Journal Halal Science, Industry, and Business, Vol. 3 No. 1 (2025)https://doi.org/10.31098/jhasib.v3i1.3126

Check for updates

Research Paper

Halal Plant-Based Gelatin Production, Authentication, and Implementation

Usman Mir Khan[1]

[1]University of Agriculture, Pakistan

Received: February 10, 2025	Revised: May 16, 2025	Accepted: May 23, 2025	Online: May 31, 2025

Abstract

The manufacture of gelatin has long been a contentious issue on a global scale. The culinary, pharmaceutical, and cosmetics sectors use gelatin extensively. However, it is considered one of the most controversial elements in Halal and Kosher food businesses. Once gelatin has been combined with food or medication, it is difficult to identify the animal from which it came. This study was carried out to develop yoghurt with transglutaminase enzyme. The yoghurt was evaluated for the rheological characteristics of yoghurt and its synthesis using the transglutaminase enzyme isolated from the fig plant as a gelatin substitute. The effects of different fig-based enzyme concentrations (5%, 10%, and 20%) and temperature settings (35°C, 45°C, and 55°C), as well as time treatments (60, 90, and 120 minutes) on gelatin-based yoghurt, were assessed. The enzymatic treatment of milk enhanced its ability to retain water following centrifugation by delaying the syneresis process during yoghurt storage at 4°C. The cross-linking of transglutaminase with milk protein improved the functional qualities of yoghurt and impacted the post-acidification process and the stability of yoghurt samples. Plant-based yoghurt exhibited higher FRAP antioxidant activity than gelatin-based yoghurt, which exhibited no antioxidant activity. Quantitative protein and fat content estimates using FTIR and Raman spectroscopy revealed better fat and protein microstructure changes. Modern scientific methods about Halal and Kosher food features must be incorporated since consumer concerns over the authenticity of Halal and non-halal food products have increased.

Keywords: *Gelatin; Halal Source; Transglutaminase Enzyme; Yoghurt*

INTRODUCTION

The debate regarding gelatin's permissibility under religious dietary regulations overshadows its many uses in food, medicine, and cosmetics. The origin of gelatin, whether bovine, porcine, or another, dictates its permissibility for halal and kosher observance. Gelatin improves the texture and lessens syneresis in food products, especially yoghurt. However, verifying the source of gelatin after processing is complex, which raises questions about ethical integrity and mislabeling. The need for trustworthy plant-based gelatin substitutes that adhere to religious principles has increased because of these problems. In order to create gelatin-free, Halal-compliant yoghurt, this study investigates the transglutaminase enzyme obtained from fig plants as a possible replacement in yoghurt manufacture (Rakhmanova et al., 2018).

Yoghurt, a fermented dairy product, has become increasingly popular worldwide because of its many health benefits, which are ascribed to its high nutritional profile and microbial content. Additives like gelatin are frequently used to improve the mouthfeel and texture of yoghurt (Kilara, 2013). Although gelatin, which is made from animal collagen, works well to achieve the right consistency, its use is restricted because of vegetarian and vegan dietary requirements and concerns about its animal-based source. Yoghurt has long been supplemented with gelatin to increase stability, decrease syneresis (water separation), and improve texture. However, the animal origin of gelatin presents problems with dietary regulations, especially in the Halal and Kosher food industries, where the acceptability of gelatin is directly impacted by its source (e.g., bovine or porcine). The animal from which gelatin is obtained determines whether or not products

Corresponding author's email: usmanmirkhan@yahoo.com

containing it are acceptable. Milk was combined with gelatin to create yoghurt to overcome the syneresis difficulty during storage. However, the source of gelatin is unknown, raising ethical questions about whether it is Halal or Haram. Consequently, there is a risk of mislabeling or adulteration driven by financial gains. This study investigates the possibility of using the transglutaminase enzyme isolated from fig plants as a plant-based and morally acceptable substitute for gelatin in yoghurt manufacturing. The main goal of this study was to ascertain how different transglutaminase concentrations, temperatures, and time treatments affect the rheological characteristics of yoghurt, emphasising water retention and syneresis (Ahmed et al., 2020).

The processing of yoghurt in Pakistan's dairy industry is promising. Using gelatin in yoghurt production is problematic because Pakistan is a Muslim country. Gelatin is made from dead animal bones and sometimes pig skin, which is not Halal. Furthermore, fragile non-covalent connections (hydrogen, electrostatic, and hydrophobic interactions) hold the protein gel in traditionally made set yoghurt together. The transglutaminase enzyme (TGase) eventually increases gel consistency while reducing syneresis. Therefore, the enzyme transglutaminase will provide yoghurt firmness and texture to enhance its physicochemical properties and nutritional value. A standard fermented dairy product people use worldwide is yoghurt (Shiby & Mishra, 2013). Together, lactic acid bacteria such as *Streptococcus thermophillus* and *Lactobacillus delbrueckii ssp. bulgaricus* ferment milk; the concentration of these bacteria plays a critical role in influencing the fermentation rate and the yoghurt's quality (Settachaimongkon et al., 2014). Local people's customs, values, rituals, and fat content vary, affecting the dairy products they choose (Inayat, 2012). While people in some nations prefer cheese and other fermented milk products, others prefer liquid milk products.

Vegetable proteases have garnered significant attention, primarily for their use in food applications. Because of the potential for disease transmission, consumers have shown resistance to animal-derived proteases. In certain nations, recombinant proteases are not permitted in human meals. In this context, ficin is an enzyme extract obtained from the latex of the fig *(Ficus carica)* and is composed of many proteases. The tree's health, surrounding environment, irrigation, etc., can all affect the precise relationship between the many active ingredients in ficin extract. For instance, when fruit ripens, latex exhibits a consistent rise in protein concentration but a drop in ficin content. During fruit ripening, distinct types of ficinia are present in varying amounts (Duran, 2024). Four proteases—A, B, C, and D—have been crystallized, and their structures are accessible. According to their investigation, all four proteins were glycoproteins with high sequence similarity to bromelain.

The following is a quick summary of some attempts to determine the primary components of ficin extract and purify it. Shortly after its discovery, attempts were made to purify and examine the makeup and role of ficin. Ficin was purified via a series of ammonium sulfate precipitations, followed by Sephadex G-100 gel filtration. Protein fractions, including ficin, were obtained. After being tagged with N-(4-dimethylamino 3,5-dinitrophenyl) (DDPS)-maleimide, the active thiol group of ficin was subjected to pepsin hydrolysis. Tyr-Ser-Gly-Val-DDPS-Cys was the peptide that included DDPS-cysteine. This ficin form's N-terminal residue of this ficin was leucine (Leal et al., 2024).

Ficin is separated from commercial products using carboxymethyl cellulose chromatography and salt fractionation. Despite discovering many ficin forms, only the primary component has been described. This protease hydrolyzed an extensive range of peptide bonds; however, peptide bonds that followed an aromatic residue appeared more effectively degraded than the others. In other words, the enzyme was very nonspecific but preferred aromatic residues (Rakhmanova et al., 2018). Later, a different study suggested purifying ficin from *Ficus carica* using an agarose-mercurial column, which would separate ficin and mercurificin. A glycosylated proteinase from *Ficus carica* var. was reported in another study. Hōraishi, known as Ficin S, was purified using CM-Sephadex C-50 and CM-cellulose. Electrophoretically, the purified Ficin S was homogenous. The ideal temperature for the enzyme was 60 °C, and its ideal pH was 8.0. The enzyme was stable for 20 hours at 4 °C and pH values between 2.0 and 8.0. Cysteine and mercaptoethanol enhanced the enzyme activity, whereas p-chloromercuribenzoate and $HgCl_2$ hindered it. The ficins A, B, C, and D from *Ficus carica var. Hōraishi* can be supplemented with this enzyme. These findings imply that the only differences between Ficin S and the other ficin isoforms are their sugar content and isoelectric point (Tanwar et al., 2014). In some instances, ficin degrades the qualities of plant derivatives intended for human consumption. Plant cysteine proteases, among the most influential enzymes, have been linked, at least in part, to cross-allergic reactions caused by fruits from various plants.

Despite the incomplete characterization of each component's specificity and mode of action, the applications of ficinia cocktails are rapidly expanding, making it one of the most widely used vegetable enzymes. Despite specific long-standing uses, a review study has not described this enzyme extract's applications. This review aims to demonstrate the various uses of this intriguing protease extract and how it has been produced for use as an industrial biocatalyst, including immobilization (Prabavathy & Nachiyar, 2011).

The hydrolysis of proteins is the most basic use of a protease. Following pretreatment with ficin and pepsin, collagen was isolated from cattle tendons; for best results, a much lower dosage of ficin is needed. Transglutaminase has been used with trypsin, ficin, and/or bromelin to proteolyze canola protein. Analysis was performed on how these treatments affected the protein's ability to gel. Although reduced proteolysis allowed for improved crosslinking with transglutaminase, resulting in a more potent gel, proteolysis caused a drop in gel strength (Sendra et al., 2010). Limited hydrolysis of barley hordein by ficin and papain enhances its foaming capability, but excessive proteolysis reduces foam stability, and neither enzyme is capable of hydrolyzing albumin. Ruminal protein degradability was predicted using the hydrolysis of soybean meal, fish, and barley proteins, which was catalyzed by papain, ficino, and *Streptomyces griseus* protease. While barley proteins hydrolyzed slowly to moderately in vitro (because of structural proteins' weak accessibility to the proteases), soybean meal nitrogen hydrolyzed nearly entirely (Thakkar & Preetha, 2016). Sugars, organic acids, minerals (like manganese, copper, magnesium, calcium, and potassium), vitamins (like vitamin K and β-carotenes), polyphenols, flavonoids, fibre, and other substances (like arabinose, glycosides, β-amyrins, β-setosterols, and xanthotoxol) that have demonstrated advantageous qualities are all abundant in figs *(Fiscus carica)* (Jeong et al., 2009; Vinson, 1999).

Thus, this study was conducted to develop yoghurt using the fig-based transglutaminase enzyme. The yoghurt was evaluated for its rheological characteristics and synthesis using the transglutaminase enzyme isolated from the fig plant as a gelatin substitute.

LITERATURE REVIEW

Gelatin

The primary purposes of gelatin in food are to decrease syneresis, increase mouthfeel, and improve textural qualities. However, given gelatin's contentious acceptance in preparing Halal and Kosher food, substitutes are increasingly sought. Transglutaminase is an enzyme that can cross-link and presents a viable substitute for gelatin, mainly when obtained from plant sources. Although little is known about plant-derived transglutaminase, prior studies have shown that the enzyme can improve dairy products' texture and water retention. Our study fills this gap by using fig transglutaminase and investigating how it affects the rheological and functional characteristics of yoghurt (Wang, 2000).

Yoghurt

Yogurt is a fermented milk product. According to O'Connell and Fox (2001), it is frequently made by fermenting *Lactobacillus delbrueckii ssp. bulgaricus*, and *Streptococcus thermophilus*. Additionally, yoghurt serves as a natural supply of probiotics, which improve digestion and absorption, improve human nutrition, and increase food safety (Sanders, 2007). Foods containing probiotics, prebiotics, and symbiotics are categorized as functional foods. Despite having high nutritional value, dairy products—including plain yoghurt—are poor sources of fibre, antioxidants, and phenolic compounds (O'Connell & Fox, 2001). This is because cattle milk contains low levels of fibre (Vasquez et al., 2015) and phenolic compounds (about 49 mg GAE/L). To satisfy customer demands, fruits or vegetables are added to dairy products to boost their fibre and antioxidant qualities, creating what is known as a "clean label" (Granato et al., 2017). According to Staffolo et al. (2017) and Tomic et al. (2017), yoghurt supplemented with dietary fibre showed increased probiotic activity.

***Ficus Carica* as a Potential Gelatin Substitute**

Figs, or *Ficus carica,* are renowned for their gelling qualities and have a distinctive composition that includes fibre, natural sugars, and phytochemicals. Because figs naturally contain pectin, they can be used as a stabilizer in dairy products. This study investigated the viability of using fig extract in place of gelatin in yoghurt, evaluating its impact on the product's texture, flavour, and general acceptability among consumers. In this regard, our research aims to raise the nutritive properties of plain yoghurt by adding figs to meet human requirements for phenolic compounds, antioxidants, minerals, and fibre. Therefore, adding fig to yoghurt may boost its probiotic activity, which could be used in human nutrition and medicine to cure and reduce obesity, hypertension, hypercholesterolemia, hyperlipemia, and gastrointestinal diseases and to encourage the formation of gut microbiota (Tosun et al., 2012).

Gelatin is a unique hydrocolloid that has various applications and serves numerous purposes. Cattle bones, hide, and pig skin were the primary sources of gelatin. The rich vegetarian and growing halal and kosher sectors have made replacing gelatin a significant issue for many years. Given its wide range of applications across numerous industries, gelatin is considered one of the most distinctive and unusual hydrocolloids used in the food industry (Lazreg-Aref et al., 2012). In the biomedical area (three-dimensional tissue regeneration and wound dressing), in the pharmaceutical industry (microspheres, hard and soft capsules), as a food ingredient (e.g., foaming and gelling agent), and in numerous other fields outside of food (photography), gelatin is utilized.

Collagen, a component of bones, connective tissue, and animal skin (Morrison et al., 1999), is hydrolyzed to produce gelatin. For commercial gelatin manufacture, animal skins, bones, and pig skins are the primary feedstock because of their low cost. The primary issue of replacing gelatin has existed for a long time because of the halal market, vegetarians, and kosher. However, it has gained more attention recently, particularly in Europe in the 1980s when "mad cow disease" first appeared (Shahinuzzaman et al., 2020). Therefore, using gelatin derived from diseased animal parts raises many concerns. Pigs or cowhides produce the most gelatin on a commercial scale.

Use of The Transglutaminase Enzyme from Figs in Yoghurt

Transglutaminase up to 0.5% was more successful in enhancing the functional qualities of goat milk-based yoghurt. Whey separation was significantly decreased, and gel consistency improved because of enzymatic cross-linking. However, Farnsworth et al. (2006) found no appreciable differences between the control and enzyme-treated yoghurt samples. There were no negative consequences on the yoghurt's fermentation process when the microbial transglutaminase was in its inactivated condition and glutathione created stirred yoghurt by covalent cross bonding. Compared with yoghurt, which is produced only by TGase, there was a significant increase in protein polymerization and perceived viscosity (Bönisch et al., 2007). Higher concentrations of the TGase enzyme decreased syneresis and increased the viscosity of the yoghurt. However, some minor issues with LAB development led to less acetaldehyde and acid generation than in the control. The optimal concentration, however, was found to be 0.3 g L^{-1}, which was identified as a good substitute for stabilizers for making non-fat yoghurt (Ozer et al., 2007). When yoghurt's composition was changed by adding liquid milk whey, transglutaminase appeared to be a more effective source for developing physical qualities. It has also been shown that the rate of syneresis is directly impacted by the cross-linking caused by TGase (Uddin et al., 2021).

Such enzyme effects on yoghurt's physiochemical and sensory properties were examined at various stages of manufacture and incubation. The results indicated that the addition of enzymes did not significantly alter the chemical makeup of yoghurt. However, adding an enzyme after pasteurization improved gel stability while decreasing syneresis (Sanli et al., 2011). Transglutaminase (TGase) forms cross-links in proteins (Motoki & Seguro, 1998). Milk's protein is cross-linked by TGase, which results in improved functional qualities like hydration ability, rheological qualities, and emulsifying qualities. (O'Sullivan et al., 2001; Motoki & Seguro, 1998; Lorenzen, 2000).

For this reason, there is much interest in identifying substitute sources for gelatin manufacture. Therefore, our research institutes are attempting to create alternate sources while considering this. This study will be planned to keep in view the following objectives:

1. Development of yoghurt by using a halal source
2. To evaluate the physicochemical and sensory attributes of yoghurt produced with fig extract as a gelatin substitute.
3. To assess the consumer acceptance of fig-stabilized yoghurt.

RESEARCH METHOD

Materials

Procurements of Milk

Milk from cows and buffaloes was bought and stored at 4°C until needed. Whole milk powder and whey protein standardize the raw milk used to make yoghurt.

Starter cultures

Commercial starter cultures were used for the manufacturing of yoghurt. These cultures were obtained from Clerici Sacco, Italy.

1. *Lactobacillus delbrueckii ssp. bulgaricus*
2. *Streptococcus thermophilus (Y350B)*

Ficus carica Extract

Prepared by processing fig pulp to obtain a pectin-rich extract.

Control Additive

Gelatin was used in the control yoghurt sample for comparison.

Preparation of Fig Extract

After cleaning and skinning, fresh figs were pulped. The natural pectin was extracted from the pulp by heating it to 80°C, then filtered and concentrated to create a thick extract. This extract was added to milk to determine the ideal quantity for yoghurt consistency (Uddin et al., 2021).

Obtaining a crude extract from the fig pulp is the first stage in extracting transglutaminase (TGase) from figs. Purification and characterization come next. After the crude extract is refined using procedures like precipitation, the enzyme is isolated using chromatographic techniques such as CM-cellulose and Sephadex G-75. A more detailed explanation will be provided in the following subsection.

Crude Extract

1. Fig Pulp

   The first step of the procedure is to prepare a crude extract from the fig pulp. Although the precise techniques of generating extracts differ, they typically combine maceration, homogenization, or blending with centrifugation to eliminate insoluble debris.
2. Purification: Precipitation:

   The crude extract is typically precipitated using ethanol precipitation or ammonium sulphate precipitation, a typical step.
   a. Chromatography
      Chromatography methods, such as CM-cellulose ion-exchange for charge-based separation and Sephadex G-75 gel filtration for size-based separation, are frequently used to enhance protein purification.
   b. Additional Purification Techniques
      Depending on the particular strategy, other purification techniques, such as dialysis and SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis), may also be used.
3. Description:
   a. Calculating Molecular Weight
      A standard method for characterizing purified transglutaminase is to measure its molecular weight, which can be accomplished using methods such as SDS-PAGE.
   b. Activity Assay
      According to the E3S Web of Conferences, particular assays are used to evaluate the purified enzyme's capacity to catalyze the creation of isopeptide linkages between proteins.
   c. Optimal conditions
      The ideal pH and temperature for the action of the purified transglutaminase are determined. The extraction consists of preparing a crude extract and performing several purification procedures to isolate and characterize the transglutaminase enzyme from figs.

Yoghurt Formulation with Transglutaminase

This study prepared yoghurt formulations with different enzyme concentrations (5%, 10%, and 20%) using transglutaminase, an enzyme extracted from fig plants. Different setting temperatures (35°C, 45°C, and 55°C) and time intervals (60, 90, and 120 minutes) were used to assess each concentration level (Table 1).

Transglutaminase catalyzes a two-step reaction in which a glutamine residue in one protein or peptide is transferred to the amine group of a lysine residue in another. It can be isolated from figs or other sources. In essence, this procedure "glues" proteins together, forming crosslinks and changing their structure and functionality. Here is a more thorough, step-by-step explanation of the procedure:

1. Activation and Thioester Formation: A glutamyl residue in the protein substrate forms a covalent intermediate with the calcium ion-dependent active form of

transglutaminase. This is due to an interaction between the glutamyl and cysteine residues in the enzyme's active site, which releases ammonia and activates the glutamine acyl moiety.

2. Acyl Transfer: An acyl transfer reaction occurs between an active thioester intermediate and a primary amine group, like the ε-amino group of a lysine residue in another protein. Because of this transfer, the glutamine and lysine residues form an isopeptide bond, which produces a covalent crosslink.
3. Protein Aggregation and Crosslinking: Proteins may aggregate because of the formation of many crosslinks, which can alter the general composition and characteristics of substrate proteins. Transglutaminase can also catalyze the hydrolysis of these isopeptide bonds, thereby reversing crosslinking.
4. Use in Food and Beyond: Transglutaminase is utilized in the food sector to improve the texture and binding of proteins, such as in cheese production, meat processing, and gluten-free baking. Also, transglutaminase has potential uses in biotechnology, materials science, and medicine outside food, including drug delivery, tissue engineering, and biosensors.

Table 1. Details of Treatments for Yoghurt Preparation from Buffalo Milk

Treatment	Transglutaminase enzyme in figs
T (control)	0%
T1	5%
T2	10%
T3	20%

Yogurt Production

After 10 minutes of heating to 85°C, the milk was cooled to 45°C. We added either gelatin or fig extract to several batches of milk. A starter culture was added to the milk, which was then incubated for four to six hours at 42°C until the pH reached 4.6. Over the course of 14 days, yogurt samples were kept at 4°C and examined (See Figure 1).

Product Development

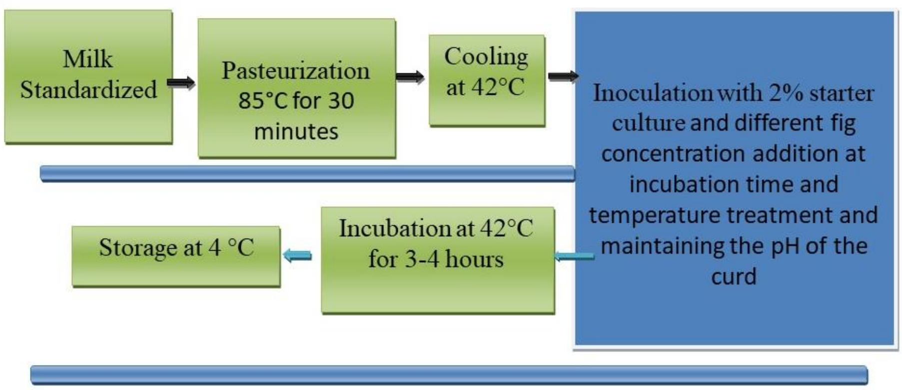

Figure 1. Development of Yoghurt Made with Transglutaminase Extracted from Fig Extract

Rheological and Functional Analysis

After centrifugation, rheological testing examined the yogurt’s capacity to hold onto water and its resistance to syneresis. Storage stability was evaluated at 4°C. FTIR and Raman spectroscopy were used to assess the microstructural changes in the protein and fat content of the yogurt samples. FRAP analysis was used to compare the antioxidant capacity of transglutaminase-treated samples with that of regular gelatin-based yogurt.

Analytical Methods

Physicochemical Analysis

The AOAC (2012) method was used to determine the following parameters: moisture, protein, fat, and ash content, and pH value.

Sensory Analysis

A sensory panel (n=50) rated the yogurt samples for texture, flavor, aroma, and overall acceptability on a 9-point hedonic scale. The semi-trained panel analyzed the samples and measured on the basis of the 9-point hedonic scale (Meilgaard & Civille, 2017).

FINDINGS AND DISCUSSION

According to the results, the 10% fig extract yoghurt had the best texture and was on par with the gelatin-stabilized yoghurt. The Fig extract may be able to stop syneresis, a typical problem in yoghurt preservation, because the water-holding capacity of fig-based yoghurt was higher. All of the samples' pH values fell between 4.0 and 4.6, which is the normal range for yoghurt and indicates efficient fermentation (Table 2). Table 1 presents yoghurt's protein, fat, and total solid compositions with various fig concentrations. Given that the moisture content of fig puree was 85%, the most significant values were observed in the T3 samples. This could be due to the high fig concentration. The high moisture content and extremely low protein and fat content in fig puree-containing samples (Soni et al., 2014) can be attributed to the decrease in protein and fat content percentage, respectively, in all samples. The high mineral contents in the fig also cause these changes (Ali et al., 2002).

The results may roughly match those of Jambi (2018), who discovered that adding date powder to yoghurt samples increased their dietary fibre content, and Sendra et al. (2010), who discovered that adding various fruit types to yoghurt could increase its fibre content and, as a result, make the samples more stable in terms of their physicochemical and rheological characteristics over time. Additionally, our findings are consistent with those of McCance and Widdowson (2014), who reported that adding fruits like wild blackberries, raspberries, and strawberries to yoghurt increases its mineral content. Lastly, we can state that adding fig puree to fermented milk will increase its K, Mg, Fe, Ca, and P content, thus increasing its nutritional value.

Table 2. Fat and Protein Content of Yoghurt Made with Transglutaminase

Treatment	Sample	Day 0	Day 7	Day 14	Day 21	Mean
Fat %	To	8.15±0.06^{d}	8.13±0.04^{d}	8.13±0.04^{d}	8.12±0.05^{c}	8.18±0.04^{d}
	T1	8.66±0.05^{c}	8.52±0.05^{c}	8.54±0.07^{c}	8.55±0.17^{b}	8.58±0.02^{c}
	T2	8.85±0.04^{b}	8.82±0.12^{b}	8.84±0.60^{b}	8.87±0.14^{a}	8.85±0.70^{ab}
	T3	8.91±0.05^{a}	8.95±0.07^{a}	8.91±0.14^{b}	8.59±0.06^{b}	8.89±0.09^{a}
Protein %	To	10.51±0.07^{d}	10.56±0.07^{d}	10.54±0.13^{d}	10.60±0.07^{d}	10.58±0.08^{d}
	T1	10.96±0.04^{c}	10.95±0.12^{c}	10.96±0.05^{c}	10.98±0.06^{c}	10.95±0.10^{c}
	T2	11.33±0.08^{b}	11.39±0.13^{b}	11.39±0.15^{b}	11.41±0.08^{b}	11.38±0.11^{b}
	T3	11.61±0.03^{a}	11.68±0.07^{a}	11.70±0.01^{a}	11.75±0.16^{a}	11.69±0.06^{a}
pH	To	4.52±0.05^{a}	4.49±0.08^{a}	4.46±0.24^{ca}	4.45±0.10^{ab}	4.48±0.05^{a}
	T1	4.49±0.04^{b}	4.48±0.19^{a}	4.47±0.05^{bc}	4.46±0.05^{a}	4.49±0.04^{a}
	T2	4.47±0.22^{b}	4.47±0.12^{ab}	4.42±0.29^{ab}	4.47±0.02^{a}	4.45±0.02^{b}

Treatment	Sample	Day 0	Day 7	Day 14	Day 21	Mean
	T3	4.42±0.07[c]	4.43±0.10[bc]	4.44±0.04[a]	4.40±0.05[c]	4.42±0.07[bc]
Syneresis %	To	1.09±0.03[c]	1.08±0.05[c]	1.15±0.10[c]	1.13±0.04[d]	1.12±0.03[b]
	T1	1.16±0.07[b]	1.15±0.10[b]	1.17±0.04[c]	1.17±0.05[c]	1.15±0.05[b]
	T2	1.19±0.03[b]	1.20±0.15[a]	1.23±0.16[b]	1.25±0.02[b]	1.27±0.01[a]
	T3	1.24±0.05[a]	1.21±0.19[a]	1.28±0.04[a]	1.29±0.01[a]	1.26±0.02[a]
Total Solids %	To	22.64±0.02[c]	22.55±0.07[c]	22.55±0.07[d]	22.52±0.07[d]	22.55±0.09[d]
	T1	23.59±0.10[b]	23.53±0.03[c]	23.56±0.09[c]	23.48±0.01[b]	23.54±0.09[c]
	T2	24.48±0.04[a]	24.46±0.03[b]	24.49±0.03[b]	24.35±0.09[c]	24.70±0.07[b]
	T3	24.98±0.25[a]	24.96±0.12[a]	24.93±0.24[a]	24.85±0.03[a]	24.92±0.04[a]

Notes: Different small letters showing significant differences among different treatments ($p<0.05$).

Antioxidant Capacity

The FRAP-antioxidant activity of the transglutaminase-treated yoghurt was higher than that of the gelatin-based yoghurt, which exhibited very little antioxidative activity. This improvement could be ascribed to plant-derived enzymes, which provide possible health advantages through antioxidant activity and enhance the yoghurt's overall functional quality. Compared with Amirdivani and Baba (2015), who showed that the TPC in plain yoghurt was 4.8 µg GAE/ml, these results were significantly greater, whereas it was 6.7 µg GAE/ml in fig-based yoghurt. Numerous factors, including varietals, extraction techniques, postharvest storage conditions, and geographical origin, could cause these variations. Numerous fruits, such as fig purée, are high in phenolic compounds added to dairy products to support their antioxidant potential (Agarwal et al., 2022). Yoghurt with varying concentrations of fig purée had an antioxidant activity of up to 10 times higher than that of yoghurt without fig extract. The T3 samples contained the most antioxidants.

FTIR and Raman Spectroscopy Comparison

According to quantitative evaluations of protein and fat structures using FTIR and Raman spectroscopy, the transglutaminase-treated yoghurt showed noticeable microstructural improvements (Figure 2 and Figure 3). Improved protein cross-linking helped preserve yoghurt's shelf life and customer appeal by reducing post-acidification effects and ensuring a steady consistency (Czaja et al., 2018).

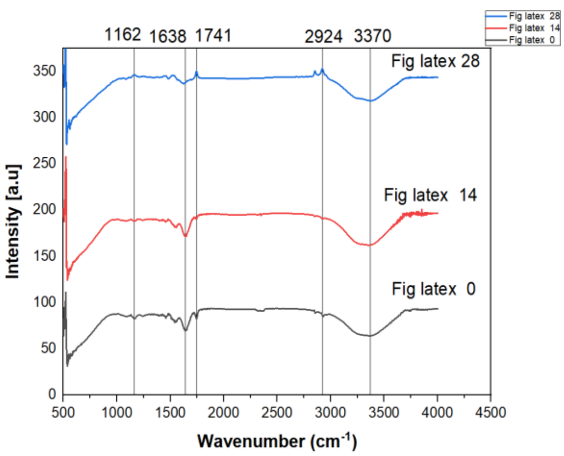

Figure 2. FTIR Spectrum of Fig-Based Yoghurt

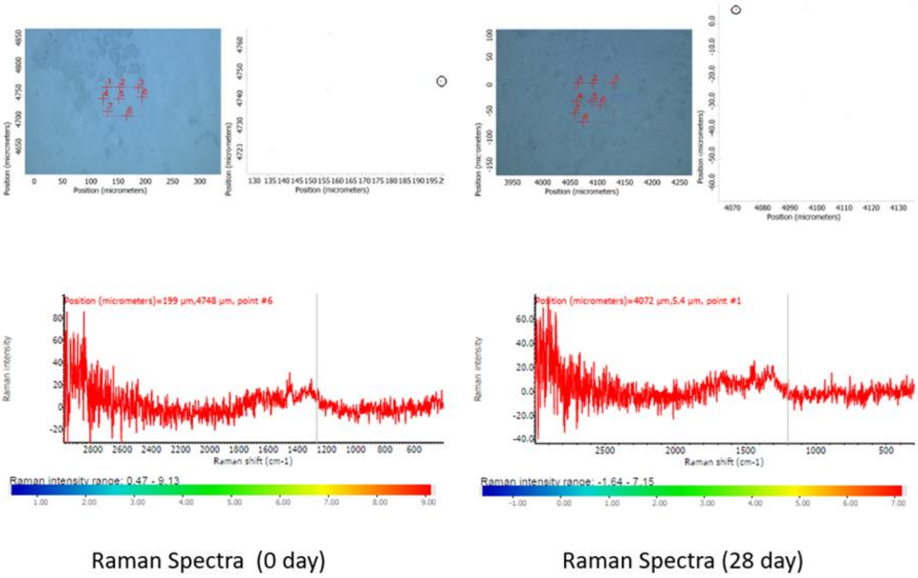

Figure 3. Raman Spectra of The Fig-Based Yoghurt

Rheological Properties and Syneresis Reduction Measurement Aspects Related to FTIR and Raman Spectroscopy

Based on the Raman and FTIR spectrum, the enzymatic transglutaminase treatment of milk greatly enhanced the yoghurt's capacity to retain water. Strengthening milk protein cross-linking, the enzyme decreased syneresis at all tested concentrations, stabilizing the yoghurt's texture. The enzymatic efficiency was directly correlated with temperature and time settings, with 45°C and 10% enzyme concentration yielding the best results (Strani, 2021).

Improved water retention and decreased syneresis in yoghurt are positively associated with higher transglutaminase concentrations. In particular, the best concentration of transglutaminase for maintaining yoghurt texture without sacrificing the product's mouthfeel and consistency was 10%. The rheological properties of the yoghurt were also greatly affected by the treatment time and temperature. The yoghurt samples exhibited the best stability at 45°C, with less syneresis at all transglutaminase doses (Wang et al., 2023). The optimal setting temperature for transglutaminase activity in yoghurt manufacturing was 45°C, whereas higher temperatures (55°C) did not further enhance syneresis control. Yoghurt samples treated with transglutaminase showed better water retention than regular gelatin-based yoghurt when stored at 4°C. Transglutaminase-induced enzymatic cross-linking improved the yoghurt's structural stability by postponing syneresis during storage (Daniloski et al., 2024).

Sensory Properties

According to sensory analysis, yoghurt containing 10% fig extract offered a distinct fruity flavour profile and high texture and mouthfeel ratings comparable to gelatin yoghurt. 2% was the ideal dosage, while the yoghurt samples with 5% fig extract were thought to be less creamy and those with 20% fig extract to be excessively thick (Table 3).

Because of its natural fig flavour and mild sweetness, which improved the yoghurt's taste profile, most sensory panel members favoured the fig-based yoghurt over the control. As a natural stabilizer, fig extract may improve product appeal in plant-based and health-conscious markets, according to this comment (Benmeziane et al., 2021). The results demonstrated changes in the sensory evaluation of yoghurt with fig puree. The statistical analysis revealed that sample T2 had the highest score for colour and flavour, whereas samples T3 and control had the lowest score for both colour and flavour. On the other hand, the T1 samples had the most significant ranking of appearance and consistency, whereas the control and T3 samples had the lowest rankings. Compared to the control, the overall acceptability of the T1 and T2 samples was very acceptable. However, throughout storage at 4±1 ºC until the end, the scores of organoleptic qualities varied across all treatments (El-Sayed & Ramadan, 2020). Teshome et al. (2017) claimed that adding fruit to yoghurt in the ideal amount enhanced the yoghurt's physic-chemical characteristics and sensory qualities of yoghurt.

Table 3. Sensory Evaluation of Yoghurt Made with Fig-Based Transglutaminase

Treatments	Samples	Day 0	Day 7	Day 14	Day 21	Mean
Sensory Evaluation (Overall Acceptability)	To	7.88±0.07^{a}	7.48±0.05^{a}	6.99±0.17^{a}	6.95±0.05^{a}	6.94±0.06^{a}
	T1	7.57±0.04^{b}	6.98±0.15^{b}	6.67±0.07^{cd}	6.33±0.08^{b}	6.09±0.08^{d}
	T2	7.34±0.08^{cd}	6.57±0.08^{d}	6.86±0.16^{b}	6.18±0.05^{c}	6.19±0.08^{c}
	T3	7.39±0.02^{c}	6.69±0.14^{c}	6.69±0.04^{c}	6.39±0.02^{b}	6.40±0.09^{b}

CONCLUSIONS

The study showed that the extract from *Ficus carica* is a good natural substitute for gelatin when making yoghurt. Without sacrificing the yoghurt's probiotic viability or microbiological quality, fig extract's pectin content effectively adds flavour, improves water-holding capacity, and enhances the desired texture. This fig-based yoghurt recipe satisfies the desire for plant-based products and appeals to customers looking for natural, animal-free substitutes. Integrating fig extract into commercial yoghurt formulas may be made easier with additional research on large-scale production and storage durability.

The new uses of this proteolytic extract (such as the production of active antibiotic fragments and promiscuous activity) and the increased focus on some traditional uses (such as milk clotting or meat tenderization) are the reasons for the academy's growing interest in ficin. This final use is because, following the "creasy cows" sickness episode, vegetable proteases align more with consumers' perceptions of their health than animal or bacterial recombinant proteases.

Ficin has long been sold commercially by Sigma (possibly at a hefty price of about 1 euro per unit). It can also be made directly from fig sap. However, this may be somewhat irreproducible depending on the makeup of the sample, meaning that its characteristics might not be entirely repeatable. Given that it is found in numerous *Ficus* species, the range of enzyme functions (pH range, stability, etc.) is enormous, and their potential uses may grow. When comparing various batches, *Ficus* cell cultures could be a smart way to obtain a comparable product in economically priced content. Since ficin extract has recently been reviewed in a new biocatalyst concept called combi enzymes, which primarily focuses on the modification of multifunctional substrates but also shows interest in simpler processes, the fact that it is actually a cocktail formed by diverse enzymes may be advantageous for some applications.

This work effectively shows that fig-based transglutaminase can be used in yoghurt instead of animal-derived gelatin, providing a functional and Halal-compliant substitute. Transglutaminase-treated yoghurt satisfies consumer quality standards and religious dietary restrictions by enhancing water retention, texture stability, and antioxidant effects. In conclusion, adding varying amounts of fig puree to yoghurt may improve its nutritional content and functional qualities. It could be used to improve the growth of *Lactobacillus* in yoghurt and boost its dietary fibre, polyphenols, antioxidant potential, and minerals. When used with probiotics, this product improves fruit's prebiotic qualities and yoghurt's probiotic qualities.

LIMITATION & FURTHER RESEARCH

In addition to the scientific findings, this study holds significant implications for the Halal and Kosher food sectors, particularly in response to the growing consumer demand for ethical sourcing and transparent labeling. By demonstrating that transglutaminase derived from fig plants can effectively replace gelatin in yoghurt production, this research offers a viable approach for producing Halal and Kosher-compliant dairy products. Transglutaminase-treated yoghurt exhibited improved texture and syneresis control at optimal concentrations (0.03%) and temperatures (45°C), aligning with the expectations of consumers seeking authentic and religiously permissible options. As consumer expectations for product authenticity continue to rise, integrating scientific techniques for ingredient verification will be essential for fostering consumer trust and supporting market growth in the Halal and Kosher sectors. Future research should explore the broader application of plant-based transglutaminase in various dairy and non-dairy food products, as well as investigate other plant-based enzyme alternatives in gelatin-dependent foods, thereby expanding the range of high-quality, ethically manufactured products available to these niche markets.

REFERENCES

Agarwal, A., Gupta, M., Kumari, A., & Gupta, A. (2022). Development and characterization of physico-chemical and functional properties of green tea yoghurt. *Journal of Microbiology, Biotechnology and Food Sciences, 11*(5), e4666. https://doi.org/10.55251/jmbfs.4666

Ahmed, M. A., Al-Kahtani, H. A., Jaswir, I., AbuTarboush, H., & Ismail, E. A. (2020). Extraction and characterization of gelatin from camel skin (potential halal gelatin) and production of gelatin nanoparticles. *Saudi Journal of Biological Sciences, 27*(6), 1596–1601. https://doi.org/10.1016/j.sjbs.2020.03.022

Ali, M. Y., Islam, M. A., Alam, M. J., & Islam, M. N. (2002). Quality of yogurt (dahi) made in laboratory and available in the market of Mymensingh Town in Bangladesh. *Pakistan Journal of Biological Sciences, 5*, 343–345. https://doi.org/10.3923/pjbs.2002.343.345

Amirdivani, S., & Baba, A. S. (2015). Green tea yogurt: Major phenolic compounds and microbial growth. *Journal of Food Science and Technology, 52*(7), 4652–4660. https://doi.org/10.1007/s13197-014-1670-6

AOAC. (2012). *Official methods of analysis* (18th ed., pp. 806–842). Association of Official Analytical Chemists.

Benmeziane, F., Raigar, R. K., Ayat, N. E. H., Aoufi, D., Djermoune-Arkoub, L., & Chala, A. (2020). Lentil (*Lens culinaris*) flour addition to yogurt: Impact on physicochemical, microbiological and sensory attributes during refrigeration storage and microstructure changes. *LWT, 140*, 110793. https://doi.org/10.1016/j.lwt.2020.110793

Bönisch, M. P., Lauber, S., & Kulozik, U. (2007). Improvement of enzymatic cross-linking of casein micelles with transglutaminase by glutathione addition. *International Dairy Journal, 17*(1), 3–11. https://doi.org/10.1016/j.idairyj.2006.01.007

Czaja, T., Baranowska, M., Mazurek, S., & Szostak, R. (2018). Determination of nutritional parameters of yoghurts by FT Raman spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 196*, 413–417. https://doi.org/10.1016/j.saa.2018.02.054

Daniloski, D., Vasiljevic, T., Freitas, D., Comunian, T. A., Brodkorb, A., & McCarthy, N. A. (2024). Physicochemical and simulated gastric digestion properties of A1/A1, A1/A2 and A2/A2 yoghurts. *Food Hydrocolloids, 157*, 110430. https://doi.org/10.1016/j.foodhyd.2024.110430

Duran, N,M. (2024). Biological activities from vegetal products in Latin America. In *Food byproducts management and their utilization* (1st ed., p. 33). Apple Academic Press. https://doi.org/10.1201/9781003377801

El-Sayed, H., & Ramadan, M. F. (2020). Production of probiotic-fermented rice milk beverage fortified with cactus pear and physalis pulp. *Zagazig Journal of Agricultural Research, 47*, 165–177. https://doi.org/10.21608/zjar.2020.70239

Farnsworth, J. P., Li, J., Hendricks, G. M., & Guo, M. R. (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. *Small Ruminant Research, 65*, 113–121. https://doi.org/10.1016/j.smallrumres.2005.05.036

Granato, D., Nunes, D. S., & Barba, F. J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. *Trends in Food Science & Technology, 62*, 13–22.

Inayat, S. (2012). Rennet Enzyme: A coagulation agent used in cheese making technology- a Review. *Journal of Animal and Plant Science, 22* (4), 634-637.

Jambi, H. A. (2018). Evaluation of physio-chemical and sensory properties of yogurt prepared with date pits powder. *Current Science International, 7*, 1–9.

Jeong, M. R., Kim, H. Y., & Cha, J. D. (2009). Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. *Journal of Bacteriology and Virology, 39*(2), 97–102. https://doi.org/10.4167/jbv.2009.39.2.97

Kilara, A. (2013). Manufacturing yogurt and fermented milks. In R. C. Chandan & A. Kilara (Eds.), *Manufacturing yogurt and fermented milks* (Chapter 13). Wiley-Blackwell. https://doi.org/10.1002/9781118481301.ch13

Lazreg-Aref, H., Mars, M., Fekih, A., Aouni, M., & Said, K. (2012). Chemical composition and antibacterial activity of a hexane extract of Tunisian caprifig latex from the unripe fruit of *Ficus carica*. *Pharmaceutical Biology, 50*(4), 407–412. https://doi.org/10.3109/13880209.2011.608192

Leal, M. R. S., Sales, P. B. S., Rodrigues, N. E. R., Silva, P. M. S., Oliveira, W. F., Correia, M. T. S., Kennedy, J. F., & Coelho, L. C. B. B. (2024). A review on the use of polysaccharides as thickeners in yogurts. *Carbohydrate Polymer Technologies and Applications, 8*, 100547. https://doi.org/10.1016/j.carpta.2024.100547

Lorenzen, P. C. (2000). Techno-functional properties of transglutaminase-treated milk. *Milchwissenschaft, 55*, 667–670.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory evaluation techniques* (4th ed.). CRC Press. https://doi.org/10.1201/b16452

McCance, R. A., & Widdowson, E. M. (2014). *McCance and Widdowson's the composition of foods*. Royal Society of Chemistry.

Morrison, N. A., Clark, R. C., Chen, Y. L., & Talashek, T. (1999). Gelatin alternatives for the food industry. In *Physical chemistry and industrial application of gellan gum* (pp. 127–131). *Progress in Colloid & Polymer Science, 114*. Springer. https://doi.org/10.1007/3-540-48349-7_19

Motoki, M., & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science & Technology, 9*, 204–210. https://doi.org/10.1016/S0924-2244(98)00038-7

O'Connell, J. E., & Fox, P. F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *International Dairy Journal, 11*(3), 103–120. https://doi.org/10.1016/S0958-6946(01)00033-4

O'Sullivan, M. M., Lorenzen, P. C., O'Connell, J. E., Kelly, A. L., Schlimme, E., & Fox, P. F. (2001). Short communication: Influence of transglutaminase on the heat stability of milk. *Journal of Dairy Science, 84*(6), 1331–1334. https://doi.org/10.3168/jds.S0022-0302(01)70162-2

Ozer, B., Kirmaci, H. A., Oztekin, S., Hayaloglu, A. A., & Atamer, M. (2007). Incorporation of microbial transglutaminase in non-fat yogurt production. *International Dairy Journal, 17*(3), 199–207. https://doi.org/10.1016/j.idairyj.2006.02.007

Prabavathy, D., & Nachiyar, C. V. (2011). Screening and characterization of antimicrobial compounds from endophytic *Aspergillus* sp. isolated from *Ficus carica*. *Journal of Pharmacy Research, 4*(6), 34–38.

Rakhmanova, A., Khan, Z. A., Sharif, R., & Lü, X. (2018). Meeting the requirements of halal gelatin: A mini review. *MOJ Food Processing & Technology, 6*(1). https://doi.org/10.15406/mojfpt.2018.06.00209

Sanders, M. E. (2007). Probiotics, strains matter. *Functional Foods Nutraceuticals Magazine, 36*, 41.

Sanli, T., Sezgin, E., Deveci, O., Şenel, E., & Benli, M. (2011). Effect of using transglutaminase on physical, chemical and sensory properties of set-type yoghurt. *Food Hydrocolloids, 25*(6), 1477–1481. https://doi.org/10.1016/j.foodhyd.2010.09.028

Sendra, E., Kuri, V., Fernández-López, J., Sayas-Barberá, E., Navarro, C., & Pérez-Álvarez, J. A. (2010). Viscoelastic properties of orange fiber enriched yogurt as a function of fiber dose, size and thermal treatment. *LWT – Food Science and Technology, 43*(4), 708–714. https://doi.org/10.1016/j.lwt.2009.12.005

Settachaimongkon, S., Nout, M. J. R., Fernandes, E. C. A., Hettinga, K. A., Vervoort, J. M., van Hooijdonk, T. C. M., Zwietering, M. H., Smid, E. J., & van Valenberg, H. J. F. (2014). Influence of different proteolytic strains of *Streptococcus thermophilus* in co-culture with *Lactobacillus delbrueckii* subsp. *bulgaricus* on the metabolite profile of set-yoghurt. *International Journal of Food Microbiology, 177*, 29–36. https://doi.org/10.1016/j.ijfoodmicro.2014.02.008

Shahinuzzaman, M., Yaakob, Z., Anuar, F. H., Akhtar, P., Abd Kadir, N. H., Hasan, A. M., Bin Rafiq, M. K., Nour, M., Sindi, H., Amin, N., Sopian, K., & Akhtaruzzaman, M. (2020). *In vitro* antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Scientific Reports, 10*, Article 11534. https://doi.org/10.1038/s41598-020-67765-1

Shiby, V. K., & Mishra, H. N. (2013). Fermented milks and milk products as functional foods—A review. *Critical Reviews in Food Science and Nutrition, 53*(5), 482–496. https://doi.org/10.1080/10408398.2010.547398

Soni, N., Mehta, S., Satpathy, G., & Gupta, R. K. (2014). Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*). *Journal of Pharmacognosy and Phytochemistry, 3*(2), 158–165.

Staffolo, M., Sato, A. C., & Cunha, R. L. (2017). Utilization of plant dietary fibers to reinforce low-calorie dairy dessert structure. *Food and Bioprocess Technology, 10*, 1988–1999. https://doi.org/10.1007/s11947-017-1872-9

Strani, L. (2021). L. (2020). *Process analytical technology approaches for dairy industry* (Doctoral dissertation, University of Milan)

Tanwar, B., Andallu, B., & Modgil, R. (2014). Influence of processing on physicochemical, nutritional and phytochemical composition of *Ficus carica* L. (fig) products. *Asian Journal of Dairying & Foods Research, 33*(1), 37–42. https://doi.org/10.5958/j.0976-0563.33.1.008

Teshome, G., Keba, A., Asefa, Z., Gebre, B., & Esho, F. (2017). Development of fruit flavored yoghurt with mango (*Mangifera indica* L.) and papaya (*Carica papaya* L.) fruit juices. *Food Science and Quality Management, 67*, 40–45.

Thakkar, U., & Preetha, R. (2016). Evaluation of fig powder as prebiotic and its utilization for development of synbiotic microcapsules. *Biosciences Biotechnology Research Asia, 13*(2), 723–729. https://doi.org/10.13005/bbra/2156

Tomic, N., Dojnov, B., Miocinović, J., Tomašević, I., Šimić, N., Djekić, I., & Vujčić, Z. (2017). Enrichment of yoghurt with insoluble dietary fiber from triticale—A sensory perspective. *LWT – Food Science and Technology, 80*, 59–66. https://doi.org/10.1016/j.lwt.2017.02.008

Tosun, M., Karlidag, H., Dzubur, A., Hadziabulic, S., & Aliman, J. (2012). Color and antioxidant characteristics of some fresh fig (*Ficus carica* L.) genotypes from northeastern Turkey. *Plant Foods for Human Nutrition, 67*(3), 271–276. https://doi.org/10.1007/s11130-012-0292-2

Uddin, S. M. K., Hossain, M. A. M., Sagadevan, S., Al Amin, M., & Johan, M. R. (2021). Halal and kosher gelatin: Applications as well as detection approaches with challenges and prospects. *Food Bioscience, 44*(Part A), 101422. https://doi.org/10.1016/j.fbio.2021.101422

Vázquez, C. V., Rojas, M. G. V., Ramírez, C. A., Chávez-Servín, J. L., García-Gasca, T., Ferriz-Martínez, R. A., García, O. P., Rosado, J. L., López-Sabater, M. C., Castellote, A. I., Andrade-Montemayor, H. M., & de la Torre-Carbot, K. (2015). Total phenolic compounds in milk from different species: Design of an extraction technique for quantification using the Folin–Ciocalteu method. *Food Chemistry, 176*, 480–486. https://doi.org/10.1016/j.foodchem.2014.12.050

Vinson, J. A. (1999). The functional food properties of figs. *Cereal Foods World, 44*(2), 82–87. https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=1723284

Wang, S. W. (2000). Gelatin replacement in yogurt application. *Abstract of IFT Annual Meeting*, Dallas, TX, USA, Paper No. 64-6, June 10–14.

Wang, W., Jia, R., Hui, Y., Zhang, F., Zhang, L., Liu, Y., Song, Y., & Wang, B. (2023). Utilization of two plant polysaccharides to improve fresh goat milk cheese: Texture, rheological properties, and microstructure characterization. *Journal of Dairy Science, 106*(6), 3900–3917. https://doi.org/10.3168/jds.2022-22195