



*The Use of St. John's Wort (Hypericum perforatum) Extract in Drinking Yoghurt Production and Determination of Changes Occuring During Storage*

*Ayran Üretiminde St. John's Wort (Hypericum perforatum) ekstraktının kullanımı ve depolama boyunca meydana gelen değişikliklerin belirlenmesi*

Fadime SEYREKOGLU<sup>1,\*</sup>, Hasan TEMİZ<sup>2</sup>

<sup>1</sup> Department of Food Processing, Suluova Vocational Schools, Amasya University, Amasya, Turkey

<sup>2</sup> Department of Food Engineering, Faculty of Engineering, Ondokuz Mayıs University, Samsun, Turkey

\*fadime.tokatli@amasya.edu.tr, ORCID: 0000-0001-9787-4115

Received/Geliş Tarihi: 03/05/2021

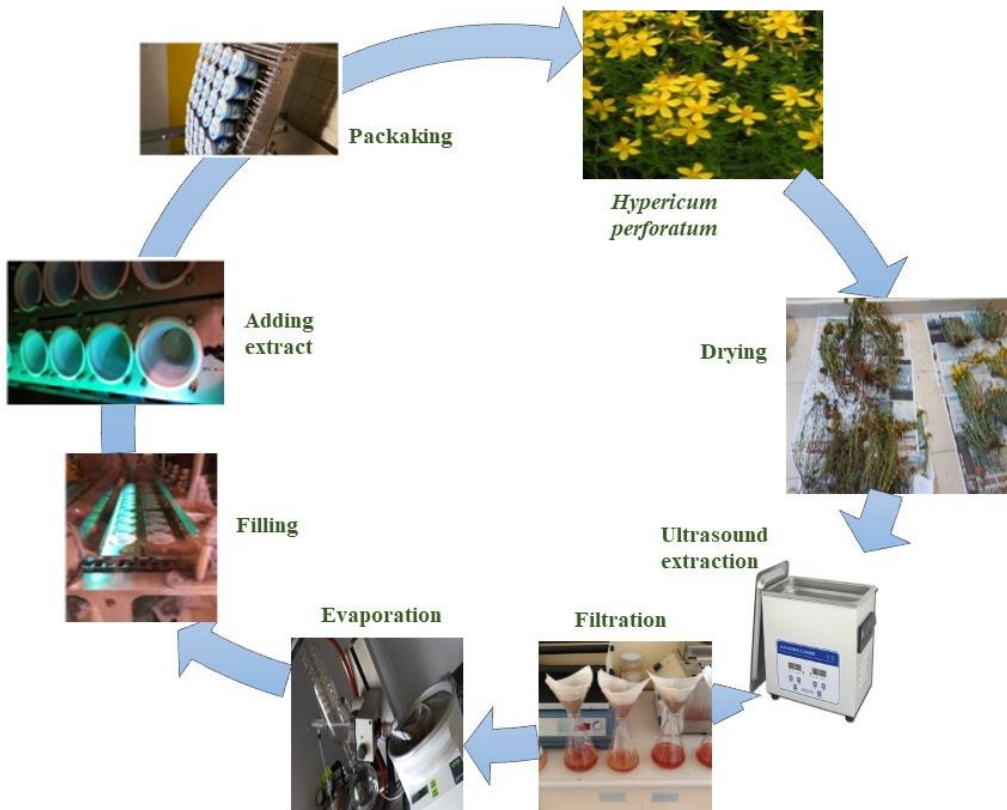
Accepted/ Kabul Tarihi: 26/06/2021

\*Corresponding author /Yazışılan yazar

doi: 10.35206/jan.931810

e-ISSN: 2667-4734

**Graphical Abstract**



## Abstract

## Özet

Medicinal and aromatic plants and extracts, essential oils obtained from these plants are widely used in many sectors as well as meeting the nutritional needs of people. Hypericum species have many biological activities such as antidepressant, antiviral, antibacterial, antioxidant and anti-inflammatory and are used as folk remedies in many areas such as sleep enhancement, rheumatic pain treatment, wound healing, skin diseases treatment among the public. One of the Hypericum species, St. John's wort (*Hypericum perforatum*) is found in almost all regions of our country and is one of the most studied plants. In our study, this plant, which has a very common medical use, was extracted and added to different amounts of drinking yoghurt. A mixture of 70% ethanol + 30% water was used as solvent during the extraction. In the study, three types of drinking yoghurt containing 1%, 2% and 3% St. John's wort extract were obtained. The control group and the drinking yoghurt samples obtained were stored for 28 days and some physicochemical and microbiological analyzes were performed on the 1st, 7th, 14th, 21st, and 28th days of the storage. According to the findings obtained; During the storage period, it was observed that the ash and salt values of the samples did not change, the dry substance, water activity and pH values decreased and the acidity values increased. In the microbiological\* analyzes performed during the storage period, the number of mesophyl bacteria groups increased statistically, while yeast – mold and coliform groups were not observed. Sensory analyzes were applied to the panelist group consisting of 10 people and when the taste – smell and general appreciation points were considered, samples containing 2% St. John's wort extract were the most liked. General appreciation scores increased statistically during storage. With this study, a functional product has been produced by adding this plant, which is not widely used alone, to a product that we consume a lot in

Tıbbi ve aromatik bitkiler ve bu bitkilerden elde edilen ekstraktlar, uçucu yağlar insanların besin ihtiyaçlarını karşılamanın yanında birçok sektörde de yaygın olarak kullanılmaktadır. Hypericum türleri antidepressan, antiviral, antibakteriyel, antioksidan, antiinflamatuvar gibi birçok biyolojik aktiviteye sahip olup, halk arasında uyku güçlendirme, romatizmal ağrı tedavisi, yara iyileştirme, cilt hastalıkları tedavisi gibi birçok alanda da halk ilacı olarak kullanılmaktadır. Hypericum türlerinden sarı kantaron (*Hypericum perforatum*) ülkemizde hemen hemen tüm bölgelerde bulunmakta ve üzerinde en çok çalışılan bitkilerdendir. Çalışmamızda tıbbi kullanımı oldukça yaygın olan bu bitkinin ekstraksiyonu gerçekleştirilmiş ve farklı miktarda ayrına ilave edilmiştir. Ekstraksiyon sırasında çözücü olarak %70 etanol + %30 su karışımı kullanılmıştır. Çalışmada %1, %2 ve %3 oranında sarı kantaron ekstraktı içeren üç çeşit ayran elde edilmiştir. Kontrol grubu ve elde edilen ayranlar 28 gün boyunca depolanmış ve depolamanın 1., 7., 14., 21. ve 28. günlerinde bazı fizikokimyasal ve mikrobiyolojik analizleri gerçekleştirilmiştir. Elde edilen bulgulara göre; depolama süresi boyunca örneklerin kül ve tuz değerlerinin değişmediği, kuru madde, su aktivitesi ve pH değerlerinin azaldığı, asitlik değerlerinin ise arttığı gözlemlenmiştir. Depolama süresi boyunca yapılan mikrobiyolojik analizlerde, toplam mezofil bakteri grubu sayısı istatistiksel olarak artış gösterirken, maya-küf ve koliform grup gözlemlenmemiştir. 10'ar kişiden oluşan panelist grubuna duyuşsal analizler uygulanmış ve tat- koku ve genel beğeni puanlarına bakıldığında en fazla beğeniyi %2 oranında sarı kantaron ekstraktı içeren örnekler almıştır. Depolama süresince genel beğeni puanları istatistiksel olarak artış göstermiştir. Bu çalışmayla tek başına kullanımı yaygın olmayan bu bitkinin günlük hayatta çok tükettiğimiz bir ürüne ilave edilmesiyle fonksiyonel bir ürün üretimi gerçekleştirilmiş ve kullanımı daha yaygın hale getirilmeye çalışılmıştır.

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daily life, and its use has been tried to be made more common.

**Key Words:** St. John's wort (*Hypericum perforatum*), extraction, drinking yoghurt, (*Hypericum perforatum*), ekstraksiyon, ayran, storage

**Anahtar kelimeler:** Sarı kantaron, depolama.

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**Abbreviations:**

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## 1. INTRODUCTION

Studies conducted for a longer life span aim to increase the quality of life as well as increase the importance of a healthy and balanced diet (Anonym, 2014). Due to the wrong nutritional rules that are known as correct, people have turned to the search for healthy and reliable food. In this context, the tendency to foods whose effects on health are scientifically proven and approved is increasing day by day (Sevilmiş, 2013). For this reason, studies in recent years have focused on food production, which is rich in nutrients and has positive effects on human health (Seçkin & Baladura, 2011). Considering the role of nutrition in health and preventing diseases, the importance of studies in this field increases even more. In the world and in our country, medicinal and aromatic plants and products produced from these plants have gained value each passing day by gaining the appreciation of the consumers. In the future, it is expected that the production of medicinal and aromatic plants, the production of the plant extracts obtained from these plants and the industrial production that processes them will increase and will be standardized in order to meet the demand of the consumer and to obtain a product of standard quality (Bayram et al., 2010).

Represented by 350 – 400 species in the world, *Hypericum* genus has 84 species in our country (Wichtl, 1986; Zeybek & Zeybek, 1994). Although *H. perforatum* L., the most common in *Hypericum* species, is used as a 'Natural Antidepressant' without side effects in European countries (Chrea et al., 2014), it also plays an active role in the treatment of symptoms such as insomnia and anxiety (Özgür Devrim, 2009). While cardiac and anticholinergic side effects were observed in patients using antidepressant drugs, no side effects were observed in studies using *Hypericum* extract (Cass, 1997; Ernst, 2003; Francis, 2005; Linde & Knüppel, 2005). Antidepressant drugs, which are becoming more common today, are widely used to prevent alcoholism and various types of addiction (Buonopane, 2005). In the studies conducted, *H. perforatum* extracts were given to experimental animals and as a result, serious decreases were observed in the alcohol intake of the experimental animals (Rezvani et al., 1999; Xu et al., 2005). Extracts of *H. perforatum* plant reduce alcohol addiction and withdrawal symptoms

caused by addiction (Abstinens Syndrome), (Coskun et al., 2006), as well as prevent withdrawal symptoms observed as a result of addictions such as nicotine (Uzbay et al., 2005; Uzbay et al., 2007) and caffeine (Uzbay et al., 2005; Uzbay et al., 2006). It has been determined that the antidepressant property of the plant is due to the secondary metabolite content of the plant, especially the hypericin molecule, and this molecule provides a significant increase in the transmission of nerve impulses in the brain (Uzbay, 2008).

Apart from the antidepressant properties of *H. perforatum* plant, it is used in the treatment of cancer, diabetes, chronic rheumatism, liver – biliary diseases, stomach ulcer and gastrointestinal diseases; In addition, it is known to be used in many areas such as jaundice, bronchitis, diarrhea and dysentery (Duke, 2002), throat infection (Tümen & Sekendiz, 1989) and the treatment of colds (Duke, 2002; Baytop, 1999). As a result of the studies, it has been determined that the compounds contributing to the pharmacological activity of *H. perforatum* are hypericin, pseudohypericin, hyperforin, flavonoids (rutin, hyperoside, and quersitrin), xanthenes and tannins (Barnes et al., 2001; Kaçar & Azkan, 2004). Studies have shown that the bioactive components of *Hypericum* species, hypericin and pseudohypericin, are highly effective against viruses. In studies conducted in in vitro environment; It has been found to be effective against Type A and B influenza, Herpes simplex, Hepatitis C viruses, Vesicular Stomatitis virus, Epstein-Barrvirus. (Cass, 1997; Mazza, 1998; Mills & Bone, 2000). It has been established that the bioactive compound hypericin exerts an antiviral effect by inactivating the HIV virus and protecting the membranes of healthy cells from virus attack (Cass, 1997; D'Hallewin et al., 2002). It has been supported by studies that the hypericin compound also shows anticancer activity in many different types of cancer (Ali et al., 2001; Blank et al., 2003; Colasanti et al., 2000; Linde et al., 1996; Martarelli et al., 2004; Mills & Bone, 2000). Hyperforin, another important bioactive ingredient, has been determined to have more antibacterial and antidepressant effects (Hölzl & Petersen, 2003). Hyperforin compound shows cytotoxic activity by inducing apoptosis in various cancer cells (Martarelli et al., 2004). This plant, which has many health benefits, has been accepted by the Council of Europe as a natural source for sweetening foods. Its aroma and fragrance has increased its use in the liquor industry (Anonym, 2000). It is used as a herbal additive in instant soups, breakfast cereals, chocolate, cakes, desserts and fruit – flavored beverages because it contains many bioactive ingredients that are beneficial for health (Anonym, 2000; Anonym, 2014).

Milk, which contains water, sugar (lactose), lipids and proteins as well as trace amounts of minerals, enzymes, vitamins, hormones and various compounds in its structure, is a very

complex liquid (Tilki, 2008). Milk is of great importance in food groups as it contains water, carbohydrates, fat, lipids, vitamins, macro and micro elements and trace elements that are necessary for our metabolism and has a high nutritional value (Souza et al., 2018). According to the Food and Agriculture Organization (FAO), global milk consumption per capita is expected to increase by 12.5% by 2025. It is known that milk and its products have an important role in preventing or treating cardiovascular disease, metabolic syndrome, osteoporosis, digestive disorders and cognitive decline (Coutinho et al., 2018).

Being in the low viscosity yoghurt class in many countries around the world and known as ‘drinkable yoghurt’ or ‘lactic drink’, drinking yoghurt is accepted as one of the most important consumption forms of yoghurt or a yoghurt derivative product that has settled in our culture (Anonym, 2009; Bölükbaşı, 2007). Drinking yoghurt is expressed as a fermented milk product created by adding water to yoghurt or adding yoghurt cultures to milk whose dry matter value is adjusted in the Turkish Food Codex Fermented Milks Communiqué (Anonym, 2009). Drinking yoghurt, with its high nutritional value, is an important alternative to carbonated beverages in our country and takes its place in the market as a food with a wide range of consumers from children to adults (Kuş, 2010; Taş, 2005). Hypericum species, which have many biological activities such as antidepressant, antiviral, antibacterial, antioxidant, anti-inflammatory, are popularly used as folk remedies in many areas such as sleep enhancement, rheumatic pain treatment, wound healing skin diseases treatment. Studies on the use of this plant, which has a very common medical use, in the food field are very few and insufficient. In the literature, various additions were made to yogurt and drinking yoghurt, but no studies were found on the use of Hypericum species in milk and its products. Thanks to the bioactive compounds it contains, *Hypericum perforatum*, which has a wide area of use in the treatment of many diseases in the medical field, has been added to our traditional drink drinking yoghurt, which plays an important role in daily nutrition; thus, a healthy, natural and useful functional product has been obtained and the deficiency in the literature has been tried to be overcome.

## 2. MATERIAL AND METHODS

### 2.1. Material

The *Hypericum perforatum* plant to be used in the study was collected from Amasya region in 2018 between June and August. Ayran samples to be used were produced in OTAT Provisions Industry and Trade LLC, Samsun/Havza according to the process of the factory and supplied from the factory.



Production was carried out by going to the Otat Provisions Industry and Trade LLC. The factory made production the ayran samples according to its own process. *Hypericum perforatum* extracts were added to ayran samples immediately after filling and before capping process. Production was carried out by adjusting the dry matter amount of the milk. The composition of the produced control group ayrans was calculated as (%): dry matter content: 7.9, fat content: 1.5, salt content: 0.5, protein content: 2.

Table 1. Composition of milk used in ayran production

Properties	Fat milk	Skimmed milk
Fat (%)	3.7	0.1
Dry matter (%)	11.25	10.5
Sh	6.8	7
pH	6.78	6.72
Briks	9.8	10

The solvent used in the extraction process and all chemicals used during the analysis were of analytical purity and were obtained from Merck (Darmstadt, Germany).

## 2.2. Preparation of Extract and Drinking Yoghurt Samples

The aerial parts of the *Hypericum perforatum* plant were dried for 3 weeks in the Suluova Vocational School of Amasya University in a cool place and in the shade. Dried plant samples were grinded in a grinder and cut into small pieces. Later, they were sealed and placed in boxes for use in the study. Ultrasonic wave assisted extraction technique was used to isolate the bioactive components of the *Hypericum perforatum* plant. The most suitable solvent to be used in ultrasonic wave assisted extraction, the temperature and time to be applied have been tried to be determined by previous preliminary tests (Seyrekoğlu & Temiz, 2019). Considering the studies carried out, the most worked temperature, time and solvent were used in *Hypericum* species. Optimum conditions found as a result of preliminary tests; 70% ethanol + 30% water as a solvent, the optimum temperature and time were found to be 30 °C and 40 minutes. The *Hypericum perforatum* plant was extracted under these conditions in the ultrasonic wave assisted bath (Çalışkan Lab. Ult 4010, Turkey) Then the ethanol remaining in the extract was removed in Rotary evaporator (Buchi). The obtained *Hypericum perforatum* extract was added to drinking yoghurt in three different proportions (1%, 2%, 3%). Drinking yoghurt samples containing *Hypericum perforatum* extract were stored in (+4) °C. Physical, chemical, microbiological and sensory analyses were performed on the 1st, 7th, 14th, 21st and 28th days of the storage.

Ayran samples production were carried out according to Otat Food Industry operating standards. According to the factory's own production process, the content of dry matter of milk was adjusted and ayran samples were produced. Then *Hypericum perforatum* extracts were added to ayran samples after filling process. The ayran production process was carried out as follows; dry matter content of milk was adjusted by mixing fat milk, skimmed milk and water and then standardization process was carried out. After evaporation and homogenization, pasteurization was applied at  $92\pm 2$  °C at  $5\pm 1$  min. Then culture was added to the milk, which was cooled to 42-43 °C temperature. After the fermentation was completed, the clot was broken and sent to the filters. *Hypericum perforatum* extract in determined proportions added to ayran samples at this stage and then were filling to the 200 mL pet cups in the filling machine. At the last stage lid closure, dating and packaging processes were carried out. The ayran samples were stored at 4 °C until analysis.

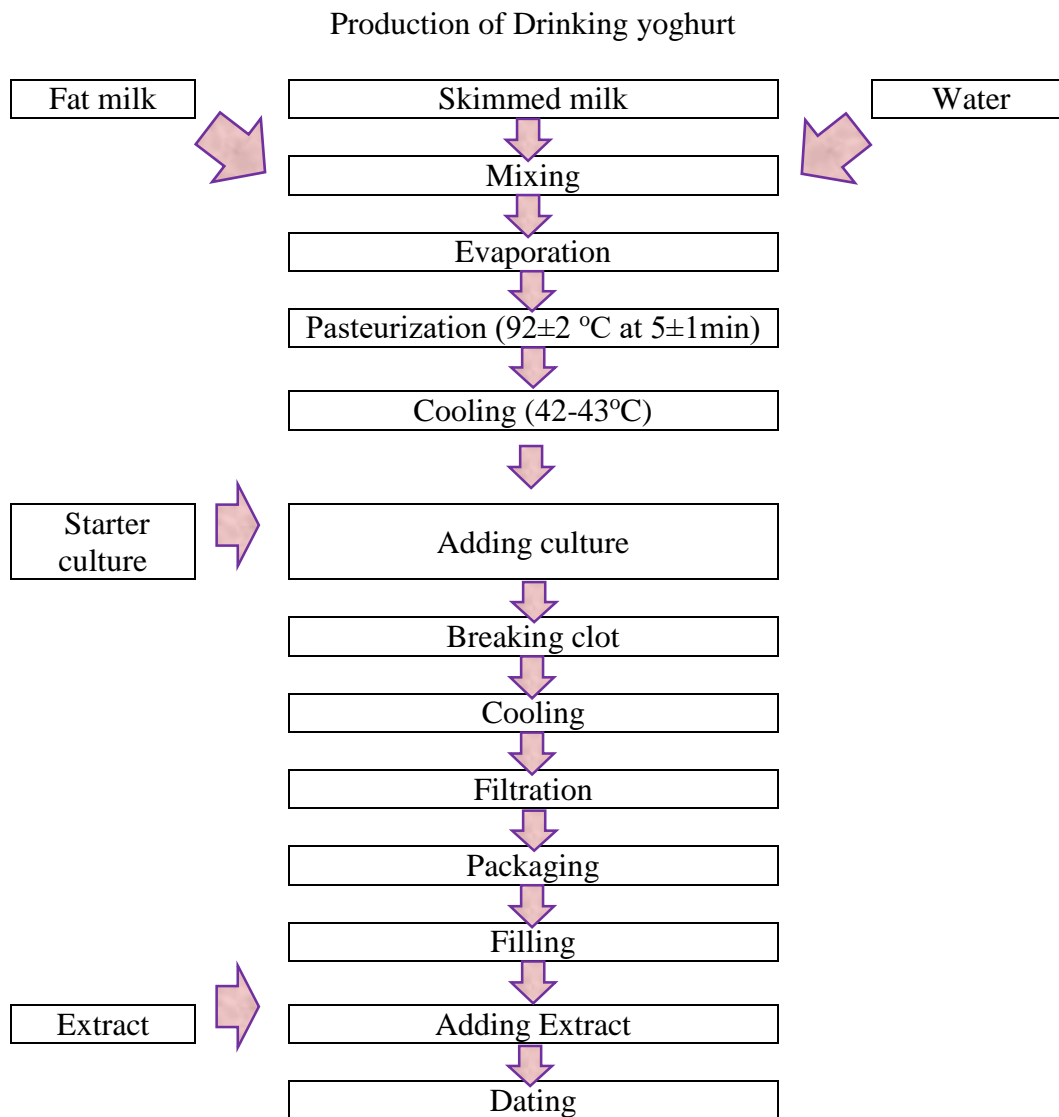


Figure 1. Production of drinking yoghurt



Figure 2. *Hypericum perforatum*

### 2.3. Physico-Chemical Analyses

The pH values of drinking yoghurt samples (at  $25 \pm 1$  °C) to which *Hypericum perforatum* extract was added were determined with an inoLab (Wellheim, Germany) brand pH meter, and the water activity amount was determined with a water activity analyzer (Novasina, LabSwift-aw). The acidity of samples was made according to TS 1018 (Anonym, 2000). Dry matter values of drinking yoghurt samples were calculated based on the method proposed by TS 1018 in the determination of dry matter (Anonym, 2000). The method made by Kezer (2013) was used in the determination of ash (Kezer, 2013). The amount of salt (%) in drinking yoghurt samples was made according to the Mohr method (Şeker & Patır, 2011).

### 2.4. Microbial Analyses

Dilutions up to  $10^{-6}$  were prepared from drinking yoghurt samples for microbiological analysis. Total mesophilic aerobic bacteria count was made according to Harrigan (1998), yeast-mold count was made according to the method specified in TS ISO 6611 (Anonym, 1996). The coliform group bacteria count in the samples was made according to the method made by Al-Kadamany et al. (2002).

### 2.5. Sensory Analyses

Sensory analyses were carried out on the basis of color and appearance, texture and consistency, taste and aroma and general taste characteristics, on the 1st, 7th, 14th, 21st and 28th days of storage with a panelist group of ten people. Before the sensory analysis, the panelists were informed about the product and the sensory analysis they would apply. Ayran samples were presented in 200 mL pet glasses with their covers closed. They were stored at 4 °C until analysis. By giving codes on the samples and was asked to fill in the characteristics corresponding to



those codes in the sensory analysis form. Drinking yoghurt samples added with *Hypericum perforatum* extract were compared among themselves and during storage. The evaluation was done in a range of points from 1 (quite bad) to 5 (quite nice) and their likelihood was checked (Anonym, 1982).

## **2.6. Statistical Analyses**

All the analyzes applied to the samples in the study were made with at least two replications and their mean standard deviations were calculated. The data, analysis of variance and comparisons between groups obtained as a result of the study were made using the SPSS 16.0 package program (Tukey Test). The significance levels of the groups were evaluated at the  $p < 0.05$  level (SPSS, 2011).

## **3. RESULTS AND DISCUSSION**

### **3.1. Physicochemical Analyses**

The results in physicochemical analyses of our drinking yoghurt samples were indicated in the Table 2.

When the physical and chemical analyses of the samples were examined, it was found that the added extracts did not cause a significant change on the physicochemical analysis (Table 2). Similarly, Şanlı et al. (2011), found that the physicochemical properties were not affected in the study in which transglutaminase was added to drinking yoghurt, and Dilek et al. (2018), similarly found that there was no difference between the control group and samples in their study where they added black carrot powder to drinking yoghurt.

Table 2. Change in physicochemical properties of drinking yoghurt samples during storage.

Properties	Storage days	Samples			
		K	P1	P2	P3
pH	1st	3.93 <sup>Ac</sup> ±0.04	3.93 <sup>Ab</sup> ±0.02	3.93 <sup>Ab</sup> ±0.01	3.94 <sup>Ab</sup> ±0.02
	7th	3.96 <sup>Abc</sup> ±0.04	3.93 <sup>ABb</sup> ±0.03	3.92 <sup>ABb</sup> ±0.01	3.91 <sup>Bb</sup> ±0.02
	14th	3.83 <sup>ABd</sup> ±0.01	3.83 <sup>Bc</sup> ±0.02	3.86 <sup>Ac</sup> ±0.02	3.82 <sup>Bc</sup> ±0.01
	21st	3.73 <sup>ABab</sup> ±0.02	3.78 <sup>Ca</sup> ±0.01	3.76 <sup>ABCa</sup> ±0.00	3.80 <sup>Ca</sup> ±0.00
	28th	3.73 <sup>Aa</sup> ±0.02	3.76 <sup>BCb</sup> ±0.01	3.66 <sup>ABCb</sup> ±0.01	3.72 <sup>Bab</sup> ±0.01
Acidity (% lactic acid)	1st	0.66 <sup>ABCb</sup> ±0.01	0.64 <sup>BCa</sup> ±0.05	0.63 <sup>Cb</sup> ±0.00	0.66 <sup>ABCa</sup> ±0.01
	7th	0.68 <sup>ABab</sup> ±0.01	0.61 <sup>Ba</sup> ±0.02	0.67 <sup>ABab</sup> ±0.04	0.67 <sup>ABa</sup> ±0.11
	14th	0.72 <sup>Aa</sup> ±0.04	0.65 <sup>Ba</sup> ±0.05	0.67 <sup>ABab</sup> ±0.01	0.65 <sup>Ba</sup> ±0.00
	21st	0.65 <sup>Cb</sup> ±0.00	0.69 <sup>Ba</sup> ±0.00	0.67 <sup>Ba</sup> ±0.01	0.71 <sup>Aa</sup> ±0.00
	28th	0.71 <sup>ABa</sup> ±0.02	0.65 <sup>Ca</sup> ±0.01	0.68 <sup>BCa</sup> ±0.00	0.68 <sup>BCa</sup> ±0.00
a <sub>w</sub>	1st	0.96 <sup>Ab</sup> ±0.00	0.97 <sup>Aa</sup> ±0.00	0.97 <sup>Ab</sup> ±0.00	0.96 <sup>Ab</sup> ±0.00
	7th	0.97 <sup>Aa</sup> ±0.00	0.97 <sup>ABa</sup> ±0.00	0.97 <sup>Aab</sup> ±0.00	0.97 <sup>Aab</sup> ±0.00
	14th	0.97 <sup>ABab</sup> ±0.00	0.97 <sup>ABa</sup> ±0.00	0.96 <sup>Bb</sup> ±0.00	0.97 <sup>ABab</sup> ±0.00
	21st	0.97 <sup>Aa</sup> ±0.00	0.97 <sup>ABa</sup> ±0.00	0.97 <sup>Aa</sup> ±0.00	0.97 <sup>Aa</sup> ±0.00
	28th	0.96 <sup>Ac</sup> ±0.00	0.96 <sup>Ab</sup> ±0.00	0.96 <sup>Ac</sup> ±0.00	0.96 <sup>Ac</sup> ±0.00
Dry matter (%)	1st	7.96 <sup>Aa</sup> ±0.46	7.98 <sup>Aa</sup> ±0.01	8.08 <sup>Aa</sup> ±0.27	8.11 <sup>Aa</sup> ±0.02
	7th	7.56 <sup>Cc</sup> ±0.03	7.80 <sup>Bb</sup> ±0.05	7.83 <sup>Ba</sup> ±0.26	7.98 <sup>ABab</sup> ±0.07
	14th	7.88 <sup>Bd</sup> ±0.22	7.64 <sup>Cc</sup> ±0.01	7.86 <sup>Ba</sup> ±0.01	8.21 <sup>Aa</sup> ±0.01
	21st	7.64 <sup>ABc</sup> ±0.06	7.79 <sup>ABb</sup> ±0.10	7.73 <sup>ABa</sup> ±0.46	7.75 <sup>ABbc</sup> ±0.03
	28th	7.84 <sup>Ab</sup> ±0.05	6.61 <sup>Cd</sup> ±0.13	7.26 <sup>ABb</sup> ±0.44	7.61 <sup>Ac</sup> ±0.41
Ash (%)	1st	1.20 <sup>Aa</sup> ±0.07	1.20 <sup>Aa</sup> ±0.46	1.09 <sup>Ab</sup> ±0.02	1.11 <sup>Ab</sup> ±0.01
	7th	1.18 <sup>Aa</sup> ±0.01	1.18 <sup>Aa</sup> ±0.01	1.14 <sup>Aab</sup> ±0.09	1.15 <sup>Aa</sup> ±0.02
	14th	1.11 <sup>Aa</sup> ±0.05	1.12 <sup>Aa</sup> ±0.01	1.10 <sup>Ab</sup> ±0.03	1.067 <sup>ABc</sup> ±0.01
	21st	1.18 <sup>Aa</sup> ±0.00	1.17 <sup>ABa</sup> ±0.01	1.18 <sup>Aa</sup> ±0.01	1.14 <sup>B<sup>C</sup>ab</sup> ±0.00
	28th	1.06 <sup>Ba</sup> ±0.00	1.11 <sup>Aa</sup> ±0.01	1.10 <sup>Ab</sup> ±0.00	1.06 <sup>Bc</sup> ±0.01
Salt (%)	1st	0.54 <sup>Aa</sup> ±0.06	0.58 <sup>Aa</sup> ±0.00	0.54 <sup>Aa</sup> ±0.06	0.58 <sup>Aa</sup> ±0.11
	7th	0.58 <sup>Aa</sup> ±0.17	0.59 <sup>Aa</sup> ±0.067	0.58 <sup>Aa</sup> ±0.13	0.54 <sup>Aa</sup> ±0.06
	14th	0.51 <sup>Aa</sup> ±0.00	0.52 <sup>Aa</sup> ±0.06	0.53 <sup>Aa</sup> ±0.06	0.53 <sup>Aa</sup> ±0.00
	21st	0.51 <sup>Aa</sup> ±0.17	0.57 <sup>Aa</sup> ±0.13	0.53 <sup>Aa</sup> ±0.00	0.51 <sup>Aa</sup> ±0.00
	28th	0.50 <sup>Aa</sup> ±0.11	0.50 <sup>Aa</sup> ±0.06	0.51 <sup>Aa</sup> ±0.06	0.50 <sup>Aa</sup> ±0.13

\*: mean standard ± deviation. A-C: For drinking yoghurt samples, the capital letters on the same line are comparable and the same letters show no statistical difference between the samples. (P> 0.05).

a-c: The lower case letters in the same column are the comparison of the storage times and the same letters show that there is no statistical difference between the samples. (P> 0.05).

K: Control drinking yoghurt, P1: Drinking yoghurt produced by adding 1 % *H. perforatum* extract, P2: Drinking yoghurt produced by adding 2 % *H. perforatum* extract, P3: Drinking yoghurt produced by adding 3 % *H. perforatum* extract.

### **3.2. pH and acidity of drinking yoghurt samples**

When the physicochemical properties of the samples are examined; While the pH values decreased with the storage, the amount of lactic acid (%) increased in accordance with this. pH values are quite close to each other. Lactic acid that formed as a result of the activities of lactic acid bacteria during storage, increased the acidity values and caused the pH values to decrease. Similar to our samples, pH values decreased with storage in the production of drinking yoghurt with pepper made by Akçay (2016), and drinking yoghurt samples with quinoa flour made by Temen (2018). In our examples, the amount of acidity is 0.61–0.72%, and this amount is quite close with the amount of 0.55–0.61% found in the study made by Avsar et al. (2001), and the amount of 0.58–0.67% in the study on the use of pectin in the production of durable drinking yoghurt by Atamer et al. (1999).

### **3.3. Dry matter content**

While the dry matter values of the samples decreased with storage, the water activity, salt and ash values did not change. While the dry matter value in our study was between 6.61% and 8.21%; Patır et al. (2006), found the dry matter amounts of 3.80%- 8.70 % in packaged drinking yoghurt samples, and Saltoğlu (2014), between 8.61 and 8.83% in fruit drinking yoghurt samples, and they are very similar to our results.

### **3.4. Water activity**

The values of the water activity of our samples wasn't observed change during the storage importantly. Since the amount of added extract was not high the amount of water activity did not change, and in paralel with the amount of water activity, the microbiological analysis of the samples were also similar. When we look at the literature, there are no studies on the amount of water activity of drinking yoghurt.

### **3.5. Ash content**

While the ash values of the drinking yoghurt with the addition of hot pepper and the control group made by Akçay (2016), varied between 0.55% and 0.61%, the amount of ash in the samples used in our study was between 1.06 – 1.20%. The difference between the ash values in our study and the values reported in the literature is due to the raw material, production method

and the added extract. Physicochemical analysis results of all samples showed similar results when compared with the literature. The differences arise from the raw materials used, auxiliary substances, initial culture, fermentation conditions and the different components and extracts added into it.

When the literature is examined, it has not been found that the plant *Hypericum perforatum* is added to drinking yoghurt. For this reason, while making comparisons, studies in which different ingredients are added to make the drinking yoghurt more functional have been looked at and the analyzes made were compared in this way. The number of studies on *Hypericum perforatum* is quite high, but the use of plant in foods is very low. *Hypericum perforatum* extract was added to ice cream and some of its properties were studied. Addition of St. John's wort extract to ice cream caused a decrease in pH values and an increase in acidity and dry matter values (Aydemir, 2015). Similarly, in our samples, it caused a increase in acidity values and decrease pH values. The fact that the extract added was the same plant provided similar results. When *Hypericum perforatum* extract was added to drinking yoghurt, it did not cause major changes on physical and chemical properties and the product preserved its unique properties.

### **3.6. Microbial Analyses**

The results in microbial analyses of our drinking yoghurt samples were indicated in the Table 3. As seen in Table 3 the total mesophylic aerobic bacteria group showed a linear increase with storage. Yeast-mold counts of the samples were similar to the control during storage. Yeast-mold was not observed in any of the samples. On the first day of storage, P3 sample (drinking yoghurt containing 3% *Hypericum perforatum* extract) had the lowest bacteria count with 4.07 log cfu / mL, while C (Control) was showed the highest number of bacteria with 4.67 log cfu / mL. On the 7th day of storage, the control sample contained the highest total mesophilic bacteria group with 4.68 log cfu, while the P3 sample contained the least bacteria with 4.13 log cfu / mL. Control and other samples were showed statistically similarity on other days of storage. *Hypericum perforatum* is showed antimicrobial effect in normally but everything could changed this antimicrobial effect. The reason for this change may be the year the plant was collected, the region where it was collected, the part used, the drying process applied before the extraction and the parameters used in the extraction.

Table 3. Change in microbial properties of drinking yoghurt samples during storage.

Microbiological Analysis	Storage days	Samples			
		K	P1	P2	P3
Total Mesophyl Bacteria (log cfu / mL)	1st	4.67 <sup>Ca</sup> ± 0.02	4.20 <sup>Ba</sup> ± 0.01	4.11 <sup>Aa</sup> ± 0.01	4.07 <sup>Aa</sup> ± 0.02
	7th	4.68 <sup>Ba</sup> ± 0.02	4.23 <sup>Aa</sup> ± 0.09	4.14 <sup>Aa</sup> ± 0.12	4.13 <sup>Aa</sup> ± 0.06
	14th	4.73 <sup>Ba</sup> ± 0.04	4.42 <sup>Ab</sup> ± 0.06	4.69 <sup>Bb</sup> ± 0.01	4.73 <sup>Bb</sup> ± 0.14
	21st	4.87 <sup>Ab</sup> ± 0.08	4.87 <sup>Ac</sup> ± 0.03	4.89 <sup>Ac</sup> ± 0.04	4.85 <sup>Ab</sup> ± 0.06
	28th	4.94 <sup>Ab</sup> ± 0.08	4.95 <sup>Ac</sup> ± 0.03	4.97 <sup>Ac</sup> ± 0.04	4.96 <sup>Ab</sup> ± 0.06
Coliform Group (log cfu / mL)	1st	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	7th	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	14th	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	21st	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	28th	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
Yeast and Mold (log cfu / mL)	1st	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	7th	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	14th	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	21st	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>
	28th	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>	<1 <sup>Aa</sup>

\*: mean standard ± deviation.

A-C: For drinking yoghurt samples, the capital letters on the same line are comparable and the same letters show no statistical difference between the samples. (P> 0.05)

a-c: The lower case letters in the same column are the comparison of the storage times and the same letters show that there is no statistical difference between the samples. (P> 0.05)

K: Control drinking yoghurt, P1: Drinking yoghurt produced by adding 1 % *H. perforatum* extract, P2: Drinking yoghurt produced by adding 2 % *H. perforatum* extract, P3: Drinking yoghurt produced by adding 3 % *H. perforatum* extract

Phenolic compounds contribute directly to the antioxidative effect and it has inhibitory effects on mutagenesis and carcinogenesis in humans (Barış et al., 2011). Also *Hypericum perforatum* extract could be affect from environment conditions undesirable reactions such as temperature, oxygen and heat during processing and storage. Everything of this situations affect antimicrobial charecteristic of plant. The antimicrobial effect of *Hypericum perforatum* was investigated by Düzgüner & Erbil (2020), and it was concluded that methanol extract was effective on all test bacteria. Likewise, Orhan et al. (2013), found that the plant has antibacterial activity. Essential oils in the structure of the plant have antimicrobial, antiproliferative and antioxidant effects (Schepetkin et al., 2020). Hyperforin, one of its bioactive components, was found to be responsible for its antibacterial effect (Hölzl & Petersen, 2003). Aydemir (2015), determined the total number of aerobic mesophilic bacteria as 8 log cfu / g in the samples he

obtained by using saffron and St. John's wort in ice cream production, and found quite higher values than our results. The difference here may be due to the plant extract used, its composition, the amount of bioactive ingredient it contains, especially the amount of hyperforin. In addition, the year, location, climatic conditions, altitude difference, the parts of the plant used for extraction and extraction methods affect the antimicrobial properties of the plant. In addition, if examined on a product basis, ayran is an acidic product and structurally, it is completely different from ice cream. The amount of secondary components used in ice cream production is quite high and this causes an increase in microbial load. Coliform group and yeast development could not be observed in any of the samples used in our study. This is an indication that the raw material, extract, production and storage conditions used are completely hygienic and a healthy functional dairy product is produced.

Ekici (1998), in a study examining the microbiological and chemical quality of ayran, found the number of yeast and mold between  $\log 4 - 7.14 \log \text{cfu/ mL}$ , and Aydemir (2015), found the average number of yeast and mold to  $\log 5 \text{ cfu / g}$ . Yeast and mold development was not observed in the samples used in our study, both at the beginning and at the end of storage, and our samples are quite healthy and microbiologically safe products within the limits given in the Microbiological Criteria Communiqué. Adding *Hypericum perforatum* to our drinking yoghurt samples has reduced the bacterial load and it is very advantageous for safe food production.

### **3.7. Sensory Analyses**

The results in sensory analyses of our drinking yoghurt samples were indicated in the Table 4.

In the sensory analysis (Table 4), when looking at the color and appearance, texture – consistency, taste – aroma scores, the control group received the highest scores, followed by P1 (Drinking yoghurt containing 1% *Hypericum perforatum* extract) sample. As the amount of extract added increases, sensory scores decrease. After the control, the most admired P1 sample got higher scores at the end of the storage in terms of taste – aroma and general taste compared to the beginning of the storage. At the end of the 28 days of storage, while the P1 sample got the same score as the control group, according to their taste and aroma scores; it was preferred by the consumers by getting higher scores than control in structure – consistency and general taste scores. With storage, the interaction of drinking yoghurt, which is a fermented dairy product and extract has increased, and at the end of the storage, a popular product has been obtained for the consumer. However, the increase in the amount of extract caused the scores to decrease, as it was out of the traditional habits of the consumer in general.



Table 4. Change in sensory properties of drinking yoghurt samples during storage.

Sensory analyses	Storage days	Samples			
		K	P1	P2	P3
Color and appearance	1st	4.71 <sup>Aab</sup> ±0.48	3.71 <sup>Bb</sup> ±0.75	3.28 <sup>Bb</sup> ±0.75	3.00 <sup>Bbc</sup> ±0.57
	7th	5.00 <sup>Aa</sup> ±0.00	4.14 <sup>Bab</sup> ±0.37	3.28 <sup>Cb</sup> ±0.75	2.71 <sup>Cc</sup> ±0.75
	14th	5.00 <sup>Aa</sup> ±0.00	4.57 <sup>ABa</sup> ±0.53	3.85 <sup>BCab</sup> ±0.69	3.28 <sup>Cabc</sup> ±1.11
	21st	4.42 <sup>ABbc</sup> ±0.53	4.71 <sup>Aa</sup> ±0.48	4.00 <sup>Bab</sup> ±0.57	3.85 <sup>Ba</sup> ±0.37
	28th	4.00 <sup>Ac</sup> ±0.57	3.85 <sup>Ab</sup> ±0.37	4.14 <sup>Aa</sup> ±0.37	3.71 <sup>Aab</sup> ±0.48
	1st	4.85 <sup>Aa</sup> ±0.37	4.42 <sup>ABa</sup> ±0.78	3.71 <sup>Ba</sup> ±1.11	3.42 <sup>Ba</sup> ±1.13
Texture – consistency	7th	5.00 <sup>Aa</sup> ±0.00	4.28 <sup>ABa</sup> ±0.75	4.00 <sup>Ba</sup> ±0.81	3.85 <sup>Ba</sup> ±1.06
	14th	4.85 <sup>Aa</sup> ±0.37	4.42 <sup>ABa</sup> ±0.53	4.42 <sup>ABa</sup> ±0.78	3.85 <sup>Ba</sup> ±1.06
	21st	4.28 <sup>Ab</sup> ±0.48	4.14 <sup>Aa</sup> ±0.89	4.14 <sup>Aa</sup> ±1.06	4.14 <sup>Aa</sup> ±0.89
	28th	3.85 <sup>Ab</sup> ±0.69	4.14 <sup>Aa</sup> ±0.69	4.00 <sup>Aa</sup> ±0.81	3.71 <sup>Aa</sup> ±0.95
	1st	4.57 <sup>Aab</sup> ±0.78	3.28 <sup>Ba</sup> ±1.25	2.71 <sup>Bb</sup> ±1.11	3.00 <sup>Ba</sup> ±1.15
	7th	4.85 <sup>Aa</sup> ±0.37	3.85 <sup>ABa</sup> ±1.21	3.14 <sup>Bab</sup> ±1.06	3.28 <sup>Ba</sup> ±1.13
Taste – aroma	14th	4.28 <sup>Aabc</sup> ±0.48	3.85 <sup>ABa</sup> ±0.69	3.28 <sup>BCab</sup> ±0.75	2.57 <sup>Ca</sup> ±0.97
	21st	3.85 <sup>Ac</sup> ±0.69	3.71 <sup>Aa</sup> ±0.48	3.28 <sup>Aab</sup> ±0.95	3.28 <sup>Aa</sup> ±0.75
	28th	4.00 <sup>Abc</sup> ±0.57	4.00 <sup>Aa</sup> ±0.00	3.85 <sup>Aa</sup> ±0.37	3.14 <sup>Ba</sup> ±0.69
	1st	4.57 <sup>Aa</sup> ±0.53	3.57 <sup>Ba</sup> ±0.53	3.28 <sup>Ba</sup> ±0.48	2.71 <sup>Ca</sup> ±0.48
	7th	4.71 <sup>Aa</sup> ±0.48	4.00 <sup>ABa</sup> ±1.00	3.57 <sup>ABa</sup> ±1.27	3.28 <sup>Ba</sup> ±1.38
	14th	4.42 <sup>Aa</sup> ±0.53	4.14 <sup>Aa</sup> ±0.69	3.28 <sup>Ba</sup> ±0.75	2.85 <sup>Ba</sup> ±0.89
General taste	21st	4.28 <sup>Aab</sup> ±0.48	3.71 <sup>ABa</sup> ±0.75	3.71 <sup>ABa</sup> ±1.11	3.14 <sup>Ba</sup> ±0.69
	28th	3.71 <sup>ABb</sup> ±0.75	4.14 <sup>Aa</sup> ±0.37	3.14 <sup>BCa</sup> ±0.37	2.85 <sup>Ca</sup> ±0.69

\*: mean standard ± deviation.

A-C: For drinking yoghurt samples, the capital letters on the same line are comparable and the same letters show no statistical difference between the samples. (P> 0.05)

a-c: The lower case letters in the same column are the comparison of the storage times and the same letters show that there is no statistical difference between the samples. (P> 0.05)

K: Control drinking yoghurt, P1: Drinking yoghurt produced by adding 1 % H. scabrum extract, P2: Drinking yoghurt produced by adding 2 % H. scabrum extract, P3: Drinking yoghurt produced by adding 3 % H. scabrum extract

#### 4. CONCLUSION

In our study, *Hypericum perforatum* extract was added to drinking yoghurt, thus an alternative and healthy functional product was obtained. Whether *Hypericum perforatum*, which is not widely used in foods, is suitable for use in ayran has been demonstrated with scientific data. The drinking yoghurt samples obtained were evaluated in terms of physicochemical and

microbiology and presented to the consumer. In addition, the sensory properties of *Hypericum perforatum* were investigated and in case such a product was produced, a demand for the product was tried to be determined. The effects of the plant on the product and shelf life during the storage period were determined and evaluated by storing longer than the normal storage period. As a result, a product that is microbiologically safe and received high scores from consumer appreciate at the end of storage was obtained. Considering the antimicrobial properties of the plant, the fact that it can be used especially in products that have a microbial risk and have a long storage time becomes clear. This plant, which is very valuable in terms of its components, grows spontaneously in nature and can be used in industrial products without any cost in order to benefit from its antimicrobial properties. This kind of herbs can be used not only as tea but also as natural additives in food formulations.

### **ACKNOWLEDGMENT**

This study was supported by the Scientific Research Projects Coordination Unit of Amasya University with the project numbered FMB-BAP 17-0273.

### **CONFLICT OF INTEREST**

The authors of the article declare that there is no conflict of interest between them.

### **AUTHOR CONTRIBUTION**

The authors declare that they have contributed equally to the article.

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