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RESEARCH ARTICLE

Sensory properties of traditional strained yogurts produced using different ratios of bacterium in yogurt culture

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Abstract: The aim of the study is to reveal the effect of different bacterium ratio in yogurt culture on the sensory properties of the product in strained yogurt samples produced in the traditional way. In the study, yogurt production was carried out by using the ratios of 20%+80%, 30%+70% and 50%+50% of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, respectively. Color and appearance, odor, consistency (with a spoon), consistency (in the mouth), taste, flavor and general taste characteristics of the strained yogurts obtained in production were evaluated and scored by the panelists sensorially. In addition, the panelists identified defects in color and appearance, taste and flavor, structure and texture in the strained yogurt samples presented to them as well as providing their personal comments about the products. In the study, the effect of bacterium ratio in strained yogurt samples is statistically significant in terms of all sensory characteristics evaluated. In this sense, strained yogurt samples produced at a bacterium ratio of 50%+50% received the highest scores in terms of preference. In the overall evaluation, the most preferred product by the panelists was strained yogurt samples produced from a bacterium ratio of 50%+50%.

Key words: Bacterium ratio, Sensory properties, Sensory test, Strained yogurt, Yogurt culture

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Introduction

Yogurt is a fermented dairy product obtained as a result of the symbiotic activity of *L. bulgaricus* and *S. thermophilus* specifically. Yogurt curd is expressed as a heat-induced acid-casein gel (Aryana and Olson, 2017;Kaur et al. 2020). The addition of starter culture to the milk to be processed into yogurt is called inoculation, and the amount of starter culture added is called inoculum amount. In production, starter culture is added after the heat-treated yogurt milk is cooled to the incubation temperature. Starter culture is a microorganism containing selected single or mixed strains that provide the desired sensory, textural and rheological properties in yogurt and provide standard quality characteristics in the final product, and *S.thermophilus* and *L. bulgaricus* mixture culture is used in different proportions in yogurt production. These strains are defined as a ubiquitous family of microbes that can ferment glucose into lactic acid as the major catabolic end product in a specific dairy environment. Generally, the ratio of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in yogurt starter cultures is 1:1 or 1:2, respectively. The different ratios of starter cultures have a significant effect on fermentation time, pH, viscosity and other characteristics of the product. The amount of culture to be added is determined according to the type of culture used and the amount of milk to be processed into yogurt (Alline et al. 2018; Dan et al. 2023; Yang et al. 2017).

Mixed fermentation with the two microorganisms gives the yogurt good textural properties. *S. thermophilus* uses the peptides and free amino acids produced by *L. delbrueckii* subsp. *bulgaricus*, while *L. delbrueckii* subsp. *bulgaricus* uses the pyruvic acid, formic acid, folic acid and long-chain fatty acids produced by *S. thermophilus*. In addition, the production of metabolites such as amino acids and short peptides contributes to the formation of flavour substances in the yogurt (Agagunduz et al. 2015; Dan et al. 2019).

Dairy farm generally first prepare the main culture from commercial culture by multiplying, then the intermediate culture. Finally, the bulk culture (enterprise culture) is prepared and an average of 2-3% of this culture is added to the milk (Kenzheyeva et al. 2022). The lyophilized culture can be used directly without multiplication

since it is frozen at very low temperatures. Therefore, the usage rate is in accordance with the information written on the packaging. The incubation process is one of the most important steps in yogurt production and this process should be terminated when the pH value of the yogurt reaches the isoelectric point of casein. The isoelectric point of casein is pH 4.5-4.6. The water holding capacity of yogurt is optimal pH 4.2-4.6 and therefore incubation in production is terminated at pH 4.5–4.6 (Zimmerman et al. 2020; Yang et al. 2017).

Despite the ease of making yogurt and its many benefits, the shelf life of it is limited. In some cases, its quality may deteriorate within a few days and become inconsumable. Taking into account this situation, various durable yogurts are made in many regions of Turkey and in some other countries, as well. In Turkey, yogurts that have long shelf life are obtained by reducing the water of normal yogurt by a certain amount in various ways (bag yogurt, winter yogurt, dry and tulum yogurt, etc.) (Kirdar and Karaca, 2017; Kirdar, 2022).

The sensory evaluation of any food is a scientific field used to measure, analyze and interpret the human responses to food characteristics perceived through the five senses (sight, taste, odor, touch, and hearing) (Ruiz-Capillas and Herrero, 2021). In addition to the many objective evaluation methods used today, sensory evaluation maintains its importance. Sensory tests are generally used to improve an existing product, to ensure quality in daily production, to develop a new product and in marketing analysis. Yogurt is a preferred food for its sensory properties such as texture, color and appearance, as well as its health benefits. Sensory features of yogurt are determined by numerous variables; milk composition, starter culture, production method, heat treatment, incubation temperature, final incubation pH and cooling process. In yogurt sensory tests, difference and quality-quantity test methods and scales are used. In the sensory analyses of yogurts, panelists evaluate external appearance, consistency with spoon and mouth, odor, taste and flavor, and valuable information about the quality of the yogurt can be obtained (Aktar, 2022). Some investigations have shown that different ratios of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in starter cultures have a critical influence on the resulting aroma of yogurt and are important for determining the overall flavour and sensory properties of the final product (Dan et al. 2017).

The aim of this study is to determine the effect of bacterium ratio in yogurt culture on the sensory properties of the product in strained yogurts produced in the traditional way.

Materials and methods

Bacterial strains

Lyophilized yogurt culture (microMilk S.r.l) used in the study was obtained from the Referans Food Industry and Foreign Trade Limited Company. The culture rate was adjusted in 3 different ways*: 20% Lactobacillus bulgaricus*+80% *Streptecoccus thermophilus*, 30% *Lactobacillus bulgaricus*+70% *Streptecoccus thermophilus* and 50% *Lactobacillus bulgaricus*+50% *Streptecoccus thermophilus*.

Materials

The research was carried out at Aydin Adnan Menderes University, Çine Vocational School, Milk and Products Application Unit. Cow milk, which was used as raw material in the study, was purchased from the factory. The milk, which came to the facility with steel transport containers, was passed through the straining cloth and the metal strainer on the milk intake scale, and its rough cleaning was ensured and the amount was measured in kg. and recorded. pH values (WTW 3310 pH Meter Set 5) were measured by taking a sample of milk from the incoming milk in a glass beaker in an amount representing the batch (Metin and Öztürk, 2017).

Production of strained yogurt

The raw milk was taken to the cooking boiler and heated with steam at 85 ºC in 1 seconds, and the milk that completed the heat treatment was cooled to 48 ºC with a heat exchanger plate in the same boiler. The cooled milk was weighed and divided into 3 batches in equal amounts, taken into separate steel churn and lyophilized yogurt culture was inoculated into the milk. The amount of culture added to the milk in each churn is 6-8 grams per 100 kg of milk. In the study, lyophilized yogurt culture was used and the amount of culture used on the packaging was taken into account (according to the amount of milk to be processed). After the addition of culture, the inoculated milk, which was transferred to the incubation cabinet, was left for incubation. The temperature of the incubation cabinet was set to 45 ºC and the incubation was terminated by pH control. Incubation was terminated when the pH values measured with a pH meter in three separate steel troughs were 4.40. Incubation period was on average 4 hours.

The products that have completed their incubation are taken to the refrigerator operating 0-4 ºC and cooled for 12 hours waiting. The most commonly used method in the production of strained yogurt in Turkey is filtering in a cloth bag. This traditional method is based on the principle of filtering the classic natural yogurt by placing it in cloth bags. The cloth bags used in straining the yogurt have very small pores. According to this traditional method, after cooling, the products were transferred separately to the straining cloths, hung on hooks and naturally filtered for 15 hours. 100% cotton press cloth was used for the filtering process. At the end of the period, pH values were determined by using a pH meter device in strained yogurt samples. In strained

yogurt samples, L*(brightness), a* (redness), b* (yellowness), c (saturation) and H (hue) values were read and recorded using a colorimeter device (3NH TECHNOLOGIES NR200) after calibration. Measurements were made in three iterations and average values were calculated.

Sensory evaluation

In the sensory analysis applied in the study, students studying at Aydin Adnan Menderes University, Department of Food Processing took part and all panelists have successfully completed the course on sensory analysis of foods. There were 14 female students between the ages of 19-25 and 6 male students between the ages of 19-21 in the panel. All the panelists are semi-trained. Before the test, brief information was shared with the panelists about the reason for the difference in the strained yogurt samples, the production method, how the test would be applied and ethical rules of testing. In the study, sensory technique scoring and hedonic scale test were used. Sensory testing was carried out in a controlled environment with standardized lighting and temperature. Three different strained yogurt samples were presented to the panelists at the same time at a temperature of +4-6 ºC, weighing 20 grams each and with transparent plastic containers and different codes.

The panelists evaluated the strained yogurt samples using a 5-point scale (1: poor, 2: moderate, 3: good, 4: very good, 5: excellent) in terms of color and appearance, odor, consistency (with a spoon), consistency (in the mouth), taste, flavor and general taste characteristics. In addition, the hedonic scale was used in the study, and the panelists marked the expressions given to them in the test such as color and appearance (non-homogeneous color and appearance, fat separation, serum separation, gas formation, porosity, presence of foreign matter, unnatural color), taste and flavor (bitter taste, absence of typical yogurt flavor, excessive acid taste, excessive sour taste, buttery taste, metallic taste) and structure and texture (too loose or too firm, too dry, rough, poor gel formation, sticky).

Statistical analysis

The data obtained from the study were analyzed by analysis of variance (ANOVA) using SPSS 18.0 statistical program and statistical difference was determined at $P < 0.05$ level with the help of Tukey-LSD multiple comparison test.

Results and Discussion

The results of the sensory test are presented in Table 1 and Figure 1. In the sensory test applied in the study, the effect of culture bacterium ratio was statistically significant in terms of all sensory characteristics evaluated in strained yogurt samples ($P<0.001$). In terms of color and appearance, odor, consistency (with a spoon), consistency (in the mouth), taste and flavor, the highest scores were obtained by strained yogurt samples produced at a bacterium ratio of 50% + 50%.

The most important difference in sensory tests was determined in the consistency (spoon) evaluation, and the average scores according to the bacterium ratio were 1.30, 2.35 and 3.75, respectively. The highest score (3.85) in terms of general appreciation was found in strained yogurt samples produced at a bacterium ratio of 50%+50% ($P<0.001$). In terms of all sensory characteristics, the total score was calculated as 12.55, 15.75 and 26.00, respectively ($P<0.001$).

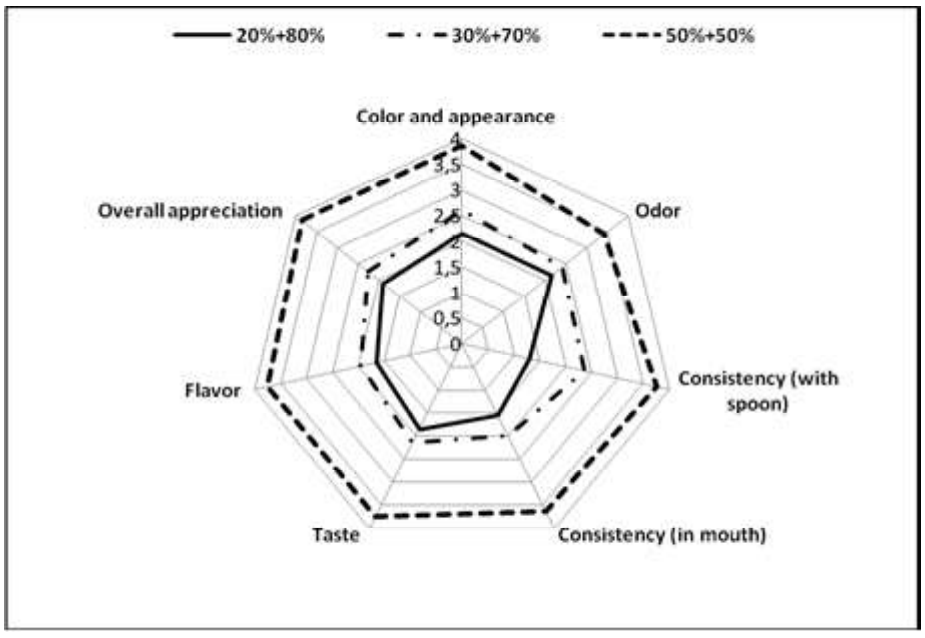

Fig 1. Sensory analysis graph of three different strained yogurt samples

The high score obtained at a bacterium rate of 50%+50% in terms of color and appearance is also directly proportional to the color measurements made with the colorimeter device. In the measurements made with the device, the highest saturation (c) ($P<0.05$) and hue (H) values were determined in the strained yogurt samples produced at a bacterium ratio of 50%+50%. The effect of culture bacterium ratio in terms of L*, a* and b* values is statistically insignificant. The effect of the bacterium ratio is important in terms of pH measurement in strained yogurt samples ($P<0.05$).

In the sensory test, 45% of the panelists detected inhomogeneous color and appearance, 35% of the panelists detected porpourity, 55% of the panelists detected lack of typical yogurt flavor and 35% of the panelists detected excessive sour taste defects in strained yogurt samples produced at a bacterium rate of 20%+80%. In strained yogurt samples produced at a bacterium ratio of 30%+70%, 35% of the panelists identified porosity, 45% of the panelists identified lack of typical yogurt flavor, and 55% of the panelists identified excessively sour taste defects. In strained yogurt samples produced at a bacterium rate of 50%+50%, the defect rate detected in terms of all features is at a very low level.

In addition, in the comments of the panelists who participated in the test, it was stated that the strained yogurt samples produced at a bacterium rate of 20%+80% were very watery, porous, very sour, bitter taste and rough, while for the strained yogurt samples produced at a bacterium rate of 30%+70%, it was stated that they had a liquid structure, sour and acid taste, acceptable flavor and dense consistency. Strained yogurt samples produced at a bacterium ratio of 50%+50% were evaluated as the most appreciated product by the panelists, and expressions of dense consistency, good taste, delicious and fluidity were included for this product.

The optimum growth temperature of *L. bulgaricus* found in yogurt culture is 40-50 ºC and this bacterium has the ability to ferment glucose, fructose, galactose and lactose into lactic acid (Wang et al 2021). The optimum growth temperature for *S. thermophilus* is 40–45 ºC, and this bacterium is capable of fermenting glucose, fructose, lactose, and sucrose (Huang et al. 2024). Yogurt fermentation consists of two stages. In the first stage, *L. bulgaricus*, which has proteolytic activity, stimulates the growth of *S. thermophilus* by releasing essential amino acids from casein (Çakmakoglu et al. 2023). In this stage, *L. bulgaricus* grows more slowly. Due to the high lactic acid concentration, the growth of *S. thermophilus* slows down at the end of the first stage. The second stage begins when *S. thermophilus* produces sufficient amounts of formic acid, which stimulates the growth of *L. bulgaricus*, and through this symbiotic effect, the desired final acidity of the yogurt is achieved (Liu et al. 2016;Singh and Mandal, 2019; Yu et al. 2021).

Table 1 The effect of different bacterium ratios on sensory properties, pH and color parameters of strained yogurt

	20%+80% Bacterium Rate	30%+70% Bacterium Rate	50%+50% Bacterium Rate	SHO	P
Color and appearance (avg.)	2.15^{b}	2.60^{b}	3.85^{a}	0.201	<0.001
Odor (avg.)	2.15^{b}	2.40^{b}	3.40^{a}	0.197	<0.001
Consistency (with spoon) (avg.)	1.30^{abc}	2.35^{abc}	3.75^{abc}	0.179	<0.001
Consistency (in mouth) (avg.)	1.55^{b}	2.00^{b}	3.65^{a}	0.184	<0.001
Taste (avg.)	1.85^{b}	2.15^{b}	3.75^{a}	0.189	<0.001
Flavor (avg.)	1.65^{b}	1.95^{b}	3.75^{a}	0.199	<0.001
Overall appreciation (avg.)	1.90^{b}	2.25^{b}	3.85^{a}	0.168	<0.001
Total score (avg.)	12.55^{abc}	15.75^{abc}	26.00^{abc}	0.901	<0.001
Strained yogurt end product pH	4.07^{a}	3.94^{b}	3.96^{b}	0.030	0.038
L* (brightness)	99.49	99.88	99.99	0.242	0.083
a* (redness)	-2.87	-3.74	-5.04	0.850	0.122
b* (jaundice)	8.92	12.71	13,43	1.370	0.058
c (saturation)	9.31^{b}	13.27^{a}	14.62^{a}	1.470	0.043
H (tint)	107.54	106.90	110.05	3.660	0.565

SHO: Standard mean of error; P: Significance; a-c: Differences between features bearing different letters on the same line are significant ($P<0.05$)

Complex biochemical reactions resulting from the activity of *L. bulgaricus* and *S. thermophilus* bacteria used in starter culture are highly effective on the sensory properties of yogurt. Appearance and color are one of the most important criteria in determining the sensory properties of yogurts. In a good quality yogurt, the appearance should be bright, the color should be white and homogeneous, and the consistency should be dark. There should be no serum separation in yogurt, there should be no gas formation and mold. In sensory tests, the dispersibility of yogurt between the tongue and the palate, whether it is plump or not, and its uniformity are among the other details emphasized. In this sense, a yogurt with a good consistency should be homogeneous and dense, and should not show a fragmented and water-released structure (Yang et al. 2017). Yogurt has a unique odor. The natural odor of yogurt, free from foreign odors, plays an important role in the evaluation of the panelists in sensory analysis. The taste parameter is another important criterion affecting the quality of yogurt and the milk from which it is obtained (Barba et al. 2017; Matejčeková et al. 2019).

Our enjoyment of yogurt is governed by our perception of aroma, taste and texture. Amongst these, flavour has the greatest effect on consumer acceptance and preference. In general, in addition to lactic acid, pyruvic acid, butyric acid, succinic acid, acetic acid, propionic acid, acetaldehyde, acetone, acetoin and a small amount of diacetyl are effective in the aroma and flavor formation of yogurt (Liu et al 2022). Among these, acetaldehyde is reported to be responsible for the typical yogurt flavor (Farag et al. 2022). When a single strain of *L. bulgaricus* or *S. thermophilus* is used in yogurt production, lactic acid and acetaldehyde production is lower compared to that in a mixed culture. *L. bulgaricus* is more capable in producing both acid and acetaldehyde, n-pentaldehyde, and 2-heptanone compared to *S. thermophilus* (Tian et al. 2019).

The viscosity of yogurt is also affected by the type of starter culture used in production (Silva et al. 2017). In studies investigating the effect of strains of *L. bulgaricus* and *S. thermophilus*, it has been reported that these bacteria produce exopolysaccharides that help increase viscosity (Tiwari et al. 2021).

In terms of sensory analysis, in many studies conducted on both fresh yogurt and strained yogurts, the product type, production method, starter cultures used in yogurt production, sensory test method, scoring and other scales used and the characteristics of the panelists participating in the test make a difference on the results. Variations in sensory evaluation results arise due to the use of sensory organs as a tool. The transformation of these tests into an objective evaluation, not a subjective evaluation, can only be achieved by careful planning of the tests and optimizing the conditions. In this study, the increases in taste, odor, flavor and consistency scores due to the increase in *L. bulgaricus* ratio are consistent with the effect of culture on sensory characteristics stated in the literature.

In a study, different ratios of *L. delbrueckii* subsp. *bulgaricus* IMAU20312 and *S. thermophilus* IMAU80809 (1:1, 1:10, 1:100, 1:1000, 1:2000) and commercial yogurt culture were used for yogurt production. The sensory panel was comprised of 15 highly experienced and screened judges who were familiar with dairy products. Colour, taste, aroma and texture of the samples were graded using the 100 point intensity scale according to the Chinese dairy industry guideline. The colours of the yogurts were determined using a ten point scale. Taste and aroma were determined between a range of 0–40, and texture was measured on a 50 point scale. In a reslut, the scores for the 1:100 bacterium ratio were higher than the other four samples and closer to the commercial control (Dan et al. 2023).

In a study, as a starter, *Lactobacillus delbrueckii* ssp. *bulgaricus* (M58) and *Streptococcus thermophilus* (S10) were used and found that the use of the M58 and S10 combination yielded synergistic benefits, positively impacting the physical, chemical, and sensory attributes of the final product (Yang et al. 2017). In another study, three starter combinations were used for the yogurt fermentation; traditional yogurt starter cultures (YF-L904, Chr. Hansen Co., Ltd., Denmark) and two co-cultures of *L. plantarum*. Twenty four panelists were selected, including 12 men and 12 women, aged between 20 and 30. Each sample was rated using a 9 point hybrid hedonic scale. The sensory assessment of yogurt used seven categories, namely milk flavor, typical yogurt flavor, sour milk, vinegar, scream flavor, off-odor, and overall acceptability. In a result, the "typical yogurt flavor," "cream flavor," and "overall acceptability" of the samples co-fermented with *L. plantarum* were stronger than those of the control. Furthermore, there were no significant differences in the other three properties ("off-odor," "vinegar," and "sour milk") of the three yogurt samples, indicating that the use of these strains did not produce excessive negative aroma compounds during fermentation (Tian et al. 2019).

A research was conducted at Akdeniz University, Department of Food Engineering to determine the physicochemical and sensory properties of yogurts. Danisco Yo-Flex 410 commercial culture was used as starter culture in this study. A 15 point hedonic scale was used in sensory analyses and 40 panelists took part. According to the sensory results, bag yogurt scored an average of 10.3 for visual consistency, an average of 11.1 for color, an average of 10.3 for consistency on a spoon, an average of 6.2 for sourness, and an average of 10.6 for overall acceptability (Aktar, 2022).

Although the results of this study are consistent with the results of the study given above, there are some differences. The reasons for these differences can be said to be the type and amount of culture used, the yogurt production method, the sensory test

method applied, the differences between the panelists and the sensory test conditions.

Conclusion

Yogurt is a product with very rich values in terms of nutritional value. Among dairy products, yogurt, which is highly preferred both in our country and around the world, is easy to digest and regulates the digestive system, strengthens the immune system and is easily consumed by people who are sensitive to lactose (intolerant). Sensory properties are characteristics determined by human senses that lead the consumer to accept or reject a food. Sensory tests performed on milk and dairy products and therefore on yogurt are an important identification tool and are an increasingly preferred method to reveal the quality of the product. Sensory analyses in yogurt are performed for purposes such as ensuring quality in daily yogurt production, determining differences in products, developing a new product, improving the quality of currently produced products and revealing consumer taste. In sensory tests, the type of yogurt samples to be tested, the type and ratio of starter culture used, the preparation methods of the samples, the selection and training of panelists, the arrangement of the panel area, the test method to be applied and the analyzing of the test results are important.

In the study, strained yogurt samples produced in the traditional way using 3 different bacterium ratios in yogurt culture were subjected to sensory testing. In terms of the sensory characteristics evaluated by the panelists participating in the test, the highest scores were obtained by the strained yogurt samples produced at a bacterium ratio of 50%+50%, and in the sensory evaluation of the defects, some negativities were detected at very low levels in the same strained yogurt samples. The most preferred product by all of the panelists participating in the test was strained yogurt samples produced at this bacterium rate.

The unique tastes and aromas of traditionally produced yogurts are affected by the quality of raw materials, but are largely due to their microflora. The chemical and physical changes caused by microorganisms during the formation of yogurt determine the taste, aroma and structure of yogurt. Therefore, by determining the microflora of the most popular yogurts in the sensory analysis results of traditionally produced yogurts, suitable culture combinations can be used for the production of yogurt suitable for taste. Usually, yogurt starter cultures are comprised of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* in a 1:1 or 1:2 ratio, but with the increasing demand for commercial starter cultures in the fermentation industry, the impact of using different ratios of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* on dairy flavour substances has been of increasing interest to the industry. According to the results of the sensory evaluation in this study, it is concluded that it would be correct to use 50% *L. bulgaricus* + 50% *S. thermophilus* bacterium ratio in yogurt production and to plan the production accordingly.

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Conflict of Interest

The authors have not any conflict of interest to declare.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

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