



The effect of adding black cummin oil (*Nigella sativa*) with different concentrations in the kefir manufacturing process on pH, viscosity and water binding capacity

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Abstract

Kefir is a fermented dairy product that involves a variety of lactic acid bacteria (LAB) in its fermentation. Lactic acid bacteria in kefir have been widely reported to provide health benefits to the human digestive system when consumed, as they help control or regulate the population of pathogenic bacteria. This study aims to determine the influence of the addition of black cummin oil with different concentrations in the kefir-making process on the pH value, viscosity, and water-holding capacity of the resulting kefir product. This research was conducted experimentally using a completely randomized design (CRD) with 5 treatments and 4 replications, involving percentages of cummin oil consisting of 1% (P1), 2% (P2), 3% (P3), 4% (P4), and 5% (P5). The research data were analyzed using analysis of variance and Duncan's multiple range test. The results of the study indicate that the addition of black cummin oil in the kefir-making process with concentrations of 1%, 2%, 3%, 4%, and 5% significantly affects the pH value, viscosity, and water-holding capacity. A higher concentration of added black cummin oil in the kefir-making process results in a decrease in the pH value, viscosity, and water-holding capacity of kefir.

Keyword: Milk, Kefir, black cummin, viscosity, water-holding capacity

Introduction

Milk is a food ingredient with high nutritional content, containing water, protein, fat, carbohydrates, minerals, and vitamins. The comprehensive nutritional content of milk makes it susceptible to spoilage due to both internal factors (from the animal) and external factors (milk collection and processing). To reduce the risk of milk spoilage, milk can be processed through pasteurization and fermentation, transforming it into kefir. Processing milk into kefir not only extends the milk's shelf life but also enhances its nutritional value.

Kefir is a fermented dairy product with a tangy flavor and a slight alcohol content, resulting from the fermentation process that utilizes lactic acid bacteria (*Lactobacillus bulgaricus* and *Lactobacillus casei*) and yeast (*Candida kefir* and *Saccharomyces cerevisiae*). These microorganisms play a role in breaking down the lactose content in milk, leading to a decrease in pH and the emergence of the tangy taste in kefir.

Kefir, a fermented dairy product derived from cow's milk, offers various health benefits, such as controlling cholesterol metabolism, providing probiotic properties, anti-tumor effects in animals, and antibacterial and antifungal properties. These benefits are due to kefir containing probiotics that promote health and having a composition of 0.65-1.33 g/l CO₂, 3.16-3.18% protein, 3.07-3.17% fat, 1.8-3.8% lactose, 0.5-1.5% ethanol, and 0.7-1.0% lactic acid (Ide, 2008).

Black cummin oil is commonly consumed by people due to its believed benefits, including preventing allergic reactions in asthma, anti-tumor effects, antibacterial properties, and anti-inflammatory effects (El-Dakhakhny *et al.*, 2002). The advantages of black cummin oil are attributed to active compounds such as *thymoquinone*, *thymohydroquinone*, *dithymoquinone*, *thymol*, *carvacrol*, *nigellidine*, *nigellimine-N-oxide*, and *alpha-hedrin* (Hutapea, 1994).

The addition of black cummin oil in the kefir-making process is expected not only to provide health benefits but also to enhance the physical quality of the resulting kefir. The physical quality includes parameters like pH level, viscosity, and water-holding capacity of the kefir product when black cummin oil is added during the fermentation process. Therefore, this research aims to determine the impact of adding black cummin oil with different concentrations in the kefir-making process on the pH, viscosity, and water-holding capacity of the kefir product.

Materials and methods

Fresh cow's milk is obtained from dairy farms in Tanjungsari, Sumedang, Indonesia. Black cummin oil is obtained from an herbal medicine store located in Tanjungsari, Sumedang, Indonesia. The kefir starter is obtained from *Yoghourmet*, a part of the Lallemand Group based in Montreal, Canada.

Production of Mother Kefir Starter

The production of the kefir starter begins by dissolving 15% skim milk in 50 ml of water, followed by pasteurization at 60°C for 30 minutes. Subsequently, the milk is cooled to a temperature of 27°C - 28°C and inoculated with 0.5% (0.25 grams) of the kefir starter. It is then incubated at 27°C for 24 hours (Setyawardani & Sumarmono, 2015).

Production of Black Cummin Kefir

Black cummin kefir is prepared by pasteurizing 50 ml of fresh milk at 60°C for 30 minutes. The milk is then cooled to a temperature of 27°C - 28°C, and black cummin oil is added with a concentration of 1-5% of the total milk volume. It is inoculated with 5% kefir starter and incubated at 27°C for 24 hours.

pH Value

The pH testing process is performed using an electronic pH meter. Before calculating the pH value, the pH meter's electrode tip needs to be prepared by cleaning it with distilled water and then drying it with a tissue. The cleaned electrode tip is calibrated by dipping it into buffer solutions with pH values of 4 and 7 (Wahyudi, 2006). Subsequently, the electrode tip is dipped into a 25 ml sample of yogurt that has been supplemented with black cumin oil. The pH reading can be observed on the pH meter.

Viscosity

Yogurt viscosity is measured using a viscometer following the method reported by Suliasih *et al.* (2018). Kefir with the addition of black cumin oil is first gently stirred in a jar using a stirring rod for 30 seconds. A cylindrical spindle is used at a speed of 30 rpm for 60 seconds. Viscosity is expressed in centipoise (dPa's).

Water Holding Capacity (WHC)

WHC is measured based on the procedure by Setyawardani *et al.* (2020) with slight modifications as follows: A 10 ml sample is weighed and then placed in a tube. It is centrifuged at a speed of 3000 rpm for 10 minutes at room temperature. The separated whey is weighed, and WHC is calculated using the formula:

$$WHC (\%) = \frac{\text{Initial sample mass} - \text{final sample mass}}{\text{Initial sample mass}} \times 100\%$$

Statistical Analysis

This research was conducted using the Completely Randomized Design (CRD) method, consisting of 5 treatments, which are the addition of black cumin oil at 1% (P1), 2% (P2), 3% (P3), 4% (P4), and 5% (P5), and each treatment was repeated four times, resulting in a total of 20 experimental units. The data for pH value, Water Holding Capacity, and Viscosity were then analyzed using Analysis of Variance (ANOVA) and further tested using the Duncan's Multiple Range Test (Gasperz, 1995).

Results and discussions

pH Value

The research results regarding the effect of the addition black cumin oil on the pH value of kefir are presented in Table 1.

Table 1: pH Values of Kefir under Various Treatments

Replications	The Treatment of Black Cumin Oil Addition				
	1%	2%	3%	4%	5%
1	4,28	4,27	4,29	4,29	4,31
2	4,26	4,28	4,29	4,30	4,31
3	4,27	4,29	4,28	4,29	4,32
4	4,28	4,27	4,30	4,31	4,30

Based on the data presented in Table 1, it is evident that kefir with the addition of black cumin oil has an average pH value ranging from 4.26 to 4.31. The pH values in kefir with added black cumin oil tend to increase. The analysis of variance (ANOVA) results indicate that the addition of black cumin oil in kefir production has a significant effect (P<0.05) on the pH value of kefir. Subsequently, a Duncan's multiple range test was conducted to determine the

differences among treatments involving the addition of black cumin oil in the kefir-making process.

Table 2: Mean pH Values of Kefir under Various Treatments

Concentration	Mean pH Values
1%	4,272 ^a
2%	4,277 ^{ab}
3%	4,290 ^{bc}
4%	4,297 ^{cd}
5%	4,310 ^d

Note: Mean values followed by different lowercase letters (subscripts) in the same row indicate statistically significant differences.

Based on Table 2, it is evident that the pH values of kefir with the addition of black cumin oil significantly differ for each treatment. The pH values of kefir with the addition of black cumin oil remain within the recommended range for kefir, as stated by Martharini and Indratiningsih (2017), which falls within the pH range of 4.2 - 4.6. This research indicates that the addition of black cumin oil has an impact on increasing the pH values of the resulting kefir. The pH elevation in the kefir is attributed to the fact that black cumin oil has a pH range of 5 (Arlin *et al.*, 2020). The higher pH values in the black cumin oil cause the pH of kefir with black cumin oil addition to increase with the rising concentration provided.

Viscosity

The research results regarding the effect of the addition of black cumin oil on the viscosity of kefir are presented in Table 3.

Table 3

Replications	The Treatment of Black Cumin Oil Addition (dPa's)				
	1%	2%	3%	4%	5%
1	0,55	0,50	0,50	0,50	0,45
2	0,60	0,55	0,55	0,55	0,40
3	0,50	0,55	0,50	0,45	0,50
4	0,55	0,50	0,50	0,50	0,45

Based on the data presented in Table 3, it is evident that kefir with the addition of black cumin oil has an average viscosity value ranging from 0.45 to 0.60 dPa's. The viscosity values in kefir with added black cumin oil tend to decrease. The analysis of variance (ANOVA) results indicate that the addition of black cumin oil in kefir production has a significant effect (P<0.05) on the viscosity values of kefir. Subsequently, a Duncan's multiple range test was conducted to determine the differences among treatments involving the addition of black cumin oil in the kefir-making process.

Table 4: Mean Viscosity Values under Various Treatments

Concentration	Mean Viscosity Values (dPa's)
1%	0,55 ^a
2%	0,52 ^{ab}
3%	0,51 ^b
4%	0,50 ^b
5%	0,45 ^b

Note: Mean values followed by different lowercase letters (subscripts) within the same row indicate statistically significant differences.

Based on Table 4, the results of Duncan's multiple range analysis show that the viscosity values of kefir with the addition of 1% black cumin oil are significantly different when compared to concentrations of 3%, 4%, and 5%, but not significantly different from the 2% concentration. Viscosity values are a measure of the thickness of a food product. The decrease in viscosity values of the resulting kefir is due to the addition of liquid black cumin oil, which can reduce the viscosity of the kefir. The viscosity values of kefir are significantly influenced by the dry matter content contained in kefir (Krisnaningsih *et al.*, 2018). The viscosity values of kefir can be affected by the protein content in milk, as milk proteins have the ability to bind water, leading to an increase in viscosity (Prastiwi *et al.*, 2018).

Water Holding Capacity (WHC)

The research results regarding the effect of the addition of black cumin oil on the water holding capacity of kefir are presented in Table 5.

Table 5

Replications	The Treatment of Black Cumin Oil Addition (%)				
	1%	2%	3%	4%	5%
1	81,72	82,17	81,57	80,02	80,90
2	82,93	82,01	82,44	80,68	80,99
3	82,93	82,92	82,75	82,50	79,81
4	83,27	83,35	82,35	81,97	80,17

Based on the data presented in Table 5, it is evident that kefir with the addition of black cumin oil has an average water holding capacity ranging from 79.81% to 83.50%. The water holding capacity values in kefir with added black cumin oil tend to decrease. The analysis of variance (ANOVA) results indicates that the addition of black cumin oil in kefir production has a significant effect (P<0.05) on the water holding capacity values of kefir. Subsequently, a Duncan's multiple range test was conducted to determine the differences among treatments involving the addition of black cumin oil in the kefir-making process

Tabel 6: Mean Water Holding Capacity Values for Various Treatments

Concentration	Mean WHC Values (%)
1%	82,71 ^a
2%	82,65 ^a
3%	82,27 ^{ab}
4%	81,29 ^{bc}
5%	80,46 ^c

Note: Mean values followed by different lowercase letters (subscripts) within the same row indicate statistically significant differences.

Based on Table 6, the results of Duncan's multiple range analysis show that the water holding capacity values of kefir with the addition of 1% black cumin oil are significantly different when compared to concentrations of 3%, 4%, and 5%, but not significantly different from the 2% concentration. The water holding capacity value represents kefir's ability to retain water when subjected to centrifugation. The decrease in water holding capacity values of the resulting kefir is due to the addition of liquid black cumin oil, which can reduce the water holding capacity of the kefir. The water holding capacity values of kefir produced represent the quality of the kefir. The higher

the quality of kefir, the greater its ability to retain free water (Aloglu & Oner, 2013). The water holding capacity values of kefir can be influenced by the fat content in milk, as the fat content in milk has the ability to bind water, leading to an increase in water holding capacity in kefir (Nguyen *et al.*, 2018).

Conclusions

The addition of black cumin oil in the kefir-making process at concentrations of 1%, 2%, 3%, 4%, and 5% significantly affects the pH value, viscosity, and water holding capacity. The higher the concentration of black cumin oil added in the kefir-making process, the lower the pH value, viscosity, and water holding capacity of kefir.

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