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Use of *Terminalia arjuna* (Kumbuk) Leaves as a Plant-derived Stabilizer to Replace Gelatin in Set Yoghurt

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***Abstract*—Stabilizers in yoghurt enhance texture and prevent whey separation in yoghurts. Gelatin is commonly used as the primary stabilizer in yoghurt. For various health and ethical reasons, consumers might opt to refrain from gelatin, often driven by concerns related to animal welfare and treatment. In this study, plant-derived stabilizers, particularly *Terminalia arjuna* (Kumbuk) leaf extract, was explored as an alternative to gelatin in set yoghurt. *Terminalia arjuna* offers additional nutritional benefits such as antibacterial, antimicrobial, and antioxidant properties while possessing stabilizing power. This study investigated the physicochemical, microbiological, and sensory characteristics of set yoghurt incorporated with kumbuk leaf extract. The experimental design followed a completely randomized design (CRD) with four treatments: a control group using 0.6% gelatin, and three experimental groups using 0.4%, 0.6%, and 0.8% kumbuk leaves extracts. Set yoghurts were assessed for pH, titratable acidity, syneresis, and sensory evaluation, and viability of fermentative bacteria (Streptococcus thermophiles and Lactobacillus bulgaricus) over 21 days at refrigerated storage.**

***Keywords*—Substitutes, gelatin, stabilization, *Terminalia arjuna*, yoghurt**

I. Introduction

Yoghurt, a widely consumed fermented dairy product known for its health benefits and nutritional value, is currently one of the fastest-growing segments in the global dairy industry. Yoghurt's stabilizing properties encompass attributes like enhancement characteristics, gel-forming qualities, emulsification properties, pH adjustment capabilities, and even potential medicinal properties [1]. Gelatin, a primary stabilizer in yoghurt, serves the dual roles of binding water and increasing viscosity. The desire to find substitutes for gelatin arises from a range of factors, such as adhering to vegan and vegetarian diets, respecting cultural or religious dietary constraints, and managing allergies and sensitivities [1]. Substitutes commonly used in yoghurt to replace gelatin include agar-agar, pectin, tapioca starch, or carrageenan. These alternatives can substitute gelatin's thickening and gelling properties while omitting animal-derived ingredients, allowing yoghurt producers to accommodate a broader spectrum of consumer preferences and dietary requirements.

Terminalia arjuna leaves possess gelling and thickening characteristics due to the presence of certain natural compounds such as hydrolysable tannins. These tannins have the ability to interact with proteins and form complexes that contribute the gel-like structure in food and pharmaceutical applications [2].

The objective of this study is to determine the applicability of kumbuk leaf extract as a natural stabilizer in set yoghurt without compromising the quality attributes.

II. Methodology

A. Preparation of Kumbuk Leaf Extract

Kumbuk leaves were collected from the Low Country Wet Zone in Sri Lanka. Leaves were thoroughly cleaned, cut into pieces, blended, and squeezed to form a gel with filtering. The collected gel was stored at 4°C after extraction.

B. Experimental Design

The experimental design was a complete randomized design (CRD) with four treatments and three replicates per treatment. Set yoghurt mixture including 0.6% of gelatin and 0%of leaf gel was used as the control group whereas the

treatment groups of yoghurts were prepared with 0.4%, 0.6%, and 0.8% of kumbuk leaf extracts.

C. Sensory Evaluation

Sensory evaluation was carried out using 30 untrained panelists to select the best concentration of leaf mucilage using 9-point hedonic scale to select the best concentration of kumbuk leaf extract to be incorporated into set yoghurt. Appearance, color, odor, taste, texture, and mouth feel of the yoghurt samples were evaluated

D. Analysis of Physicochemical Properties

After selecting the best treatment group out of the three concentrations of kumbuk leaf extracts, the selected treatment group was further analyzed along with the yoghurt of the control group.

E. pH Value and Titratable Acidity

The pH, and titratable acidity of the yoghurts were measured according to the method described in AOAC, (2005). The yoghurt sample dissolved in distilled water and mixed well. The amount of 1 ml of Phenolphthalein indicator will be introduced into 10 ml of the mixed solution. It will be then titrated against 0.1 N Sodium Hydroxide solution until a pink color emerges for complete neutralization.

Calculations

$$\text{TTA \%} = \frac{(\text{N x V x Molecular weight of lactic acid}) \times 100\%}{\text{Weight of the sample}}$$

Where;

TTA %= Total titratable acidity

N = Normality of NaOH

V = Volume of NaOH

F. Determination of Syneresis

Syneresis of the yoghurt samples was measured using undisturbed yoghurt samples (without mechanical disturbances) during cold storage at 4°C. The syneresis measurement was carried out using a 25-ml measuring cylinder by draining out the expelled whey carefully [3].

G. Viability of Fermentative Bacteria in Yoghurts

The viability of Lactobacillus bulgaricus and Streptococcus thermophiles bacteria in yoghurts was measured as described by Almusallam et al. [4]. In order to assess the effect of functional ingredients on the fermentation process.

III. RESULTS AND DISCUSSION

This study focused on evaluating the potential of kumbuk as a plant-derived stabilizer to replace gelatin in set yoghurt and employed a multifaceted approach, incorporating sensory evaluation, physicochemical analysis, and microbiological assessment to determine the optimal concentration of kumbuk in yoghurt production.

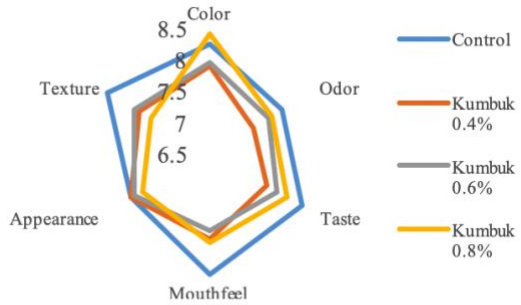

Fig. 1. Sensory evaluation of yoghurt incorporated with Terminalia arjuna (Kumbuk) leaves.

Significantly, the inclusion of 0.6% kumbuk in the yogurt led to a distinct variation in its texture, setting it apart from other yogurt samples containing different levels of kumbuk. Consequently, the yogurt group incorporated with 0.6% kumbuk was chosen for more extensive analysis. According to the Tab. 1. Over the storage period of yoghurt samples, their pH levels decreased in control samples. However, the kumbuk extract incorporated yoghurt sample exhibited some fluctuations during the storage period. In contrast, titratable acidity (Tab. 2.) increased progressively throughout the storage duration. The pH decreases and acidity increase is attributed to the high metabolic activity of lactic acid bacteria in the yoghurt [5]. Furthermore, adding kumbuk at 0.6% concentration increased syneresis (Tab. 3), which was observed to rise in both the control and treatment groups as the storage period continued. Similar findings have been observed in previous investigations [6], and have been linked to milk acidification, which causes a decrease in the charges on caseins, weakening the electrostatic forces that keep micelles together and lower steric stability. This reduces the attractive force between micelles, which become 'adhesive' or sticky and form a weak particle gel with serum separation [7]. Incorporating kumbuk leaf extract at a concentration of 0.6% reduced L. bulgaricus (Tab. 4) counts throughout the storage period. Further, while the yoghurts of the control group exhibited an increase in L. bulgaricus growth over the storage period, the treatment group indicated a decline in L. bulgaricus counts. These might be due to the available compounds in the yoghurt medium and produced compounds to the medium during the storage period [8]. The introduction of kumbuk at a 0.6% concentration had a contrasting effect on the growth of S. thermophiles (Tab. 5). The counts of S. thermophiles increased notably on Day 14 and Day 21 in the treatment group, demonstrating a growth enhancement during these specific time points, and this growth trend continued over the course of the storage period.

TABLE 1. PH VALUES (MEAN±STANDARD DEVIATION) OF YOGHURT INCORPORATED WITH KUMBUK LEAF EXTRACT

Treatment	*Day 1*	*Day 7*	*Day 14*	*Day 21*
Control (0.6% gelatin)	4.32 ± 0.01^{bA}	4.25 ± 0.01^{bB}	4.23 ± 0.01^{aC}	4.18 ± 0.01^{D}
Kumbuk (0.6% leaf gel)	4.36 ± 0.01^{aB}	4.40 ± 0.01^{aA}	4.17 ± 0.01^{bC}	4.18 ± 0.01^{C}

[a-b] Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[A-D] Mean values in the same row with different superscripts differ significantly ($p<0.05$)

TABLE 2. TITRATABLE ACIDITY (MEAN±STANDARD DEVIATION) OF YOGHURT INCORPORATED WITH KUMBUK LEAF EXTRACT

Treatments	*Day 1*	*Day 7*	*Day 14*	*Day 21*
Control (0.6% gelatin)	0.56 ± 0.01^{C}	0.58 ± 0.01^{bC}	0.63 ± 0.01^{bB}	0.73 ± 0.01^{A}
Kumbuk (0.6% leaf gel)	0.55 ± 0.01^{D}	0.64 ± 0.01^{aC}	0.72 ± 0.01^{aA}	0.71 ± 0.01^{B}

[a, b] Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[A-D] Mean values in the same row with different superscripts differ significantly ($p<0.05$)

TABLE 3. SYNERESIS (MEAN±STANDARD DEVIATION) VALUES OF YOGHURT INCORPORATED WITH KUMBUK LEAF EXTRACT

Treatment	*Day 1*	*Day 7*	*Day 14*	*Day 21*
Control (0.6% gelatin)	32.78 ± 0.74^{bC}	32.52 ± 0.35^{bC}	37.43 ± 0.43^{bB}	38.92 ± 0.15^{bA}
Kumbuk (0.6% leaf gel)	35.87±0.49aD	37.23±0.66aC	42.43±0.80aB	44.25±0.35aA

[a, b] Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[A-D] Mean values in the same row with different superscripts differ significantly ($p<0.05$)

TABLE 4. VIABILITY OF L.BULGARICUS (MEAN±STANDARD DEVIATION) IN YOGHURT INCORPORATED WITH KUMBUK LEAF EXTRACT

Treatment	*Day 1*	*Day 7*	*Day 14*	*Day 21*
Control (0.6% gelatin)	7.96 ± 0.15^{aC}	9.40 ± 0.10^{aB}	9.86 ± 0.25^{aB}	11.86 ± 0.32^{aA}
Kumbuk (0.6% leaf gel)	5.30 ± 0.20^{bB}	6.63 ± 0.20^{bA}	4.66 ± 0.20^{bC}	1.22 ± 0.05^{bD}

[a-b] Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[A-D] Mean values in the same row with different superscripts differ significantly ($p<0.05$)

TABLE 5. VIABILITY OF S.THERMOPHILES (MEAN±STANDARD DEVIATION) IN YOGHURT INCORPORATED WITH KUMBUK LEAF EXTRACT

Treatment	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Control (0.6% gelatin)	4.23 ± 0.15^{C}	7.13 ± 0.15^{aAB}	7.50 ± 0.10^{aA}	6.66 ± 0.41^{bB}
Kumbuk (0.6% leaf gel)	4.66 ± 0.20^{C}	6.10 ± 0.11^{bB}	7.26 ± 0.15^{aA}	7.70 ± 0.20^{aA}

[a-b] Mean values in the same column with different superscripts differ significantly ($p<0.05$)
[A-D] Mean values in the same row with different superscripts differ significantly ($p<0.05$)

IV. CONCLUSION

This study exhibited, the replacing of gelatin with *Terminalia arjuna* (kumbuk) leaf extract at the concentration of 0.6%. The results showed that replacing gelatin with kumbuk leaf extract accomplished positive results on the pH, acidity, and syneresis. The replacement of gelatin with 0.6% kumbuk leaf extract also showed positive impact directly on the pH, acidity, and some microbiological qualities. However, the effect on the S. thermophiles while indicating some antimicrobial action on L.bulgaricus. Furthermore, kumbuk leaf extract incorporation caused some higher syneresis and showed some drawbacks during this work. This should be addressed and investigated in near future works to confirm the suitability of plant-derived stabilizers for replacing the gelatin.

REFERENCES

[1] Farag, M.A., et al., Dissecting yogurt: The impact of milk types, probiotics, and selected additives on yogurt quality. 2022. 38(sup1): p. 634-650.
[2] Amalraj, A., S.J.J.o.t. Gopi, and c. medicine, Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: a review. 2017. 7(1): p. 6578.
[3] Seo, M., et al., Physicochemical, microbial, and sensory properties of yogurt supplemented with nanopowdered chitosan during storage. 2009. 92(12): p. 5907-5916.
[4] Almusallam, I.A., et al., Effect of date palm (Phoenix dactylifera L.) spikelets extract on the physicochemical and microbial properties of set-type yogurt during cold storage. LWT, 2021. 148: p. 111762.
[5] Ahmad, I., et al., Studying the influence of apple peel polyphenol extract fortification on the characteristics of probiotic yoghurt. 2020. 9(1): p. 77.
[6] García-Pérez, F., et al., Effect of orange fiber addition on yogurt color during fermentation and cold storage. 2005. 30(6): p. 457-463.
[7] Asiimwe, A., J.B. Kigozi, and J.J.A.F.S.J. Muyonga, Physicochemical Properties, Sensory Acceptance and Storage Stability of Yogurt Flavored with Refractance Window Dried Passion Fruit Powder. 2021. 20(5): p. 38-49.
[8] Gueimonde, M., et al., Quality of plain yoghurt made from refrigerated and CO2-treated milk. Food Research International, 2003. 36(1): p. 43-48.