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PHYSICOCHEMICAL PROPERTIES OF YOGHURT PROCESSED FROM THE MILK OF COWS FED CLOVE BUD (*Syzygium aromaticum*) AND BLACK SEED (*Nigella sativa*) POWDER AS AFFECTED BY STORAGE

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ABSTRACT

This study evaluated the physicochemical properties of yoghurt processed from the milk of cows fed clove bud powder (CBP) and black seed powder (BSP). Twelve Romanian spotted dairy cows were divided into four groups: negative control, positive control (synthetic antioxidants), CBP and BSP (1.5 % DM) in a completely randomized design. The cows were fed for 21 days, milk collected and processed into yoghurt. The yoghurts were analysed over 28 days of refrigerated storage. Results showed that pH (4.208 to 4.112) and titratable acidity (1.22 to 0.88 %) decreased, while ash (0.640 to 1.300 %), protein (3.068 to 3.435 %), moisture (89.075 to 94.675 %), and fat content (2.478 to 3.850 %) ($p < 0.05$) significantly increased with storage days (day 0 to 28, respectively). The study concludes that supplementing cow diets with CBP and BSP improved the nutritional profile offering a natural alternative to synthetic additives. For a more nutrient-dense yoghurt, it is recommended that dairy cows be fed up to 1.5 % DM of clove bud and black seed powders.

Keywords: Yoghurt, Clove bud powder, Black seed powder, Physicochemical, Antioxidant

INTRODUCTION

For a long time, people have believed that fermented foods are healthy and nutritious, and they may even increase the availability of some nutrients (Abdul Hakim *et al*., 2023; Horlacher *et al*., 2023). There is a lot of interest in and a new approach to product development presented by a number of initiatives to make yoghurts enriched with herbs and spices from natural sources (Gahruie *et al*., 2015; Caleja *et al*., 2016). Around the world, livestock diets have been supplemented with plant extracts to boost the milk's nutritional benefits, which has many positive health effects. Black seed, basil, turmeric, cinnamon, clove, dill, ginger, mint, oregano, rosemary, saffron, sage, and thyme are some of the most often used herbs and spices (Carlsen *et al*., 2010). According to Milind and Deepa (2011), clove buds contain about 5.98% protein, 20% total fat, 61.21% carbs, 34.2% fiber, and 5.88% ash. The black seed (*Nigella sativa*) powder (BSP) contains about 18.09 - 26.7 % protein, 28.5 - 45.4 % fat, 5.11 – 18.96 % fiber, 4.39 – 7.1 % moisture and 9.52 – 40.0 % carbohydrates (Kabir *et al*., 2019; Karrar *et al*., 2022; Uddin *et al*., 2024).

The global demand for functional foods, particularly dairy products with enhanced health benefits and extended shelf-life, is steadily increasing (Abdi-Moghadam *et al*., 2023). Phytogenic extracts can help in extending the shelf life of dairy products (especially fermented dairy products like yoghurt) through their suppressing effect on fungi and bacteria (Aljobair, 2024). Clove (*Syzygium aromaticum*) bud powder and black seed (*Nigella sativa*) powder are rich sources of bioactive compounds, including potent antioxidants and antimicrobial agents and novel food ingredients enriched with polyphenolic compounds (Obeidat *et al*., 2023; Bikheet *et al*., 2025; Adetoro *et al*., 2025). Eugenol and thymoquinone are abundant in clove and black seeds serving as their principal constituents (Ulanowska and Olas, 2021; Aljobair, 2024), harnessing secondary plant metabolites, like eugenol and thymoquinone as antimicrobial presents a novel approach to developing a functional yoghurt by increasing its shelf-life (Ferrando *et al*., 2024). Butylated hydroxy toluene (BHT) is a well-established synthetic antioxidant widely used in animal feed to prevent lipid oxidation and preserve feed quality (Bampidis, *et al*., 2022). A direct comparison of the effects of natural supplements (clove and black seed) with a synthetic antioxidant like BHT, when administered through the cow's diet, is crucial. Such a comparison will provide valuable insights into the relative effectiveness and potential advantages of natural alternatives in enhancing dairy product quality. The objective of this study was to evaluate the effect of storage period on the physicochemical properties of yoghurt stored for a period of 28 days at 4 ºC.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the Microbiology Laboratory of the Department of Veterinary Medicine, University of Life Sciences, King Mihai I of Timisoara, Romania. The University of Life Sciences, King Mihai I of Timisoara, Romania lies in the Western part of the country – Romania. It is located between longitude $45^0$78'83.84" N and latitude 21^0 22'60.11" E (Nistor *et al*., 2018).

Experimental Materials

Clove buds and black seeds were purchased from the metropolitan market in Kano state, Nigeria. Dairy cows in a private farm at Giulvaz County, Timisoara were used for the experiment. Fresh milk collected from the dairy cows was processed into yoghurts.

Clove Bud Powder (CBP) and Black Seed Powder (BSP)

Healthy and matured buds of clove (*Syzygium aromaticum*) were cleaned and the dust free samples were air dried at 30 °C for 30 minutes in a ventilated drying oven with a model no. (BOV-T50F BIOBASE). The samples were ground using laboratory hammer mill (HWM Series - Infitek) into a fine ground form. Exposure to direct sunlight was carefully avoided to prevent the loss of active components (Uddin *et al*., 2017). The same process was applied to the black seed.

Experimental Animals and Management

A total of 12 dairy cows, Romanian spotted breed were divided randomly into four (4) treatments group of 3 cows per treatment. Each group was randomly assigned to one of the treatments. Namely; negative control, positive control (mixture of gallates, BHT and citric acid), CBP and BSP. The cows were fed for 21 days (2 weeks' adaptation period and 1-week feeding). After which milk was collected and processed into yoghurt. Table 1 shows the supplemented diet fed to dairy cows in a private farm at Giulvaz county in Timisoara (Romania).

Table 1: Gross Composition of Supplemented Diet fed to Dairy Cows in Timisoara Farm

Ingredients	Inclusion (%)			
	Neg control	BHT (+ve)	CBP	BSP
Wheat bran	30.00	29.99	29.85	29.85
Barley	27.50	27.50	27.50	27.50
Wheat	27.50	27.50	27.50	27.50
Sunflower meal	12.00	12.00	12.00	12.00
Common salt	1.00	1.00	1.00	1.00
$CaCO_3$	1.00	1.00	1.00	1.00
Premix (dairy)	1.00	1.00	1.00	1.00
Neg control	0.00	0.00	0.00	0.00
BHT (+ve)	0.00	0.01	0.00	0.00
CBP	0.00	0.00	0.15	0.00
BSP	0.00	0.00	0.00	0.15
Total (%)	100.00	100.00	100.00	100.00

Neg control – negative control; BHT – butylated hydroxytoluene; CBP – clove bud powder; BSP – black seed powder

Yoghurt Processing

Milk was processed into yoghurt in an industry located at Timis County, Masloc communer about 37 km from the University of Life Sciences, Timisoara, Romania as described by Nguyen *et al*. (2017). Fresh milk was pasteurized to a temperature of 85 ^{o}C for 30 minutes. Then, cooled to incubation temperature of 42 ^{o}C after which starter culture (a mixed strain of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) was inoculated. Fermentation took place for about 6 to 8 hours in a warm place (38 ^{o}C). The yoghurt samples were then labeled, packaged into 300ml plastic containers and stored in refrigerator at 4 ^{o}C for analysis.

Determination of Yoghurt Physicochemical Properties

The physicochemical properties; pH, titratable acidity, total fat, total solids, ash content and total protein were determined as described by AOAC (2000) during all the storage days (0, 7, 14, 21 and 28) at 4 ^{o}C.

Experimental Design and Statistical Analysis

The experiment was conducted in a 4x5 factorial arrangement of a completely randomized design. The factors were four yoghurt types {negative control, positive control (Butylated hydroxytoluene, BHT), clove bud powder yoghurt (CBPyog) and black seed powder yoghurt (BSPyog)} and five storage periods (day 0, 7, 14, 21 and 28). Data collected on physicochemical properties of yoghurt samples were analysed for variance (ANOVA) and differences among means were separated using Tukey-HSD in a JMP version 17.0 package.

RESULTS AND DISCUSSION

Table 2 shows the physicochemical properties of yoghurt processed from cows fed diets supplemented with CBP and BSP as affected by storage period. During storage in this study, there was a decrease in the pH values of yoghurts as the storage period progressed up to 28 days. The decrease could be attributed to the lactic acid production from bacterial cultures as highlighted by Ouazib *et al*. (2023). This observation was similar to what Ouazib *et al.* (2023) reported for day 0 (4. 61) and day 21 (4.14). Similarly, Al Shaikh *et al*. (2022) found a decrease in pH values of yoghurt fortified with extracts of black seed from the first day of storage up to 21 days as the storage period increases with the control yoghurt having 4.37 while the yoghurt fortified with extracts of black seed had 4.36. pH is an important factor in the processing of yoghurt, as it offers information about the shelf life of yoghurts. Its decrease during storage is mainly due to the action of lactic acid bacteria, which continue to transform lactose into lactic acid (Abdalla and Ahmed, 2010). Gulbas and Saldamli (2005) realized a decrease in the pH values of fortified cheese after two months from 4.71 at day 0 to 4.54, which is in agreement with the present study. The measurement of TA is an indicator of bacterial metabolic activity (Ouazib *et al*., 2023) in fermented dairy products. Al Shaikh *et al*. (2020) and Aljobair (2024) realized an increase in TA up to 21 days as the storage period increases with the highest values 1.21 % and 0.95 %, this was not in accordance with this study. This might be linked to the buffering capacity of peptides and amino acids released from proteolysis, which would require more alkali to titrate to the endpoint, thus giving a lower TA. The TA of the yoghurt added with mulberry leaves extract (MLE) increased throughout the storage with the highest value, 1.04 % on day 21 stored at 4 ^{o}C (Tang *et al*., 2024). This is not in agreement with this research. This could be attributed to the effect of black seed powder which causes an increase in the growth of lactic acid bacteria but since the powder was fed to the cows, its effect might be reduced.

Al Shaikh *et al*. (2020) observed that as the storage period progressed, the moisture contents in yoghurt decreased in yoghurt supplemented with black seed extract with the lowest 85.51 % at storage day 21. This is not in line with this study. The increase in moisture content recorded in this study could be due to the coagulated gel phase being analysed on days 7, 14, 21 and 28 without mixing back the expelled whey into the yoghurt. The results of Ali *et al*. (2022) in cheese fortified with black seed extracts does not compare favourably with the current investigation. The reason for this increase maybe due to the low evaporation rate of moisture content during storage.

Atwaa *et al*. (2022) recorded the protein of yoghurt supplemented with fennel extracts at day 0 (3.68 %) and day 21 (3.92 %) increase as the storage period progresses. This is similar to the result of this finding. The protein of yoghurt produced from milk of cows fed fodder supplemented with herbal mixture (oregano, thyme, cinnamon, purple coneflower) reported a decrease in protein content with initial (day 0) 3.52 % and final (day 21) 3.30 % as the storage period progresses (Stobiecka *et al*., 2023). This is contrary to what was found in this research. This could be traced to the fact that protein became more concentrated in the gel as water was lost. However, some authors, Paskudska *et al*. (2018); El-Sayed and Youssef (2019); Walkenhorst *et al*. (2020); Radzikowski *et al*. (2020) reported that the use of herbal blends in cow nutrition improved the protein content resulting in an increase in the content of bioactive components such as whey proteins in dairy products which is in agreement with this study. Khalil *et al*. (2022) and Nakov *et al*. (2024) worked on chia seeds enriched yoghurt and observed that the protein content remains stable during storage up to 21 days with initial day 1 (3.92 %) and final day 21 (4.59 %) which is not in line with this investigation. This might be caused by factors such as enzymatic activity or microbial growth, which can affect protein solubility or lead to degradation (Nakov *et al*., 2024). The fat content of yoghurt during storage was observed to remain constant (Nakov *et al*., 2024) which was contrary to what was reported in the current research. Stobiecka *et al*. (2023) found a decrease in fat content as the storage period progresses which is not in agreement with the present study. Paskudska *et al*. (2018); El-Sayed and Youssef (2019); Walkenhorst *et al*. (2020); Radzikowski *et al*. (2020) worked on herbal blends fed to cows and observed an increase in the fat contents of yoghurts with an increase in unsaturated fatty acids, this was similar to the present investigation. The increase in fat is due to the relative concentration effect caused by the loss of other components such as water and dissolved solids.

Furthermore, Atwaa *et al*. (2022) recorded an increase in the ash content of yoghurt added fennel extracts as affected by storage up to 21 days at 4 °C, with day 0 (0.78 %) and day 28 (0.94 %), this compared with the results of this findings with increased ash content as the storage time progressed. In a research conducted by Sanusi *et al*. (2023) on yoghurt fortified with soursop puree, the ash content values increased with an increase in the storage period up to 28 days with the initial (0.59 %) and the final (0.69 %), this is similar to the results of this study although the values reported in this research were higher. This is attributed to the fact that yoghurt bacteria break down casein proteins, they release minerals that are bound within the protein micelles, which increases the measurable mineral content in the yoghurt sample. Hamid and Abdelrahman (2012) reported an increase in ash content values in white cheese fortified with various cinnamon, cardamom and fenugreek during storage. Although, the values were higher than those revealed in the current research. This could be due to the chemical composition of CBP, BSP and the mode of supplementing the herbs in the diet of cows as most of the minerals goes to the body system, not all is remitted in the milk.

Table 2: Main Effect of Storage Period on Physico-Chemical Properties of Yoghurt

Storage Period (days)	pH	TA (%)	Ash (%)	Protein (%)	MC (%)	Fat (%)
0	4.21^{a}	1.22^{a}	0.64^{e}	3.07^{b}	89.08^{e}	2.48^{c}
7	4.19^{ab}	1.19^{b}	0.74^{d}	3.18^{ab}	89.81^{d}	3.08^{bc}
14	4.17^{ab}	1.10^{c}	0.80^{c}	3.32^{ab}	91.13^{c}	3.45^{ab}
21	4.16^{bc}	1.04^{d}	1.02^{b}	3.37^{ab}	92.90^{b}	3.67^{ab}
28	4.11^{c}	0.88^{e}	1.30^{a}	3.44^{a}	94.68^{a}	3.85^{a}
SEM	0.012	0.201	0.006	0.084	0.078	0.152
P-VALUE	<0.001	<0.001	<0.001	0.024	<0.001	<0.001

^{abcd} means with different superscript in a column are significantly different; TA – titratable acidity; MC – moisture content; SEM – standard error of the mean; P-VALUE – probability value (P<0.05).

CONCLUSION

The research revealed that supplementing dairy cow diets with clove bud powder (CBP) and black seed powder (BSP) influenced the physicochemical properties of yoghurt. pH and titratable acidity (TA) decreased over the 28-day storage period, while ash, protein, moisture, and fat content increased. CBP and BSP can serve as effective natural alternatives to synthetic antioxidants like BHT in cow nutrition. Yoghurt from cows fed CBP and BSP exhibited improved nutritional profiles, characterized by higher protein and fat content compared to the control. This enhances the product's marketability as a nutrient-dense functional food.

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