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Substituting Gelatin with Stabilizer Derived from Fiddleheads of *Acrostichum aureum* (Karan koku) in Set Yoghurt

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Abstract*—Stabilizers play a prominent role in improving the texture of yoghurt and preventing undesirable whey separation. Moreover, stabilizers rich in hydrophilic colloids possess the capability to bind water, increase viscosity, and ultimately enhance the overall quality of yoghurt. This research investigated the potential of utilizing plant-derived stabilizers, specifically the extract from *Acrostichum aureum,* known as Karan Koku (KK) as a promising substitute for gelatin in the production of set yoghurt. The experimental design was developed with four treatments: a control group using 0.6% gelatin, and three experimental groups using 0.4%, 0.6%, and 0.8% Karan Koku fiddlehead extracts. Sensory properties were analyzed for yoghurt samples containing KK fiddlehead extract at these three concentrations and the best treatment group was selected. The chosen treatment group, along with the control group of yoghurt made with 0.6% gelatin underwent and investigation of their physicochemical and microbiological properties. Yoghurt prepared with 0.6% KK exhibited the most favorable sensory attributes compared to the others. While a decrease in pH was observed in KK added yoghurt during storage, it remained within the acceptable pH range in yoghurt. The titratable acidity was notably higher in KK added yoghurt in first two weeks. Additionally, there was a significant increase in syneresis in the KK added yoghurt over the storage period. Streptococcus thermophilus showed significantly higher levels in KK added yoghurt. KK fiddlehead extract, at a concentration of 0.6% can be used as stabilizer without having much detrimental effects on quality attributes, however further studies are required to reduce the syneresis.

Keywords—Substitutes, gelatin, stabilization, Acrostichum aureum, yoghurt

I. INTRODUCTION

Stabilizers are essential ingredients in yoghurt production which involves in improving the texture, consistency, and shelf life of yoghurts. Common stabilizers used in yoghurt production include gelatin, pectin, agar, carrageenan, and guar gum. These stabilizers help prevent issues such as syneresis (whey separation) and maintain the desired thickness of yoghurt. Gelatin is widely used but poses challenges for vegans, vegetarians, and those with dietary restrictions, leading to the exploration of alternative stabilizers [1]. *Acrostichum aureum*, also known as Karan koku, is a fern species that has gained attention as a potential natural stabilizer in yoghurt production. Its properties make it a promising alternative to gelatin. This fern offers stabilizing attributes, binding water, and increasing the thickness of yoghurt [1].

Unique combination of its stabilizing properties and additional nutritional benefits would be beneficial in using it as a major ingredient in yoghurt manufacturing. Apart from its stabilizing power, KK possesses antibacterial, antimicrobial, and antioxidant properties. These added health benefits can enhance the overall appeal of yoghurt, making it an attractive choice for both producers and health-conscious consumers [1].

The main objective of this study was to investigate the effect of KK extract as a stabilizer on physicochemical, sensory, and microbiological properties of set yoghurt.

II. METHODOLOGY

A. Preparation of Acrostichum aureum Fiddlehead Extract

KK fiddleheads were collected from the Low Country Wet Zone in Sri Lanka. Fiddleheads 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 experiment was designed with four experimental groups and three replicates per treatment. Set yoghurt prepared with 0.6% of gelatin and 0% of fiddlehead gel was used as the control group whereas the treatment groups of yoghurts were prepared with 0.4%, 0.6%, and 0.8% of KK fiddlehead extracts [2].

C. Sensory Evaluation

Sensory evaluation was carried out using 30 semi-trained panelists to select the best concentration of fiddlehead mucilage using 9-point hedonic scale to select the best concentration of KK fiddlehead 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 KK fiddlehead extracts, the selected treatment group was further analyzed along with the yoghurt of 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 was then titrated against 0.1 N Sodium Hydroxide solution until pink color emerged for complete neutralization.

Calculations

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

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 25ml measuring cylinder by draining out the expelled whey carefully [3].

G. *Viability of Fermentative Bacteria in Yoghurts*

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

III. Results and Discussion

This study focused on evaluating the potential of KK as a plant-derived stabilizer to replace gelatin in set yoghurt.

The sensory assessment findings (Figure 1), clearly highlighted a significant variation in certain sensory attributes when 0.6% KK was introduced into the yoghurt. This observation held great importance, prompting the choice of 0.6% KK for subsequent analysis.

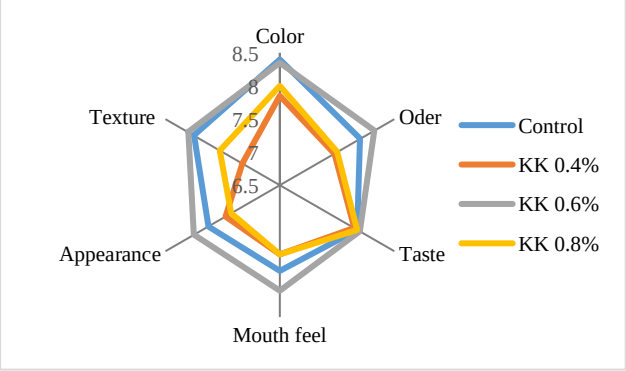

Fig. 1. Sensory evaluation results of set yoghurt incorporated with KK

As indicated in Table 1, the pH values of both the control and treatment groups did not exhibit any significant differences, except on Day 14. Although the pH value showed a decline during the storage period in the yoghurt with KK addition, all the samples consistently maintained their pH within the range of 4.0 to 4.6. This consistency suggests that the product quality remained stable and stayed within the crucial acidity range required for yoghurt, ensuring its quality and safety [5].

Table 2 revealed increase in titratable acidity of the yoghurt samples over the storage period despite the addition of KK. This finding demonstrates that the acidity of the yoghurt remained on an upward trend during storage, which can be attributed to various factors such as microbial activity and fermentation [6]. Minimum titratable acidity level of 0.15% had been established as a quality criterion for yoghurt [7] while all the samples in this study exceeded this predetermined threshold. This means that even with the incorporation of KK the acidity levels in the yoghurt remained well above the minimum requirement for yoghurt quality.

Information on syneresis provides valuable insights into the water-holding capacity and stability of the yoghurt during the storage period. Syneresis refers to the separation of whey or liquid from the yoghurt's gel-like structure. In this study, the addition of KK. resulted in a significant increase in stabilizing property.

The study involved monitoring the growth of two essential yoghurt cultures, L. bulgaricus and S. thermophilus over a 21 days' storage period (Table 4, Table 5). It was observed that the growth of these critical yoghurt cultures did not exhibit any decline until Day 14. This suggests that both L. bulgaricus and S. thermophilus maintained their populations reasonably well during the initial two weeks of storage. The presence of KK led to a decrease in the count of L. bulgaricus on Day 1 and Day 21 compared to the control group, indicating a reduction in the population of this specific bacteria. In contrast, the addition of KK had a different effect on S. thermophilus, as it contributed to an increase in its growth.

This finding is significant as it suggests that the addition of 0.6% KK did not negatively impact the viability of these essential bacteria. The viability of these cultures is crucial for yoghurt fermentation and quality because they are responsible for the fermentation process [6].

TABLE 1. CHANGE OF PH VALUES (MEAN±STANDARD DEVIATION) IN SET YOGHURT INCORPORATED WITH KK

Treatment	Day 0	Day 7	Day 14	Day 21
Control (0.6% gelatin)	4.32±0.01	4.25±0.01	4.23 ± 0.01^{a}	4.18±0.01
KK (0.6%				
fiddlehead gel)	4.32 ± 0.01^{A}	4.26 ± 0.01^{B}	4.20 ± 0.01^{bC}	4.18 ± 0.01^{D}

[a, b] Differing superscripts within columns indicate means that were significantly different (P<0.05).

[A, D] Differing superscripts within row indicate means that were significantly different (P<0.05).

TABLE 2. CHANGE OF TITRATABLE ACIDITY (MEAN±STANDARD DEVIATION) IN SET YOGHURT INCORPORATED WITH KK

Treatments	Day 0	Day 7	Day 14	Day 21
Control (0.6%) gelatin)	0.56 ± 0.01^{bC}	0.58 ± 0.01^{bC}	0.63 ± 0.01^{B}	0.73 ± 0.01^{A}
KK (0.6% fiddlehead gel)	0.60 ± 0.01^{aC}	0.63 ± 0.01^{Ab}	0.63 ± 0.05^{B}	0.71 ± 0.01^{A}

[a, b] Differing superscripts within columns indicate means that were significantly different (P<0.05).

[A, C] Differing superscripts within row indicate means that were significantly different (P<0.05).

TABLE 3. SYNERESIS (%) (MEAN±STANDARD DEVIATION) IN SET YOGHURT INCORPORATED WITH KK

Treatment	Day 0	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}
KK (0.6% fiddlehead gel)	42.93 ± 0.53^{aB}	42.24 ± 0.84^{aC}	42.13 ± 0.29^{aC}	46.36 ± 0.15^{aA}

[a, b] Differing superscripts within columns indicate means that were significantly different (P<0.05).

[A, C] Differing superscripts within row indicate means that were significantly different (P<0.05).

TABLE 4. *LACTOBACILLUS BULGARICUS* GROWTH (LOG CFU/G) IN SET YOGHURT INCORPORATED WITH KK

Treatment	Day 0	Day 7	Day 14	Day 21
Control (0.6% gelatin)	7.96 ± 0.15^{aC}	9.40 ± 0.10^{B}	9.86 ± 0.25^{B}	11.86 ± 0.32^{aA}
KK (0.6%				
fiddlehead gel)	3.66 ± 0.15^{bC}	9.53 ± 0.25^{A}	9.63 ± 0.30^{A}	8.06 ± 0.30^{bB}

[a, b] Differing superscripts within columns indicate means that were significantly different (P<0.05).

[A, C] Differing superscripts within row indicate means that were significantly different (P<0.05).

TABLE 5. *STREPTOCOCCUS THERMOPHILUS* GROWTH (LOG CFU/G) IN SET YOGHURT INCORPORATED WITH KK

Treatment	Day 0	Day 7	Day 14	Day 21
Control (0.6% gelatin)	4.23 ± 0.15^{bC}	7.13 ± 0.15^{aAB}	7.50 ± 0.10^{bA}	6.66 ± 0.41^{bB}
KK (0.6% fiddlehead gel)	5.33 ± 0.21^{aD}	6.56 ± 0.25^{bC}	11.33 ± 0.07^{aA}	9.73 ± 0.45^{aB}

[a, b] Differing superscripts within columns indicate means that were significantly different (P<0.05)

[A, D] Differing superscripts within row indicate means that were significantly different (P<0.05).

IV. CONCLUSION

The findings from the current study support the replacement of gelatin with selected plant-derived stabilizers, which is with 0.6% KK fiddlehead extract. Results indicated that addition of KK at 0.6% concentration did not have any adverse effects on pH and titratable acidity values. Furthermore, findings of this study demonstrated that the addition of KK specifically at a concentration of 0.6% had a positive effect on S. thermophilus, ultimately supporting the overall quality of yoghurt production. By observing the increase in syneresis due to KK addition, this research highlights a potential challenge that needs to be addressed to ensure the product's stability and consumer appeal. It may prompt further investigations into optimizing the formulation or processing conditions to mitigate this issue while still harnessing the benefits of KK as a stabilizer.

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