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***Research Article***

# Investigation of the Physical and Chemical Properties of Traditional Homemade Yogurt with Different Rates of Pollen Additions

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**Abstract**

Fermented milk products are widely consumed as healthy foods worldwide (Matumoto-Pintro et al., 2011). Due to the widespread consumption in our country the production amount of yogurt, which is the most processed product of cow milk after drinking milk, increases every year. Yoghurt production increased by 2.2% from 2018 and reached 1.19 million tons (TÜİK, 2018). In addition to its nutritional properties and the presence of living microorganisms, it has some quality flaws such as low viscosity, stickiness, loose structure, serum separation (water release), as well as its benefits for human health. In this study, physical and chemical properties of yoghurt produced using conventional home-made yeast-milled bee pollen-added yeast in which 1 control and 6 different concentrations (%0.5, %1, %1.5, %2, %2.5, %3) were investigated. The acidity, titratable acidity, mineral matter content and color analysis of the use of pollen at different rates in yoghurt samples were evaluated. In the yoghurt samples evaluated within the scope of the study, it was concluded that the acidity, titration acidity, mineral substance content and color change of the addition of pollen in different rates were statistically significant ($p < 0.01$).

**Keywords:** Bee pollen, yoghurt, minerals, color, pH, SH.

# Farklı Oranlarda Polen İlaveli Geleneksel Ev Yoğurdunun Fiziksel ve Kimyasal Özelliklerinin İncelenmesi

**Öz**

Dünya genelinde fermente süt ürünleri sağlıklı besinler olarak yaygın şekilde tüketilmektedir (Matumoto-Pintro ve ark., 2011). Ülkemizde de tüketiminin yaygın olmasından dolayı sanayiye aktarılan inek sütünün, içme sütünden sonra en çok işlendiği ürün olan yoğurdun üretim miktarı her yıl artış göstermektedir. Yoğurt üretimi 2018 yılından % 2,2 oranında artarak 1.19 milyon tona ulaşmıştır (TÜİK, 2018). Besleyici özellikleri ve canlı mikroorganizmaların varlığı ile insan sağlığına olan yararlarının yanı sıra, fermente bir süt ürünü olan yoğurdun düşük viskozite, yapışkanlık, gevşek yapı, serum ayrılması (su salma) gibi bazı kalite kusurları bulunmaktadır (Domagała ve ark., 2013). Bu çalışmada 1 kontrol ve 6 farklı oranda (% 0.5, %1, %1.5, %2, %2.5, %3) öğütülmüş polen ilaveli geleneksel ev yoğurdu mayası kullanılarak üretilen yoğurtların fiziksel ve kimyasal özellikleri araştırılmıştır. Yoğurt örneklerinde farklı oranlarda polen kullanımının asitlik, titrasyon asitliği, mineral madde içeriği ve renk değerleri üzerindeki etkisi değerlendirilmiştir. Araştırma kapsamında değerlendirilen yoğurt örneklerinde farklı oranlarda polen ilavesinin asitlik, titrasyon asitliği, mineral madde içerik ve renk değişimi istatistiksel olarak anlamlı olduğu sonucuna varılmıştır ($p<0.01$).

**Anahtar Kelimeler:** Polen, yoğurt, mineraller, renk, pH, SH.

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# 1. Introduction

According to Fermented Dairy Products Communique No. 2009/25 of the Turkish Food Codex, yogurt is a fermented milk product using the symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. Additionally, according to TS 1330 the yogurt standard, yogurt defined as a "product obtained pasteurization of cow milk, sheep milk, buffalo milk, goat milk or their mixtures or homogenising the pasteurized milk with the addition of milk powder, or without homogenisation adding the yogurt culture consisting of *Lactobacillus delbrueckeii subsp bulgaricus* and *Streptococcus thermophilus* after proper processing". Yogurt is a milk product produced by adding starter culture or wild yeast after pasteurization process (78 °C, 15 minutes).

Since yogurt is a fermented dairy product, digestion is easier than milk (Emirdağı, 2014) and the dry matter components are richer than milk (Eren Karahan, 2016). Yogurt is widely consumed nutritious food because of consisting high rate of calcium, riboflavin, protein, phosphorus, vitamin A, vitamin B1 (Thiamine), vitamin B12 (Riboflavin) and Vitamin B6. In addition, in yogurt folic acid, niacin, magnesium and zinc values are also higher than milk. Therefore, with a regular consumption of yogurt, especially for children and youngsters most of the daily required proportion of vitamin A, folic acid, vitamin B12, calcium and magnesium can be taken (Peker, 2012: 4). Another food that has an important role in the growth of children and youngsters is pollen. It is medically known that pollen is used in the treatment of many diseases such as infectious diseases and gastric bleeding. Using pollen in children contributes to child development (Karataş and Şerbetçi, 2008). Although pollen composition varies according to plant resources and production methods (Şahinler, 2000), in general, it consists protein (25-30%), carbohydrate (30-55%), lipid (4.8%), vitamins and minerals (12.13%) (Sandıkçı Altunatmaz and Yılmaz Aksu, 2016), and starch (15%, 50) (Erdoğan and Dodoloğlu, 2005). It is a natural antioxidant due to phenolic substances (gallium, caffeic, ferulic, chlorogenic, coumaric acid) and flavonoids (myrisetin, camferol, galangin) found in pollen structure (Sandıkçı Altunatmaz and Yılmaz Aksu, 2016).

Additionally in pollen, the basic amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine) (Erdogan and Dodologlu, 2005), different minerals, in addition to all of the B group vitamins C, D, E vitamins, natural hormones, enzymes, coenzymes, pigments, and carbohydrates have been reported (Karagözoğlu et al., 2012). There are many researches on physical, chemical, sensory characteristics and microbiological quality of yogurt produced in Turkey (Koçhisarlı and Ergül, 1987; Öz, 1990; Özdemir and Bodur, 1994; Tunçtürk et al., 2000; Herdem, 2006; Duraket et al., 2008; Coşkun and Şenoğlu, 2011; Koçak, 2013; Demirkaya and Ceylan, 2013; Biberoğlu and Ceylan, 2013; Köse and Ocak, 2014; Bakırcı et al, 2015; Eren Karahan, 2016). In addition to this, studies are continuing to improve the quality of yogurt and produce yogurt with different characteristics (Peker, 2012). Although studies on the physical, chemical and sensory properties of yogurt were conducted, limited number of studies was found in the literature regarding pollen-added yogurt (Yerlikaya, 2014; Lomova et al., 2014; Glušacet et al., 2015; Atallah, 2016; Zlatevet et al., 2018). At this point, especially in terms of new product development, the aim of this study is to examine the physical and chemical properties of traditional homemade yogurt with different rates of pollen additions.

# 2. Material and Method

The yoghurt samples that constitute the material of the study were obtained by applying the traditional fermentation method of milk supplied from the different villages of Konya. After the fat-free dry matter ratio of milk to be used in making yoghurt was adjusted to 12%, it was heated at 90 °C for 10 minutes, then it was allowed to cool down to 43 °C, and 3% of the traditional yoghurt culture was added. The samples with yoghurt culture were incubated for 3 hours at 43 °C and the results were kept in the refrigerator at 4 °C for 1 day, and the analysis was started the other day. Pollen were added to to traditional yogurt samples other than the control sample of the yoghurt samples using the traditional method before incubation at the rates of 0.5%, 1%, 1.5%, 2%, 2.5% and 3%.

## 2.1. Physical and Chemical Analysis

Physical and chemical measurements were conducted in duplicate. Viscosity measurement and color determination analyses were performed within the scope of physical analyses. The viscosity measurement was determined using the Poulten RY-8 model viscometer at 50 rpm with the cap 6 (sample temperature 3 ± 1 °C) and the results were read directly in centipoise (cP) on the digital display of the device. Color values were measured using the Minolta Meter CR 400 (Konica Minolta, Inc. Osaka, Japan). Before the measurement, the device was calibrated against the white surface calibration plate and the $L^*$, $a^*$ and $b^*$values were determined according to the CIE lab color scale.

After taking yogurt samples on a white background, color of the samples were measured from 4 different points (Pagliarini and Rastelli, 1994). As chemical analyses, the pH and titratable acidity and minerals were evaluated separately for each sample. For pH analysis, 10g sample was taken and 100 ml of pure water was added and mixed until homogenous. The pH value was determined by immersing NEL brand 821 model pH meter glass electrode to the samples at 20 ° C. For the titratable acidity analysis, pH of yogurt samples was transferred to the 250 ml flask and transferred to the line with pure water. The well stirred samples were then filtered through a filter paper and taken up 25 ml of the filtrate. The glass electrode of the pH meter used in the pH determination was immersed into this sample and with continuous stirring with a magnetic stirrer; the sample was titrated with 0.1 N NaOH until the pH reached 8.1. The result is indicated in terms of lactic acid (Batu et al., 2007). After weighing samples for the determination of the mineral substances 0.15-0.20 grams on the precision scale, mixture of 5 ml of 65% nitric acid ($HNO_3$) and 2 ml hydrogen peroxide ($H_2O_2$) burned in closed system microwave oven 180 °C for 30 minutes and pure water added to this mixture up to 20 ml. The samples were filtered through filter papers and reading was done by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) (Varian-Vista Model) device. Results obtained (values read on device) were multiplied by the 'volume/initial weight' coefficient determined for each sample and the mineral substances of the samples were calculated in mg/kg (Skujins, 1998).

$$\text{Mineral Concentration} = \frac{\text{Volume}}{\text{Amount of Sample}} \times \text{Value read}$$

## 2.2. Statistical Evaluation

The effect of different proportion of pollen addition on the physical and chemical parameters of yogurts was determined by ANOVA using the MINITAB package program and the

differences between the groups were determined by Tukey Multiple Comparison Test. p values <0.05 were considered statistically significant and all analyses were performed twice.

## 3. Results and Discussion

During the viscosity analysis, 7 different cp values (1, 2,5, 5, 10, 20, 50, 100) were measured. Each application was applied to 7 different yogurt samples separately. The viscosity of yogurt samples was evaluated statistically and the results of the analysis are shown in Table 1. When the viscosity values given in Table 1 are examined, it is determined that the lowest value (50±70,7) belongs to the sample F (50cP) and sample D (100 cP). It was determined that the highest value belongs to the C sample (40250 cP) at 1cP. There was no statistically significant difference between yogurt samples according to viscosity value of 1, 2,5, 20 and 50 cp (p> 0.05). When reading at 5, 10, 100 cP values, two statistically different groups were found. When reading at 5 cP, the highest viscosity was found in yogurt that has 1% pollen added at a score of 3550±70,7. When the results of 10 cp were examined, the highest viscosity was found in yogurt that has 1% pollen added at a score of 3000. It was determined that the addition of pollen and different viscosity measurement had an effect on the viscosity values. When these results are taken into consideration, having the higher viscosity in pollen-added yogurt than the control group can be attributed to the higher protein content of pollen (Yerlikaya, 2014: 273).

*Table 1. Viscosity Values of Yogurt Samples*

| Samples | Viscosity (cP) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2,5** | **5** | **10** | **20** | **50** | **100** |
| **A** | 5650±1909$^{a}$ | 1750±1344$^{a}$ | 1750±212,1$^{ab}$ | 1100±141,4$^{b}$ | 1800±707,1$^{a}$ | 3100±2121,3$^{a}$ | 4900±0,0$^{a}$ |
| **B** | 100±0,0$^{a}$ | 4550±6010$^{a}$ | 2450±919,2$^{ab}$ | 2150±495,0$^{ab}$ | 2500±989,9$^{a}$ | 1000±0,0$^{a}$ | 900±1131,4$^{b}$ |
| **C** | 40250±35002$^{a}$ | 4300±566$^{a}$ | 3550±70,7$^{a}$ | 3000±282,8$^{a}$ | 1900±1272,8$^{a}$ | 1150±212,1$^{a}$ | 1500±1414,2$^{ab}$ |
| **D** | 5550±4172$^{a}$ | 100±0,0$^{a}$ | 1250±353,6$^{b}$ | 950±70,7$^{b}$ | 700±282,8$^{a}$ | 500±0,0$^{a}$ | 50±70,7$^{b}$ |
| **E** | 15600±16829$^{a}$ | 1650±2333$^{a}$ | 2250±636,4$^{ab}$ | 1800±424,3$^{ab}$ | 1300±141,4$^{a}$ | 900±141,4$^{a}$ | 200±282,8$^{b}$ |
| **F** | 9450±13223$^{a}$ | 100±0,0$^{a}$ | 950±212,1$^{b}$ | 1100±282,8$^{b}$ | 850±70,7$^{a}$ | 600±141,4$^{a}$ | 50±70,7$^{b}$ |
| **G** | 8250±4596$^{a}$ | 1550±2192$^{a}$ | 1700±848,5$^{b}$ | 1350±212,1$^{ab}$ | 1100±282,8$^{a}$ | 700±141,4$^{a}$ | 100±0,0$^{b}$ |

**Note:** A: Control Group (Pollen-Free Yogurt), B: 0.5% pollen-added yogurt, C: 1% pollen-added yogurt, D: 1.5% pollen-added yogurt, E: 2% pollen-added yogurt, F: 2.5% pollen added yogurt, G: 3% pollen added yogurt.

a, b, c, d, e, f, g: For each analysis, the values given in lower case in the same column show statistically significant differences (p <0.05).

Color determination in foods is one of the most important parameters of physical analysis. L*, a*, b* values are examined in yogurt samples. The results of the color determination analysis of yogurt samples examined are given in Table 2. Significant statistical differences are found (p <0.05). L* value indicates whiteness and two different groups were determined when samples were analysed statistically. The highest L* value was determined as 88.37 in control sample. It was determined that L* value decreases when pollen concentration is increased. a* represents redness or greenness. When the results of pollen added yogurt samples were examined, three different statistical groups were emerged. The highest a* value was determined in G sample. b* means yellowness or blueness. Six different groups were emerged when the result of the analysis was evaluated statistically. The highest b* value was evaluated in G sample. Similar to L*, it was identified that a* and b* values of samples were decreased as pollen addition increased. Similar to the results of Tarakçı (2010), the pollen addition to yogurt causes color change in the yogurt samples.

*Table 2. Colour Determination of Yogurt Samples*

| Samples | L* | a* | b* |
|---|---|---|---|
| **A** | 88,37±0,5374$^{a}$ | -4,235±0,1061$^{c}$ | 9,14±0,2828$^{f}$ |
| **B** | 84,34±3,4648$^{ab}$ | -3,435±0,5586$^{bc}$ | 11,55±0,0283$^{ef}$ |
| **C** | 85,61±0,2192$^{ab}$ | -3,555±0,1061$^{c}$ | 14,10±0,2970$^{de}$ |
| **D** | 86,43±0,9405$^{ab}$ | -3,885±0,5869$^{c}$ | 15,44±0,7495$^{cd}$ |
| **E** | 82,97±0,7425$^{ab}$ | -2,735±0,1626$^{bc}$ | 17,40±0,8485$^{bc}$ |
| **F** | 78,89±1,2799$^{ab}$ | -1,695±0,0354$^{ab}$ | 20,38±0,8980$^{ab}$ |
| **G** | 79,98±1,8597$^{b}$ | -0,885±0,3041$^{a}$ | 22,56±0,3253$^{a}$ |

**Note:** A: Control Group (Pollen-Free Yogurt), B: 0.5% pollen-added yogurt, C: 1% pollen-added yogurt, D: 1.5% pollen-added yogurt, E: 2% pollen-added yogurt, F: 2.5% pollen-added yogurt, G: 3% pollen-added yogurt.

a, b, c, d, e, f, g: For each analysis, the values given in lower case in the same column show statistically significant differences (p <0.05).

The titratable acidity and pH values of the samples are shown in Table 3. When the pH of the yogurt samples were analyzed statistically, two different groups were formed. These groups were divided into two groups as pollen-free yogurt and pollen-added yogurts. The highest pH value was determined in the control sample. Significant differences were found between these

two groups ($p < 0.05$). The addition of pollen in samples resulted in a decrease in pH level. Küçükçetin (2008), Yerlikaya (2014) and Glušac et al. (2015) suggested that the addition of pollen to yogurt and dairy products would decrease the pH level. The findings of the research have reached similar results with the studies conducted in the literature. When the results of titration analysis in yogurt samples were evaluated statistically, three different groups were obtained. Titratable acidity levels ranged from 1,780 to 1,080, and the highest titratable acidity was determined in the control sample. On the other hand, the lowest value was determined in the D sample. It was observed that the titratable acidity level decreased in pollen-added yogurt. The reason for this is thought due to the inhibitory effect of pollen on the vital activity of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* and thus the proper implementation of lactic acid fermentation (Zlatev et al., 2018: 135). These results regarding titratable acidity are supported by studies in the literature (Lomova et al., 2014; Zlatev et al., 2018).

*Table 3. Titratable Acidity and pH Values of Yogurt Samples*

| Samples | pH | Titratable Acidity (SH) |
|---|---|---|
| **A** | 4,465±0,06364$^{a}$ | 1,780±0,02828$^{a}$ |
| **B** | 4,170±0,01414$^{b}$ | 1,180±0,05657$^{bc}$ |
| **C** | 4,180±0,00000$^{b}$ | 1,130±0,04243$^{bc}$ |
| **D** | 4,085±0,00707$^{b}$ | 1,080±0,02828$^{c}$ |
| **E** | 4,095±0,00707$^{b}$ | 1,230±0,04243$^{bc}$ |
| **F** | 4,130±0,02828$^{b}$ | 1,280±0,07071$^{bc}$ |
| **G** | 4,125±0,00707$^{b}$ | 1,330±0,01414$^{b}$ |

**Note:** A: Control Group (Pollen-Free Yogurt), B: 0.5% pollen-added yogurt, C: 1% pollen-added yogurt, D: 1.5% pollen-added yogurt, E: 2% pollen-added yogurt, F: 2.5% pollen added yogurt, G: 3% pollen added yogurt.

a, b, c: The values given in lower case in the same column for each analysis show statistically significant differences ($p < 0.05$).

The analysis of minerals for yogurt samples and their results are given in Table 4. When the amount of mineral substances was taken into consideration, statistically significant differences were observed ($p < 0.05$). Differences occurred depending on the amount of pollen added. It was determined that pollen addition had a significant effect on yogurt samples and presents in statistical groups. When the mineral results were analyzed statistically, the highest value of Mo, Cu, Mn, Zn, Fe, S was determined in the G sample which had 3% pollen supplemented. When the Mg, Ca, K, P values were examined, the highest value was observed in the control group which was pollen-free yogurt. When mineral B was evaluated, the highest value was determined in the F sample with 2.5% pollen supplemented. Similar to Atallah (2016), study was concluded as the amount of pollen added in the yogurt increases significantly, the minerals of the yogurt also increase ($p < 0.05$).

*Table 4. Minerals Values of Yogurt Samples*

| Samples | Macro Nutrients (mg kg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | **Mo** | **Cu** | **B** | **Mn** | **Zn** | **Fe** |
| **A** | 0,3350±0,007071$^{c}$ | 2,955±0,007071$^{f}$ | 0,000±0,00000$^{e}$ | 0,000±0,000000$^{g}$ | 28,41±0,00707$^{c}$ | 25,81±0,007071$^{f}$ |
| **B** | 0,3450±0,007071$^{c}$ | 3,155±0,007071$^{e}$ | 0,000±0,00000$^{e}$ | 0,285±0,007071$^{f}$ | 27,03±0,00000$^{e}$ | 28,02±0,007071$^{e}$ |
| **C** | 0,3250±0,007071$^{c}$ | 1,935±0,007071$^{g}$ | 0,000±0,00000$^{e}$ | 0,645±0,007071$^{e}$ | 23,29±0,00707$^{g}$ | 23,76±0,007071$^{g}$ |
| **D** | 0,3350±0,007071$^{c}$ | 4,255±0,007071$^{d}$ | 2,115±0,00707$^{d}$ | 1,235±0,007071$^{d}$ | 25,66±0,00707$^{f}$ | 34,14±0,007071$^{d}$ |
| **E** | 0,0800±0,000000$^{d}$ | 4,875±0,007071$^{b}$ | 2,345±0,00707$^{c}$ | 1,535±0,007071$^{c}$ | 28,14±0,00000$^{d}$ | 41,50±0,007071$^{b}$ |
| **F** | 0,3850±0,007071$^{b}$ | 4,715±0,007071$^{c}$ | 5,855±0,00707$^{a}$ | 2,415±0,007071$^{b}$ | 32,34±0,00707$^{b}$ | 39,16±0,007071$^{c}$ |
| **G** | 0,7250±0,007071$^{a}$ | 7,595±0,007071$^{a}$ | 5,505±0,00707$^{b}$ | 2,840±0,000000$^{a}$ | 40,57±0,00000$^{a}$ | 42,52±0,007071$^{a}$ |
| | **S** | **Mg** | **Ca** | **K** | **P** | |
| **A** | 2143±0,00707$^{f}$ | 1081,7±0,00000$^{a}$ | 11443±0,00707$^{a}$ | 9429±0,00707$^{a}$ | 7177±0,00707$^{a}$ | |
| **B** | 2162±0,00000$^{e}$ | 973,9±0,00707$^{e}$ | 10233±0,00707$^{b}$ | 8516±0,00707$^{d}$ | 6591±0,00707$^{c}$ | |
| **C** | 1980±0,00707$^{g}$ | 793,3±0,00707$^{g}$ | 8411±0,00000$^{g}$ | 7598±0,00707$^{g}$ | 5889±0,00707$^{g}$ | |
| **D** | 2177±0,00000$^{d}$ | 1008,9±0,0000$^{d}$ | 9531±0,00707$^{c}$ | 8782±0,00707$^{b}$ | 6719±0,00707$^{n}$ | |
| **E** | 2189±0,00707$^{c}$ | 867,1±0,00707$^{f}$ | 8986±0,00000$^{f}$ | 8154±0,00707$^{f}$ | 6267±0,00707$^{f}$ | |
| **F** | 2199±0,00000$^{b}$ | 1067,2±0,00000$^{b}$ | 9498±0,00707$^{d}$ | 8322±0,00707$^{e}$ | 6415±0,00707$^{d}$ | |
| **G** | 2337±0,00707$^{a}$ | 1024,5±0,00707$^{c}$ | 9085±0,00707$^{e}$ | 8547±0,00707$^{c}$ | 6320±0,00707$^{e}$ | |

**Note:** A: Control Group (Pollen-Free Yogurt), B: 0.5% pollen-added yogurt, C: 1% pollen-added yogurt, D: 1.5% pollen-added yogurt, E: 2% pollen-added yogurt, F: 2.5% pollen added yogurt, G: 3% pollen added yogurt.

a, b, c, d, e, f, g: For each analysis, the values given in lower case in the same column show statistically significant differences ($p < 0.05$).

## 4. Conclusions and Recommendations

In this study, physical and chemical analysis of the functional product obtained from traditional yogurt with various amounts of pollen were investigated. It was determined that as the level of pollen addition increased, the viscosity of the product increased and significant changes occurs on L*, a*, b* values ($p < 0.05$). When looking at the results of physical analysis of pollen-added yogurt samples, this study shows similar results with some studies in literature (Tarakçı, 2010; Yerlikaya, 2014). In addition, when chemical analyses were evaluated, it was found that the addition of pollen caused a decrease in pH and titratable acidity and caused an increase in some minerals (Mo, Cu, Mn, B, Zn, Fe, S, Mg, Ca, K, P). These results confirm the results of the studies in the literature (Küçükçetin, 2008; Yerlikaya, 2014; Lomova et al., 2014; Glušac et al., 2015; Atallah, 2016; Zlatev et al., 2018). On the other hand, considering this study, physicochemical and sensory properties can be compared by adding pollen to yoghurt samples before and after incubation, and by increasing the concentration ratio, sensory properties can be examined at high rates. Microbiological effects of pollen supplementation can be studied extensively and its effect on shelf life can be investigated. The product range can be developed by developing a new functional product by adding another food substance instead of pollen. The same and additional analyzes can be applied by producing two different options as fat-free yogurt and fatty yogurt. By examining the fatty acid profile and organic acid profile, the effect of pollen addition can be examined and the effects of yogurt with pollen can be examined from one side. Additionally, *in vitro* bioavailability can be examined and post-consumption mineral and antioxidant usefulness can be examined in a wide range.

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