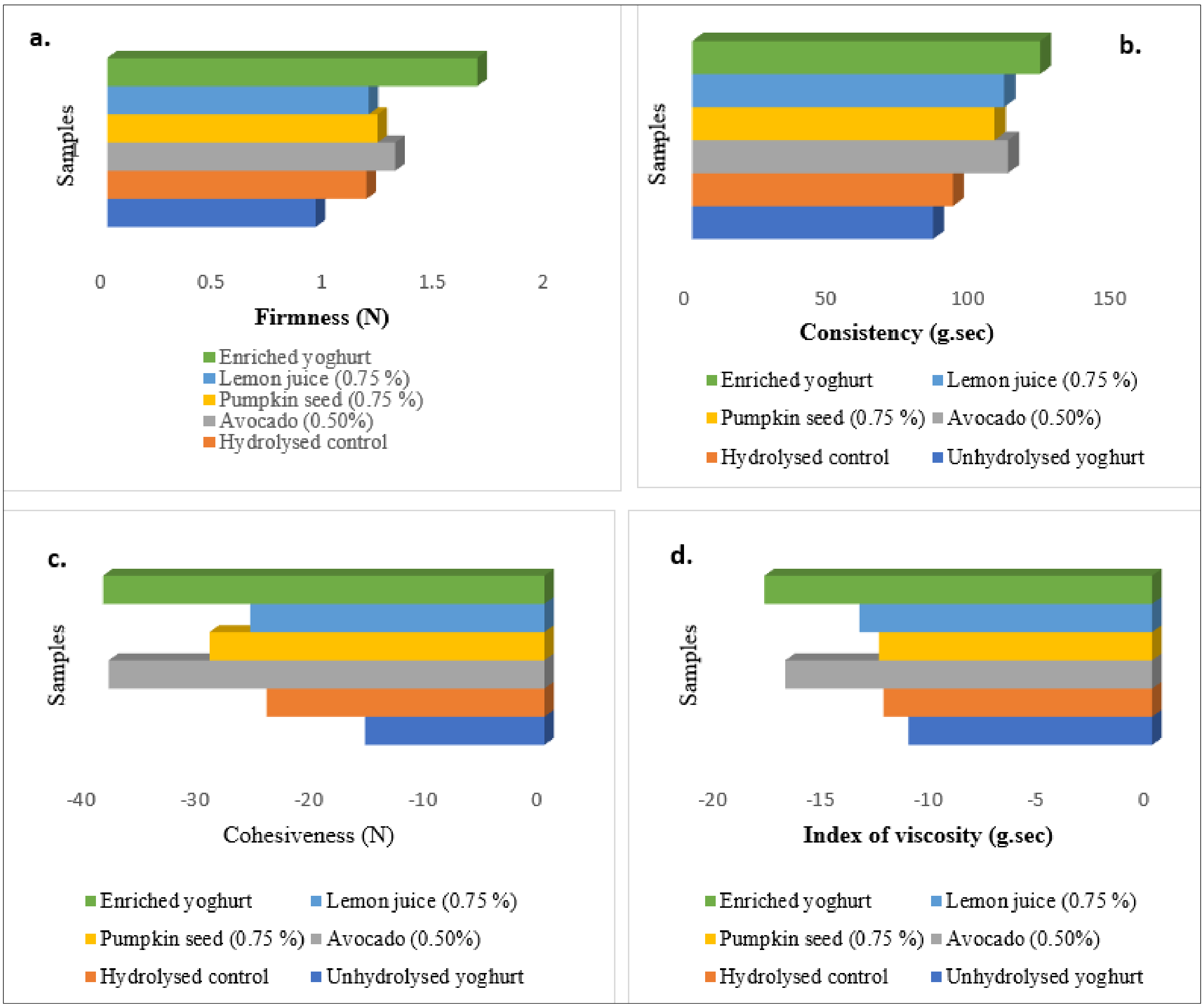

Fig 3: Effect of fortification on rheological characteristics of low lactose yoghurt samples, a. Firmness b. Consistency c. Cohesiveness d. Index of viscosity

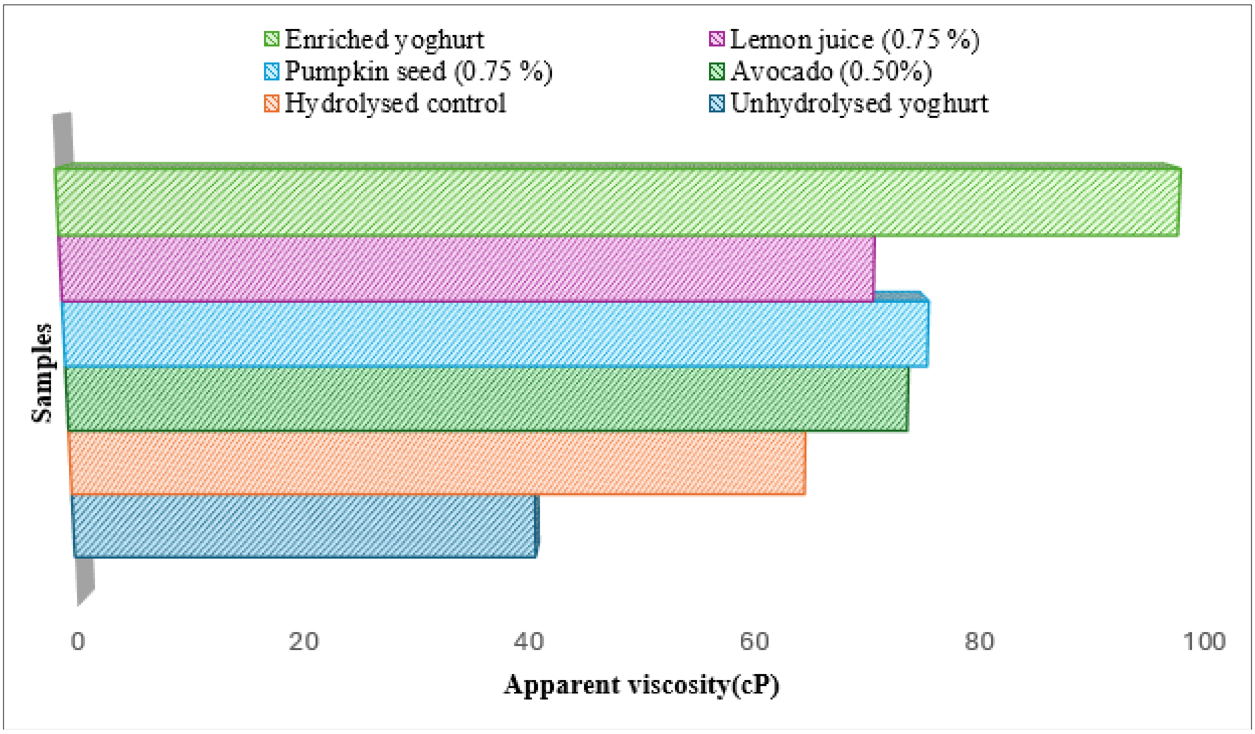

Fig 4: Effect of fortification on apparent viscosity of the yoghurt samples

Table 1: Physico-chemical characteristics of micronutrient rich low lactose yoghurt

Samples	Titratable acidity (%LA)	Syneresis (ml/100 g)	Setting time (min)
Yoghurt			
Control 1	0.62[a]	27.46[b]	224.67[e]
Control 2	0.67[a]	29.50[c]	191.00[d]
Avocado (0.50%)	0.79[ab]	25.90[b]	178.33[c]
Pumpkin seeds powder (0.75%)	0.78[ab]	26.06[b]	175.00[c]
Lemon juice (0.75%)	0.82[b]	26.23[b]	126.67[a]
Developed product	0.72[ab]	21.43[a]	154.33[b]
CD(P≤0.05)	0.12	1.60	10.62

Note

All values are average of three trials.

Similar superscripts indicate non-significant at the corresponding critical difference.

Control 1: Lactose unhydrolyzed control

Control 2: Lactose hydrolysed control

Table 2: Gross composition of micronutrient rich low lactose yoghurt

Constituents (%) / Samples	Total solids	Fat	Protein	Ash	Vitamin C (Per L)
Control 1	15.08±0.22[a]	3.53±0.03[a]	3.89±0.06[a]	0.84±0.03[a]	0.02±0.01[a]
Control 2	15.21±0.26[ab]	3.53±0.03[a]	3.89±0.06[a]	0.86±0.03[a]	0.02±0.01[a]
Avocado (@ 0.50%)	16.07±0.07[b]	4.23±0.06[b]	4.11±0.06[ab]	1.09±0.05[b]	0.04±0.02[ab]
Pumpkin seeds (@ 0.75%)	16.20±0.15[b]	3.86±0.08[a]	4.37±0.07[bc]	1.16±0.03[b]	0.02±0.01[a]
Lemon juice (@ 0.75%)	15.31±0.19[ab]	3.53±0.06[a]	3.92±0.06[a]	1.03±0.06[ab]	0.05±0.03[bc]
Developed product	17.26±0.27[c]	4.45±0.11[b]	4.59±0.08[c]	1.48±0.04[c]	0.07±0.03[c]
CD(P≤0.05)	0.52	0.17	0.16	0.11	0.04

Note

Data is presented as mean ± SEM (n = 3)

Similar superscripts indicate non-significant at the corresponding critical difference

Control 1: Lactose unhydrolyzed control

Control 2: Lactose hydrolysed control

Conclusion

Based on the findings of the current study, it is deduced that the enzymatic breakdown of lactose in milk experienced a notable decrease in lactose as the concentration of lactase enzyme increased, resulting in a reduction of lactose content in yoghurt. This phenomenon also exerted discernible effects on the sensory and physicochemical properties. To enhance the lactose-reduced milk, micronutrient-rich natural additives such as avocado fruit powder, pumpkin seed powder, and lemon juice were incorporated into the optimized formulation. This enrichment markedly ameliorated both the sensory attributes and rheological properties of the yoghurt when compared to the control sample. Consequently, this lactose-reduced and enriched yoghurt presents a viable option for individuals with lactose intolerance, providing essential nutrients.

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