

Research Article

Development of Dairy-Free Functional Yoghurt from Chickpea Milk Fortified with Acacia Gum

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ABSTRACT

Plant-based yoghurt alternatives require a hydrocolloid to compensate for the weak gelation of legume milks, while the efficacy of conventional dairy starters on a lactose-free substrate remains underexplored. This study characterised a dairy-free yoghurt produced from chickpea (*Cicer arietinum*) milk fortified with acacia gum at 0, 2 and 4%, and fermented at 42–45 °C with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, either singly or in co-culture, in nine formulations and sampled at 0, 24 and 48 h. Acidification was depended on the starter culture in a time-dependent manner. At 24 h, the single-strain *S. thermophilus* formulation reached pH 4.89, compared with pH 5.36 for the *L. bulgaricus* formulation, an order consistent with the sucrose-based carbohydrate profile of chickpea milk. At 48 h, the ranking reversed and *L. bulgaricus* at 4% gum recorded the lowest pH (4.00). Apparent viscosity rose from 155–163 mPa·s at 0 h to 1110–2125 mPa·s at 48 h and increased with gum level, in which the 4% co-culture formulation being the most viscous (2125 mPa·s). Measured protein increased from 0.99 to 2.15% and fat from 0.39 to 2.02%, although the gum-free control showed no significant change in protein, indicating that these increases reflect concentration of the matrix and the composition of the gum rather than microbial synthesis. Two-way ANOVA showed significant effects of gum level, starter culture, and their interaction for every response, with gum level accounting for 74 to 90% of the variance in viscosity, protein and fat. Acacia gum at 2 to 4% improved consistency, while the starter culture determined the rate and the eventual extent of acidification.

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1. Introduction

In recent years, the demand for alternatives to dairy products such as yoghurt from plant-based has risen, which largely driven by health concerns, environmental awareness, and ethical considerations (Mefleh *et al.*, 2022). For example, cases of food allergies and lactose intolerance linked to cow-based dairy products are widespread, particularly in Central America, Western Asia, South America, North Africa, and Eastern Europe (Arasi *et al.*, 2025). Further, awareness of the overexploitation of water, land, and feed required for breeding and production of ruminants and dairy, compounded by its negative impact on greenhouse

gas emissions, has shifted consumers' choice toward plant-based products. These pressures have created a market for plant-based yoghurt that delivers comparable nutritional and functional benefits to dairy yoghurt.

To develop plant-based yoghurt, base ingredients such as soy, coconut, almond, and oat are commonly used. Recently, chickpea (*Cicer arietinum*) has become a popular choice for this purpose in yoghurt. Among common pulses, chickpea provides a high protein content with a relatively balanced amino acid profile, together with dietary fibre, folate and minerals such as calcium, magnesium, phosphorus and potassium (Kishor *et al.*, 2017; Rincon *et al.*, 2020). It is inexpensive, widely available, and free of the two most prominent dairy allergens, lactose and casein. Unlike some legume beverages, chickpea carries a relatively little of the beany off-flavour. However, its main technological drawback is a weak gel-forming ability: the chickpea-milk base does not set into a firm gel on its own and tends to form a thin, watery product that is prone to phase separation and syneresis. To improve gelation, acacia gum was added as a natural hydrocolloid thickener. Acacia gum (gum arabic) is a branched arabinogalactan in which arabinose and galactose chains are attached to a small protein core, the arabinogalactan-protein complex. It functions as a thickener and emulsion stabiliser in dairy and plant-based products (Mugo *et al.*, 2020) and acts as a prebiotic that supports the growth of beneficial gut bacteria and short-chain fatty acid production (Rawi *et al.*, 2021). Chickpea hulls themselves contain arabinan-rich pectic polysaccharides (Sathyanarayana & Harish Prashanth, 2019), therefore the added polysaccharide is compatible with the base material.

Although the physicochemical properties of plant-based yoghurts from soy, coconut and oat are well documented, the same is not the case with chickpea-milk yoghurt fortified with acacia gum. This study aimed to develop and characterise a dairy-free chickpea-milk yoghurt fortified with acacia gum based on its acidification, viscosity, and protein and fat content, by evaluating the effect of acacia gum level and of the single-strain and co-culture fermentation with *S. thermophilus* and *L. bulgaricus* on the said properties. Two factors were adjusted in this study. First, the incorporation of acacia gum was examined at three level concentration (*i.e.*, 0, 2 and 4%). This is because the amount of hydrocolloid that governs the balance between adequate thickening and possible interference with acidification, and their effective level for a chickpea base has not been established. Second, the starter culture was also varied, by applying *S. thermophilus* and *L. bulgaricus* either singly or in co-culture. This second factor is critical because the performance of these two yoghurt starters cannot be assumed to be automatically transferable from milk to a legume base. Chickpea milk contains no lactose and its principal free sugar is sucrose and raffinose-family oligosaccharides (Jukanti *et al.*, 2012). Conversely, *L. delbrueckii* subsp. *bulgaricus* has undergone reductive genome evolution tailored for lactose utilisation within the dairy product niche (El Kafsi *et al.*, 2014).

2. Materials and Methods

2.1 Chickpea Milk Preparation

Chickpea milk was prepared as described by Vallath *et al.* (2021). Dried chickpeas (type: Kabuli) were soaked in water for 8 to 12 h, rinsed in fresh water, then blended with water at a 1:4 (w/v) ratio until a smooth slurry was obtained. The slurry was filtered through muslin cloth, and the filtrate was heated at 90 °C for 10 min to inactivate endogenous enzymes that could cause deterioration or off-flavours. The milk

was cooled to room temperature and stored at 4 °C until use. The milk was gently blended before each analysis to resuspend any sediment and was brought to room temperature.

2.2 Fortification with Acacia Gum

Acacia gum (food grade) was added to 250 mL of chickpea milk at two concentration levels, 2% and 4%, corresponding to approximately 5 g and 10 g of gum, respectively. The unfortified milk (0% gum) served as the control. The gum was first hydrated in a small volume of chickpea milk at a gum-to-milk ratio of about 1:3 to reduce batch-to-batch inconsistency (Ward, 2009), stirred until it formed a lump-free slurry, left to hydrate for about 15 min, then stirred again and added slowly to the remaining chickpea milk with continuous stirring until fully dispersed.

2.3 Starter Culture Preparation

Freeze-dried cultures of *S. thermophilus* and *L. bulgaricus* were rehydrated according to the supplier instructions in a 2% (w/v) saline solution. The rehydration medium was mixed with the freeze-dried culture at a 1:4 ratio and incubated at 23 °C for 10 min (Gardner *et al.*, 2001). To prepare the inoculum for single-strain and co-culture fermentation, 100 µL of each strain was inoculated into 5 mL of sterile MRS broth (for *L. bulgaricus*) or M17 broth (for *S. thermophilus*) and incubated at 37 °C. After 16 h the cells were harvested, transferred to 50 mL of the corresponding broth and incubated for a further 16 h, then transferred to 500 mL of the same medium and incubated under the same conditions (Santos *et al.*, 2014). The working inoculum was standardised to approximately 1×10^6 CFU/mL.

2.4 Fermentation

Chickpea milk (250 mL per formulation) was inoculated with *S. thermophilus*, *L. bulgaricus* or a 1:1 mixture of the two strains according to Table 1, resulting in nine formulations across three samples (0, 2, and 4% acacia gum). The inoculated milk was incubated at 42–45 °C. Samples were withdrawn aseptically from each vessel at 0, 24, and 48 h and analysed for pH and viscosity; protein and fat were determined at 0 and 48 h. Each formulation was produced as independent fermentation batches (three for pH, two for viscosity, protein and fat).

Table 1. Formulation of the yoghurt samples.

Formulation	Chickpea milk (mL)	Acacia gum (%)	<i>Streptococcus thermophilus</i> inoculum (mL)	<i>Lactobacillus bulgaricus</i> inoculum (mL)
Sample 1 (control, 0% gum)				
F1	250	–	5.0	–
F2	250	–	–	5.0
F3	250	–	2.5	2.5
Sample 2 (2% gum)				

Formulation	Chickpea milk (mL)	Acacia gum (%)	<i>Streptococcus thermophilus</i> inoculum (mL)	<i>Lactobacillus bulgaricus</i> inoculum (mL)
F4	250	2	5.0	–
F5	250	2	–	5.0
F6	250	2	2.5	2.5
Sample 3 (4% gum)				
F7	250	4	5.0	–
F8	250	4	–	5.0
F9	250	4	2.5	2.5

F1–F3, F4–F6, and F7–F9 correspond to Samples 1, 2, and 3, respectively.

2.5 pH Analysis

pH was measured with a digital pH meter (FiveEasy F20, Mettler Toledo) at 0, 24, and 48 h. The meter was calibrated according to the manufacturer's procedure before each set of readings.

2.6 Viscosity Determination

Apparent viscosity was measured with a rotational viscometer fitted with an appropriate spindle. At each sampling time, the sample was transferred to the measuring container, and the viscosity was recorded at a fixed spindle speed once the reading had stabilised, with the temperature maintained at 25 ± 0.1 °C. Each sample was measured in duplicate. Apparent viscosity, reported in mPa·s, was determined at 0, 24, and 48 h.

2.7 Protein Analysis

All reagents used in the analyses were of analytical grade (Sigma-Aldrich, USA). Protein content of the chickpea-milk base was determined by the Kjeldahl method (Kjeltec 2300, FOSS, Germany) before inoculation (0 h) and following 48 h fermentation. One gram of sample was weighed on the nitrogen-free filter paper and transferred into a digestion tube containing two selenium catalyst tablets and 25 mL of concentrated sulfuric acid (H₂SO₄). The tube was digested at maximum heat for about 1 h 30 min until the solution cleared, then cooled to room temperature. For distillation, 50 mL of boric acid with eight drops of mixed indicator (methyl red and bromocresol green) was placed in a conical flask, and the digest was distilled into it. The distillate was titrated with 0.1 M hydrochloric acid (HCl) to a brilliant scarlet endpoint (Ezeonu *et al.*, 2016). The nitrogen content obtained was converted to crude protein using the factor 6.25 (Equation 1).

$$\text{Protein content (\%)} = [(0.00014 \times \text{Titre} \times 50) / \text{Weight of sample}] \times 100 \times 6.25$$

Equation 1. Percentage of protein content.

2.8 Fat Analysis

Fat content was determined by Soxhlet solvent extraction (AOAC, 2005) (Soxhtec 8000, FOSS, Germany) on the chickpea-milk base (0 h) and the fermented yoghurt (48 h). Extraction beakers were dried at 105 °C for 1 h, cooled in a desiccator for 1 h and weighed (M1). Samples (2 g, M0) were placed in thimbles plugged with cotton wool. Extraction was carried out with petroleum ether at 40–60 °C for 8 h. After extraction, the beakers holding the extracted fat were dried at 105 °C for 30 min, cooled in a desiccator and weighed (M2) (Nielsen & Carpenter, 2017). Fat content was calculated with Equation 2.

$$\text{Fat content (\%)} = [(M2 - M1) / M0] \times 100$$

Equation 2. Percentage of fat content.

where M0 is the sample weight, M1 the weight of the empty beaker, and M2 the weight of the beaker with extracted fat.

2.9 Statistical Analysis

All data were expressed as mean $\pm$ standard deviation, where n denotes the number of independent fermentation batches (n = 3 for pH; n = 2 for viscosity, protein and fat). Three analyses were performed. First, differences between formulations at each sampling time were tested by one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test; because the three formulations within each sample share a common milk base. However, data for the 0 h sampling time were analysed as three bases rather than between the nine formulations. Second, differences between sampling times within each formulation were tested by one-way ANOVA with Tukey HSD test. Because samples were withdrawn repeatedly from the same fermentation vessel, treating sampling times as independent groups is conservative: it retains between-batch variation in the error term and therefore reduces rather than inflates the likelihood of declaring a difference. Third, the nine formulations were analysed as a 3 $\times$ 3 factorial (three acacia gum levels $\times$ three starter culture regimes) by two-way ANOVA at 24 and 48 h, with eta-squared (η^2) reported as a measure of effect size and the interaction interpreted before the main effects. Analyses were performed in IBM SPSS Statistics version 29, and significance was accepted at $p < 0.05$ (Zimmerman *et al.*, 2019).

3. Results and Discussion

3.1 pH

Acidity is a defining characteristic of yoghurt and gives it its sour, refreshing taste. The pH of each formulation over fermentation is shown in Table 2. Before fermentation, the three bases were near-neutral (pH 6.30, 6.28, and 6.27 for 0, 2, and 4% acacia gum). The gum-free base was marginally but statistically higher than the two fortified bases; a difference of 0.03 pH units is analytically negligible and is not interpreted further.

By 24 h, all formulations had acidified, and the starter culture effect was pronounced. The single-strain *S. thermophilus* formulation (F1) reached the lowest pH (4.89), while the *L. bulgaricus* formulation (F2) remained considerably higher at 5.36; the co-culture (F3) fell between the two at 5.06. This ordering is the reverse of that typically reported in bovine milk, but it is consistent with the carbohydrate composition of the substrate. Chickpea milk contains no lactose, its principal free sugar being sucrose alongside the raffinose-family oligosaccharides raffinose, stachyose, verbascose and ciceritol (Jukanti *et al.*, 2012). *L. delbrueckii* subsp. *bulgaricus* has undergone reductive genome evolution towards efficient lactose utilisation in the dairy niche (El Kafsi *et al.*, 2014), whereas *S. thermophilus* metabolises plant-derived sucrose and fructose as readily as lactose and is the primary driver of rapid acidification in mixed yoghurt culture (Iyer *et al.*, 2010). A screen of 276 lactic acid bacteria on the principal legume sugars found all *Streptococci* to be sucrose-positive against a minority of *Lactobacilli*, with *S. thermophilus* as the most effective acidifier of soy juice (Harlé *et al.*, 2020). Jan *et al.* (2022) reported the same asymmetry directly: in soy milk, *L. bulgaricus* achieved only limited acidification, failed to grow, and showed a marked stress response in its proteome. The slow early acidification of F2 is therefore best interpreted as substrate limitation rather than as loss of culture viability.

The level of acacia gum also modulated acidification. After 24 h, formulations with 2% gum (F4–F6) ranged from 5.06 to 5.16 and those with 4% gum (F7–F9) from 5.27 to 5.44, higher than the corresponding gum-free formulations. This is consistent with partial fermentation of the gum by the lactic acid bacteria and with the buffering contribution of the added polysaccharide (Rawi *et al.*, 2021).

By 48 h, however, the ranking reversed. Averaged across gum levels, the *L. bulgaricus* formulations reached the lowest mean pH (4.19) against 4.29 for *S. thermophilus* and 4.39 for the co-culture, and F8 recorded the lowest value of the study (4.00), consistent with the strong lactic-acid-producing capacity of this species once established (Karnaouri *et al.*, 2020). *L. bulgaricus* therefore adapted to the chickpea substrate over an extended fermentation rather than being excluded from it. Analysed as a 3 × 3 factorial, acidification depended on both independent variables and on their interaction (Table 6). At 24 h the gum × starter interaction accounted for the largest share of the variance ($\eta^2 = 0.459$), slightly exceeding the main effect of gum level ($\eta^2 = 0.443$) and far exceeding that of the starter culture ($\eta^2 = 0.067$); the pattern persisted at 48 h (interaction $\eta^2 = 0.422$). The main effects therefore cannot be read in isolation: the starter culture's influence on pH depended on acacia gum level, and the gum level's influence depended on the culture used. One plausible contributor is that the arabinogalactan-protein fraction of acacia gum supplies nitrogen in a matrix that lacks casein, partially relieving the nutritional constraint on this proteolysis-dependent species; this remains a hypothesis and would require nitrogen-source and viable-count data to test.

Table 2. pH of yoghurt samples during fermentation.

Sample	Formulation	pH		
		0 h	24 h	48 h
Sample 1	F1	6.30 ± 0.006 ^{A,a}	4.89 ± 0.092 ^{D,b}	4.25 ± 0.003 ^{E,c}
	F2	6.30 ± 0.006 ^{A,a}	5.36 ± 0.000 ^{AB,b}	4.32 ± 0.009 ^{C,c}
	F3	6.30 ± 0.006 ^{A,a}	5.06 ± 0.015 ^{C,b}	4.58 ± 0.009 ^{A,c}
Sample 2	F4	6.28 ± 0.010 ^{B,a}	5.16 ± 0.025 ^{C,b}	4.30 ± 0.006 ^{D,c}
	F5	6.28 ± 0.010 ^{B,a}	5.06 ± 0.015 ^{C,b}	4.26 ± 0.003 ^{E,c}
	F6	6.28 ± 0.010 ^{B,a}	5.13 ± 0.015 ^{C,b}	4.26 ± 0.007 ^{E,c}

Sample	Formulation	pH		
		0 h	24 h	48 h
Sample 3	F7	6.27 ± 0.000 ^{B,a}	5.44 ± 0.021 ^{A,b}	4.32 ± 0.006 ^{C,c}
	F8	6.27 ± 0.000 ^{B,a}	5.33 ± 0.036 ^{B,b}	4.00 ± 0.009 ^{F,c}
	F9	6.27 ± 0.000 ^{B,a}	5.27 ± 0.015 ^{B,b}	4.34 ± 0.003 ^{B,c}

Values are mean ± SD (n = 3). Within a column, means not sharing an uppercase superscript differ significantly between formulations at the same sampling time. Within a row, means not sharing a lowercase superscript differ significantly between sampling times for that formulation. All separations were by Tukey HSD at p < 0.05. n is the number of independent fermentation batches. At 0 h, the three formulations within a sample share a common milk base, so the 0 h column was compared as three bases rather than between the nine formulations.

3.2 Viscosity

Apparent viscosity increased with both fermentation time and acacia gum concentration (Table 3). At 24 h the gum-free formulations reached 235 to 278 mPa·s, indicating a contribution from microbial activity even without added gum. Increasing the acacia gum level raised viscosity further, to 290.5–319 mPa·s at 2% gum (F4–F6) and 351–388 mPa·s at 4% gum (F7–F9), a clear concentration-dependent effect consistent with the thickening role of acacia gum in fermented foods (Mugo *et al.*, 2020). By 48 h, all formulations had thickened markedly, from 1110 mPa·s in the control (F1) to 2125 mPa·s in the 4% co-culture formulation (F9).

Two mechanisms explain the rise during fermentation. As pH falls toward the isoelectric region of the chickpea proteins, the proteins lose net charge and aggregate into a gel network, so viscosity increases even in gum-free formulations. In parallel, *S. thermophilus* and *L. bulgaricus* can produce exopolysaccharides, particularly in co-culture, which further strengthen the network (Tong *et al.*, 2023); this is consistent with the higher 48 h values of the co-culture formulations (F3, F6, F9). Acacia gum adds to this network as a hydrated thickener, and its effect is amplified as the matrix concentrates over time.

At 0 h, the three bases were only partially separated: the 4% base (163 mPa·s) was significantly more viscous than the unfortified base (155 mPa·s), while the 2% base (160 mPa·s) did not differ significantly from either. This small difference is attributable to the reading being taken shortly after gum addition, before the polysaccharide had fully hydrated and developed its network. A measurement taken after complete hydration would be expected to resolve the three bases more clearly, and this is noted as a limitation of the present dataset.

The factorial analysis identifies acacia gum level as the dominant determinant of viscosity, accounting for 89.6% of the variance at 24 h and 74.3% at 48 h, against 6.9% and 14.0% for the starter culture (Table 6). The gum × starter interaction was significant but small ($\eta^2 = 0.032$ and 0.117), indicating that gum level and culture acted largely additively at 24 h, with a modest interaction emerging by 48 h as the co-culture formulations gained disproportionately at the highest gum level. Averaged across gum levels, the co-culture reached the highest 48 h viscosity (1665 mPa·s) compared with the two single-strain regimes (1418 and 1553 mPa·s), consistent with a contribution from exopolysaccharide production during mixed-culture fermentation and with the complementary activity of the two species reported for optimised yoghurt starters (Ge *et al.*, 2024).

Table 3. Apparent viscosity (mPa·s) of yoghurt samples during fermentation.

Sample	Formulation	Apparent viscosity (mPa·s)		
		0 h	24 h	48 h
Sample 1	F1	155 ± 1.414 ^{B,c}	235 ± 1.414 ^{H,b}	1110 ± 0.707 ^{H,a}
	F2	155 ± 1.414 ^{B,c}	278 ± 0.707 ^{F,b}	1421 ± 0.000 ^{F,a}
	F3	155 ± 1.414 ^{B,c}	260 ± 2.560 ^{G,b}	1387 ± 7.071 ^{G,a}
Sample 2	F4	160 ± 1.414 ^{AB,c}	290.5 ± 2.121 ^{EF,b}	1466 ± 0.000 ^{E,a}
	F5	160 ± 1.414 ^{AB,c}	295.5 ± 4.243 ^{E,b}	1461 ± 7.071 ^{E,a}
	F6	160 ± 1.414 ^{AB,c}	319 ± 0.000 ^{D,b}	1483 ± 7.778 ^{D,a}
Sample 3	F7	163 ± 1.414 ^{A,c}	351 ± 7.071 ^{C,b}	1678 ± 0.707 ^{C,a}
	F8	163 ± 1.414 ^{A,c}	365 ± 4.950 ^{B,b}	1778 ± 0.707 ^{B,a}
	F9	163 ± 1.414 ^{A,c}	388 ± 1.414 ^{A,b}	2125 ± 0.000 ^{A,a}

Values are mean ± SD (n = 2). Within a column, means not sharing an uppercase superscript differ significantly between formulations at the same sampling time. Within a row, means not sharing a lowercase superscript differ significantly between sampling times for that formulation. All separations were by Tukey HSD at p < 0.05. n is the number of independent fermentation batches. At 0 h the three formulations within a sample share a common milk base, so the 0 h column was compared as three bases rather than between the nine formulations.

3.3 Protein

Crude protein content (nitrogen × 6.25) is reported in Table 4. It rose with acacia gum level and, apparently, with fermentation time. This pattern requires careful interpretation, because lactic acid bacteria hydrolyse existing proteins into peptides and amino acids through proteolysis (Harper *et al.*, 2022) rather than synthesising new protein, so a true increase in protein during a closed fermentation is not expected.

Two effects account for the observed rise. First, gel formation is accompanied by syneresis and moisture loss, which concentrates the solids and raises the protein measured per unit mass. Second, acacia gum is itself an arabinogalactan-protein complex and contributes nitrogen of its own: on a dry-weight basis *Acacia senegal* gum contains about 2.7% protein, most of it in the arabinogalactan-protein and glycoprotein fractions (Lopez-Torrez *et al.*, 2015). Together with gum-protein interactions that stabilise the network (Chen *et al.*, 2019), these effects explain the concentration-dependent increase.

The factorial analysis confirms that acacia gum level, rather than the starter culture, drove the protein values ($\eta^2 = 0.776$ versus 0.150), with a small but significant interaction ($\eta^2 = 0.060$; Table 6). The gum-free control (F1) showed no significant change in protein between 0 and 48 h, whereas every gum-containing formulation did, consistent with gum addition rather than fermentation as the source of the increase. The magnitude at 0 h, however, is smaller than gum-derived protein alone would predict: at 2% gum the measured rise was 0.005 percentage points, against roughly 0.03 expected from an arabinogalactan-protein content of 2.7%. The gum used here may therefore be closer in composition to *Acacia seyal*, which carries appreciably less protein, and the rise from 0 to 48 h is better attributed to concentration of the matrix than to the gum itself. Confirmation on a dry-matter basis would separate the two contributions.

Table 4. Protein (%) of yoghurt samples before and after fermentation.

Sample	Formulation	Protein (%)	
		0 h	48 h
Sample 1	F1	0.9930 ± 0.0000 ^{C,a}	1.0745 ± 0.1153 ^{C,a}
	F2	0.9930 ± 0.0000 ^{C,b}	1.1560 ± 0.0000 ^{C,a}
	F3	0.9930 ± 0.0000 ^{C,b}	1.1560 ± 0.0000 ^{C,a}
Sample 2	F4	0.9978 ± 0.0000 ^{B,b}	1.1844 ± 0.0122 ^{C,a}
	F5	0.9978 ± 0.0000 ^{B,b}	1.2885 ± 0.0459 ^{C,a}
	F6	0.9978 ± 0.0000 ^{B,b}	1.7514 ± 0.1392 ^{B,a}
Sample 3	F7	1.0031 ± 0.0001 ^{A,b}	1.7574 ± 0.0006 ^{B,a}
	F8	1.0031 ± 0.0001 ^{A,b}	1.8815 ± 0.0559 ^{B,a}
	F9	1.0031 ± 0.0001 ^{A,b}	2.1525 ± 0.0177 ^{A,a}

Values are mean ± SD (n = 2). Crude protein was calculated as total nitrogen × 6.25. Within a column, means not sharing an uppercase superscript differ significantly between formulations at the same sampling time. Within a row, means not sharing a lowercase superscript differ significantly between sampling times for that formulation. All separations were by Tukey HSD at p < 0.05. n is the number of independent fermentation batches. At 0 h the three formulations within a sample share a common milk base, so the 0 h column was compared as three bases rather than nine formulations. At 0 h the within-base standard deviations approach zero, so the separation of the three bases indicates only that they differed and is not a measure of the size of that difference.

3.4 Fat

Table 5 shows fat content, measured by Soxhlet extraction. As with protein, the measured value increased with acacia gum level, from 0.3850% in the gum-free base to 0.5025% (2% gum) and 1.1561% (4% gum) at 0 h, and rose further after fermentation to 2.0150% in F9. This trend cannot reflect fat synthesised by the bacteria, and acacia gum is a polysaccharide rather than a lipid, so it does not directly add fat.

The most plausible explanations are concentration and co-extraction. Moisture loss during gelation concentrates the solids, and the gum improves emulsion stability so that lipophilic material is retained in the matrix rather than lost; petroleum ether may also co-extract some gum-associated, emulsified material, inflating the apparent fat value. Acacia gum, like other stabilisers, helps emulsions form and resist phase separation (De Souza Paglarini *et al.*, 2020), which is consistent with the higher and more reproducible fat readings at higher gum levels. It should be noted that the increase at 4% gum is proportionally larger than that at 2% gum, which a simple additive contribution would not predict, and this warrants verification against the original extraction records.

Acacia gum level accounted for 85.1% of the variance in fat content and the starter culture for 9.7%, with a small significant interaction ($\eta^2 = 0.051$; Table 6). Since acacia gum contains essentially no lipid, this reinforces the interpretation that the measured increase reflects retention and co-extraction of emulsified material within a concentrating matrix rather than any lipid contributed by the gum or synthesised by the cultures. These apparent increases should therefore be read as changes in retained, extractable lipid, and confirmation on a dry-matter basis would remove the concentration effect.

Table 5. Fat (%) of yoghurt samples before and after fermentation.

Sample	Formulation	Fat (%)	
		0 h	48 h
Sample 1	F1	0.3850 ± 0.0070 ^{C,b}	0.4900 ± 0.0000 ^{H,a}
	F2	0.3850 ± 0.0070 ^{C,b}	0.5151 ± 0.0140 ^{H,a}
	F3	0.3850 ± 0.0070 ^{C,b}	0.6175 ± 0.0106 ^{G,a}
Sample 2	F4	0.5025 ± 0.0000 ^{B,b}	0.7088 ± 0.0123 ^{F,a}
	F5	0.5025 ± 0.0000 ^{B,b}	0.8945 ± 0.0064 ^{E,a}
	F6	0.5025 ± 0.0000 ^{B,b}	0.9788 ± 0.0017 ^{D,a}
Sample 3	F7	1.1561 ± 0.0001 ^{A,b}	1.2438 ± 0.0017 ^{C,a}
	F8	1.1561 ± 0.0001 ^{A,b}	1.7400 ± 0.0000 ^{B,a}
	F9	1.1561 ± 0.0001 ^{A,b}	2.0150 ± 0.0000 ^{A,a}

Values are mean ± SD (n = 2). Within a column, means not sharing an uppercase superscript differ significantly between formulations at the same sampling time. Within a row, means not sharing a lowercase superscript differ significantly between sampling times for that formulation. All separations were by Tukey HSD at $p < 0.05$. n is the number of independent fermentation batches. At 0 h, the three formulations within a sample share a common milk base, so the 0 h column was compared as three bases rather than nine formulations. At 0 h, the within-base standard deviations approach zero, so the separation of the three bases indicates only that they differed and is not a measure of the size of that difference.

3.5 Effects of Acacia Gum Level and Starter Culture

Because the nine formulations constitute a complete 3×3 factorial, the contributions of the two independent variables can be partitioned directly (Table 6). Acacia gum level was the dominant influence on viscosity, protein and fat, accounting for 74 to 90% of the variance in those responses, while the starter culture accounted for 7 to 15%. For pH, the balance was different: at 48 h the starter culture and the gum $\times$ starter interaction each explained more variance than gum level alone. The interaction was significant for every response measured, so the two factors are interpreted jointly rather than as independent contributions.

Table 6. Two-way ANOVA of acacia gum level and starter culture on the measured responses.

Response	Source	df	F	p	η^2
pH, 24 h	Acacia gum level	2	129.3	<0.001	0.443
	Starter culture	2	19.5	<0.001	0.067
	Gum $\times$ Starter	4	67.0	<0.001	0.459
	Error	18	–	–	0.031
pH, 48 h	Acacia gum level	2	1437.1	<0.001	0.236
	Starter culture	2	2072.4	<0.001	0.341
	Gum $\times$ Starter	4	1282.9	<0.001	0.422
	Error	18	–	–	0.001
Viscosity, 24 h	Acacia gum level	2	1541.7	<0.001	0.896
	Starter culture	2	118.9	<0.001	0.069
	Gum $\times$ Starter	4	27.8	<0.001	0.032
	Error	9	–	–	0.003

Response	Source	df	F	p	η^2
Viscosity, 48 h	Acacia gum level	2	27030.9	<0.001	0.743
	Starter culture	2	5099.8	<0.001	0.140
	Gum $\times$ Starter	4	2131.9	<0.001	0.117
	Error	9	–	–	0.000
Protein, 48 h	Acacia gum level	2	233.1	<0.001	0.776
	Starter culture	2	44.9	<0.001	0.150
	Gum $\times$ Starter	4	9.0	0.003	0.060
	Error	9	–	–	0.015
Fat, 48 h	Acacia gum level	2	35859.2	<0.001	0.851
	Starter culture	2	4105.0	<0.001	0.097
	Gum $\times$ Starter	4	1082.6	<0.001	0.051
	Error	9	–	–	0.000

η^2 is the proportion of total variance attributable to each source. Error df = 18 for pH ($n = 3$) and 9 for viscosity, protein and fat ($n = 2$). The interaction is interpreted before the main effects.

4. Conclusion

Chickpea milk fortified with acacia gum and fermented with *S. thermophilus* and *L. bulgaricus* produced a dairy-free yoghurt whose properties depended on acacia gum level, starter culture, and their interaction. Increasing the acacia gum level raised apparent viscosity in a concentration-dependent manner, reaching about 2125 mPa·s at 4% gum after 48 h; gum level accounted for 74 to 90% of the variance in viscosity and for 78 and 85% of the variance in the measured protein and fat, which are best explained by concentration of the matrix and by the composition of the gum itself rather than by microbial synthesis.

The starter culture drove acidification in a time-dependent way. *S. thermophilus* acidified the chickpea substrate the fastest over the first 24 h, consistent with its capacity to use the sucrose of a legume base, whereas *L. bulgaricus* acidified slowly at first and reached the lowest pH of the study by 48 h. The co-culture gave intermediate acidity together with the highest 48 h viscosity. Acacia gum at 2 to 4% therefore improved the consistency of chickpea-milk yoghurt, while the starter choice determined the rate and eventual extent of acidification.

Several limitations should be recognised. Only two starter strains at fixed inoculum ratios were examined; protein and fat were determined in duplicate and at two sampling times only; viable counts, residual sugars, sensory properties and storage stability were not assessed; and differences between sampling times were tested by treating those times as independent groups, which is conservative but not equivalent to a repeated-measures analysis. Further work should confirm the compositional changes on a dry-matter basis, quantify viable counts and residual sugars, and add sensory evaluation and storage-stability testing before the suitability of the product as a dairy alternative can be established.

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