

## Research Article

# Assessment of the Antioxidant Activity and Quality Attributes of Yogurt Enhanced with Wild Herbs Extracts

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The milk and yogurt products assortment has expanded by their enhancement with herb extracts, fibres extracted from by-products of the fruit processing industry and also fresh spices. The aim of the present study was to test to what extent the addition of different herb extracts in yogurt will improve its qualitative characteristics and antioxidant activity. The aqueous extracts obtained from the four plants are considered in this study, respectively, thistle (*Silybum marianum* L.), hawthorn (*Crataegus monogyna*), sage (*Salvia officinalis* L.), and marjoram (*Origanum vulgare* L.). It was examined the effect of aqueous extracts prepared from four herbs (0.25/1%) (w/w) on yogurt's qualitative characteristics (pH, titratable acidity, syneresis, water holding capacity, antioxidant activity, colour parameters, and rheological parameters) on both one day and 28 days after preparation. The final results show that the physicochemical and rheological properties of the yoghurt with herb extracts addition were improved compared to the control sample after 28 days of storage. The best results in terms of antioxidant properties were obtained when marjoram extract (*Origanum vulgare* L.) was incorporated. According to the data obtained, the best quality in terms of the physicochemical and rheological properties were in the case of the sample with 0.5% thistle extract (*Silybum marianum* L.) addition, while from point of view of the nutritional value, the best quality was in the case of the sample with 1% marjoram extract (*Origanum vulgare* L.) addition. The present study leads to the conclusion that yogurts enhanced with natural extracts may serve as functional food products, with significant health benefits.

## 1. Introduction

Yogurt is a fermented dairy product, and it is obtained and consumed massively in many countries and also highly appreciated, being considered important in human diet [1]. Yogurt is a source of bioactive peptides which are formed during fermentation, but generally has a limited content of antioxidant activity. For this reason, several attempts to produce yogurts fortified with natural antioxidants from natural sources have a considerable interest and present a novel approach for product development [2, 3].

In this regard, extracts of many plants, herbs, fruits, and mushrooms rich in bioactive compounds are increasingly used as additive in yogurt for better nutritional and functional improvement. For example, extracts have been used for

preparation of yogurt by many researchers: crude extracts from artichoke [4], grape and grape callus extracts [5, 6], tea infusions [7], *Lycium barbarum* water extract [8], seaweed extracts [9], spirulina [10], *Pleurotus ostreatus* aqueous extract [11], black tea extracts [12], and mangosteen rind (*Garcinia mangostana* Lin.) extract [13].

Herbal nutraceuticals are commonly used by people who seek alternative health care for prevention and treatment of disease. Therefore, in the recent past, there has been rapid growth in demand for herbal medicines in food products like yogurt.

Thistle (*Silybum marianum* L.), in family *Asteraceae*, is one of the important hepatoprotective crops. The active ingredient in milk thistle is silymarin, which is composed of flavonolignans that include silydianin, silychristin, and

silybin [14]. Silymarin is a strong antioxidant, which can promote liver cell regeneration, reduce blood cholesterol, and help in the prevention of cancer [15, 16]. Recently, silymarin has been widely used in commercial preparations, herbal teas, and as a biologically active ingredient in food supplements and medical products. These products are currently among the most rapidly growing sectors in the food product industry [17]. In addition, thistle fruit extracts have antiviral and antitumor activities, and their constituents are under intense research in the clinical therapy of cancer for chemoprevention, treatment, and amelioration of chemotherapy associated side effects [18, 19].

Hawthorn (*Crataegus monogyna*), a member of the *Rosaceae* family, is native to northern temperate zones, including those of North America, East Asia, Central Asia, and Europe [20, 21]. Hawthorns grow as large shrubs or small trees and are usually armed with thorns. Many studies have demonstrated the beneficial effect of extracts of hawthorn fruits on the heart, blood circulation system, and they prevent myocardial dysfunction, improve coronary circulation, and possess hypolipidemic effects. Hawthorn contains a variety of biological active substances, among which polyphenols are a class of the most important and effective components [22, 23].

Sage (*Salvia officinalis* L.) is the most widespread species of the *Lamiaceae* family and encompasses about 900 species distributed throughout the world, which has been recognized for many medicinal plants with designated radical scavenger activity [24, 25]. Its biomass before flowering has been extensively used not only in food processing as a spice but also in pharmaceutical preparations showing a broad range of biological and medicinal activities [26]. Traditionally, it has been widely used as herbal tea, spice, and food flavouring agent, while industrially it found application as fragrance agent in cosmetics, perfumery, and pharmaceutical industry. Different sage species are reported to show many biological activities and medicinal properties, such as antimicrobial, antioxidant, antibacterial, anti-inflammatory, antitumoral, anxiolytic, antidiabetic, antifungal, antiplasmodial, hypoglycaemic, and anticarcinogenic effects [27, 28].

Marjoram (*Origanum vulgare* L.), popularly known as oregano, is a very versatile plant and although it has been used in folk medicine as diaphoretic, carminative, anti-inflammatory and tonic, only now it has been recognized for its antimicrobial property. Marjoram has been traditionally used for the treatment of gastrointestinal disturbances, cough, and bronchial diseases [29]. Marjoram is used in mouthwashes for oral hygiene and also applied topically to relieve symptoms of the common cold, such as nasal congestion. Several studies reported that extracts of marjoram had high antioxidant capacity mostly due to the polyphenolic compounds present in them. This herb has aroused interest among researchers in recent times because it shows biological activities including antimicrobial, antifungal, and antioxidant, and it may have the greatest potential for use in industrial food applications [30, 31].

This study proposed the use of aqueous extracts of some herbs in manufacture of yogurt, plants which are specific to

spontaneous flora in Romania. The objective of this study was to investigate the effect of adding natural ingredients, such as herbs aqueous extracts (ranging concentration of 0.25/1%), on the functionality and the structural properties of yogurt in comparison with a yogurt sample with no additions. The production of yogurt with antioxidant properties has a promising potential for utilization as functional product. The novelty of this study is the choice of these plants to be used as an addition in yogurts, the choice that has been made taking into account the health benefits of the bioactive compounds present in these plants.

## 2. Materials and Methods

**2.1. Materials.** The yogurt samples were obtained in laboratory conditions, using the following raw materials: cow's fresh milk with 3.5% fats, 4.5% carbohydrates, 3% proteins; lactic bacteria cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) supplied by Danisco Romania S.R.L. The herbs used in the experiments were supplied by Fares Bio Vital Laboratories, Romania: thistle (*Silybum marianum* L.)- $Y_{\text{thistle}}$ , hawthorn (*Crataegus monogyna*)- $Y_{\text{hawthorn}}$ , sage (*Salvia officinalis* L.)- $Y_{\text{sage}}$ , and marjoram (*Origanum vulgare* L.)- $Y_{\text{marjoram}}$ . These herbs were milled and made into a fine powder. These herbs were stirred in 200 mL distilled water for 30 minutes at 70°C, stored at  $4 \pm 0.5^\circ\text{C}$  for 12 h, and filtered before use in yogurt production.

**2.2. Yogurt Preparation.** The production of yogurt with different formulations has been carried out by following a traditional process on cow's milk, including pasteurization at 90°C for 15 minutes, cooling to 41°C, inoculation with 0.02% (w/v) starter culture, dosing in packages (yogurt jars), adding herb extracts (0%, 0.25%, 0.5%, 0.75% and 1.0%) (v/v), and incubation at 41°C, until the samples reached a pH of 4.6 [32]. The finished yogurt samples were stored at  $4^\circ\text{C} \pm 0.5^\circ\text{C}$  for 24 h, and then the analyses were performed in order to determine the physicochemical and rheological properties of samples.

### 2.3. Physicochemical Analysis

**2.3.1. Titratable Acidity and pH of Yogurt Samples.** Titratable acidity of yogurt samples was determined by titration with NaOH 0.1N and expressed in Thörner degrees. pH was measured with a pH-meter (Mettler Toledo, Germany) in different stages of samples preparation: during fermentation, in finished product, and during storage.

**2.3.2. Colour Evaluation.** Colour parameters were determined using a CR400 Chroma Meter (Konica Minolta, Japan), with illuminate D65 as a reference:  $L^*$  (100 = white; 0 = black),  $a^*$  (+, red; - green),  $b^*$  (+ yellow; - blue). Samples were analyzed in triplicate.

**2.3.3. Susceptibility to Syneresis (S) and Water Holding Capacity (WHC).** Syneresis of the different yogurt samples was determined according to the methodology proposed by

Barkallah et al. [10]. Therefore, 100 mL of each sample was placed in a funnel lined with Whatman filter paper number 1. After 6 h of drainage, the volume of whey was measured and the following formula was used to calculate susceptibility of syneresis:

$$S = \left( \frac{V1}{V2} \right) \times 100, \quad (1)$$

where  $V1$  is the volume of whey collected after drainage and  $V2$  is the volume of yogurt sample.

Water holding capacity of yogurt samples was determined by the centrifugation of 5 g of yogurt at 4500  $\times$ g for 15 minutes at 4°C (Spin MPW 223E Centrifuge, MPW Med. Instruments, Warsaw, Poland). The WHC was calculated according to the following equation:

$$\text{WHC} (\%) = \left( 1 - \frac{W1}{W2} \right) \times 100, \quad (2)$$

where  $W1$  is the weight of whey after centrifugation and  $W2$  is the weight of yogurt [10].

#### 2.4. Determination of Antioxidant Activity

**2.4.1. Determination of the Total Polyphenol Content (TPC).** Total polyphenol content was determined by an assay applied by Maksimović et al. [33]. TPC was expressed as mg gallic acid equivalent (GAE)/g.

**2.4.2. Radical Scavenging Activity (RSA %) Assay.** Free radical scavenging activity (RSA) of the samples was measured using the method of Brand-Williams et al. [34]. 100  $\mu$ m of the sample solution was mixed with 2.9 mL of DPP (2,2-diphenyl-1-picrylhydrazyl) 60  $\mu$ M in methanol solution. The reaction mixture was left in the dark for 30 minutes, after which the absorbance was measured at 517 nm using a spectrophotometer (Spectrophotometer UV-3600). Methanol was used as blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$\text{RSA} \% = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100. \quad (3)$$

**2.5. Rheological Analysis.** The Modular Advanced Rheometer System (Thermo Haake Mars, Germany) was used for studying the rheological properties of yogurt samples. The samples were allowed to rest for 10 minutes at 4°C on the Ti 40 mm geometry plate, before conducting the analyses. The samples were subjected to frequency dependency experiments from 0.1 to 10.0 Hz, at 4°C. The storage module ( $G'$ ) and the loss module ( $G''$ ) at 1 Hz frequency were monitored. Also, there were conducted viscosity tests depending on time (10 minutes at a constant shear rate of 100  $\text{s}^{-1}$ ). Three determinations of the two tests were conducted for each sample [32]. The Haake RheoWin Data Manager software was used for all the analyses to obtain the graphical

representation of viscosity curves and dynamic tests [35, 36].

**2.6. Statistical Analysis.** The statistical analysis was done using XLSTAT (free trial version 2016, Addinsoft, Inc., Brooklyn, NY, USA) at a significance level at  $p < 0.05$ . The graphical representation of the principal component analysis (PCA) allows the data to be analyzed on a two-dimensional  $F1/F2$  map and to identify the trends between variables at a significance level  $p < 0.05$ . When two variables are far from the centre and close together, they correlate significantly. If they are on the opposite side of the centre, then they are significantly negatively correlated.

### 3. Results and Discussion

**3.1. Physicochemical Characteristics and Antioxidant Activity.** The yogurt samples were analyzed after two storage periods (1 and 28 days) at 4°C. The results are shown in Table 1 and are expressed as the mean value of each analysed parameter of the samples, conducted in triplicate for the two storage periods considered.

An overall decrease of pH values of yogurt samples occurred during storage in refrigeration conditions. The pH for all the yogurt samples decreased during storage period, for example, in the case of  $Y_{\text{thistle-1\%}}$  sample, the pH decreases from 4.62 in the first day to 4.25 in the last day of storage. The addition of herbs aqueous extracts did not seem to significantly affect the pH of the analysed samples compared to the control sample. The results presented above are in agreement with those obtained by Pelaez Vital et al., who reported that pH for control yogurt was approximately the same as pH of yogurts with *Pleurotus ostreatus* aqueous extract [11]. According to Caleja et al., the analysis of this parameter in yogurts is very important in terms of product safety [3].

Titrate acidity of all yogurt samples was between 88 and 111°T on the first day of storage, and between 101 and 116°T, after 28 days of storage in refrigeration conditions for all the analyzed samples. Similarly, Barkallah et al. concluded that additional *Spirulina platens* increased the buffering capacity that required additional acid development by starter cultures during the whole storage period (28 days) at 4°C [10]. The results obtained shows all yoghurt samples with herb extracts have lower pH values ( $p < 0.05$ ) and a higher acidity ( $p < 0.001$ ) than the control sample. One explanation for this aspect is that the herb extracts improve the growth of bacteria contained in the yogurt samples.

Syneresis is considered by many researchers as one of the most important parameters indicating the quality of yogurt during storage [10, 36–39]. The control yogurt samples showed a higher syneresis compared to yogurt samples with different levels of herbs aqueous extracts addition, after the 28 days of the storage. The lowest syneresis after 28 days of storage was observed for yogurt with 0.50% thistle (*Silybum marianum* L.). In the same time, an improvement of the WHC parameter after 28 days of storage was observed for all yogurt samples containing extracts compared to the control

TABLE 1: Physicochemical characteristics and antioxidant activity of yogurt samples.

Storage day	Sample	Characteristics					
		pH	Acidity (T)	Syneresis (%)	WHC (%)	TPC (mg-GAE/g)	RSA (%)
Day 1	$Y_{\text{Control}}$	4.41 ± 0.01	105 ± 0.2	54.4 ± 0.1	35.77 ± 0.1	0.99 ± 0.02	10.56 ± 0.1
	$Y_{\text{thistle-0.25\%}}$	4.50 ± 0.01	101 ± 0.1	53.6 ± 0.2	32.44 ± 0.1	0.98 ± 0.02	10.14 ± 0.1
	$Y_{\text{thistle-0.50\%}}$	4.38 ± 0.02	107 ± 0.2	48.0 ± 0.1	34.11 ± 0.3	0.88 ± 0.01	12.80 ± 0.1
	$Y_{\text{thistle-0.75\%}}$	4.56 ± 0.01	97 ± 0.1	49.8 ± 0.3	34.56 ± 0.1	0.90 ± 0.01	11.02 ± 0.2
	$Y_{\text{thistle-1\%}}$	4.62 ± 0.01	88 ± 0.1	54.2 ± 0.1	30.78 ± 0.1	1.27 ± 0.01	12.67 ± 0.1
	$Y_{\text{hawthorn-0.25\%}}$	4.52 ± 0.02	100 ± 0.1	51.6 ± 0.1	34.86 ± 0.1	3.46 ± 0.01	19.23 ± 0.1
	$Y_{\text{hawthorn-0.50\%}}$	4.36 ± 0.03	111 ± 0.1	49.8 ± 0.1	36.04 ± 0.3	3.88 ± 0.01	21.60 ± 0.1
	$Y_{\text{hawthorn-0.75\%}}$	4.44 ± 0.01	106 ± 0.3	48.0 ± 0.2	35.60 ± 0.1	4.02 ± 0.02	32.02 ± 0.2
	$Y_{\text{hawthorn-1\%}}$	4.50 ± 0.01	104 ± 0.1	53.6 ± 0.2	31.28 ± 0.1	4.34 ± 0.01	33.38 ± 0.1
	$Y_{\text{sage-0.25\%}}$	4.49 ± 0.01	110 ± 0.1	55.0 ± 0.1	37.61 ± 0.2	2.18 ± 0.01	23.14 ± 0.1
	$Y_{\text{sage-0.50\%}}$	4.52 ± 0.03	102 ± 0.3	54.2 ± 0.1	35.02 ± 0.1	3.88 ± 0.03	22.30 ± 0.1
	$Y_{\text{sage-0.75\%}}$	4.51 ± 0.01	105 ± 0.1	54.8 ± 0.1	35.72 ± 0.1	4.17 ± 0.01	20.23 ± 0.3
	$Y_{\text{sage-1\%}}$	4.54 ± 0.02	101 ± 0.1	54.8 ± 0.1	33.92 ± 0.1	4.08 ± 0.01	21.21 ± 0.1
	$Y_{\text{marjoram-0.25\%}}$	4.45 ± 0.3	107 ± 0.2	52.4 ± 0.1	33.21 ± 0.3	3.21 ± 0.03	33.14 ± 0.1
	$Y_{\text{marjoram-0.50\%}}$	4.51 ± 0.01	97 ± 0.1	53.6 ± 0.3	30.90 ± 0.1	5.88 ± 0.01	42.30 ± 0.1
	$Y_{\text{marjoram-0.75\%}}$	4.48 ± 0.01	108 ± 0.1	52.0 ± 0.1	30.38 ± 0.1	5.11 ± 0.01	34.38 ± 0.1
$Y_{\text{marjoram-1\%}}$	4.41 ± 0.01	106 ± 0.2	48.8 ± 0.3	31.67 ± 0.1	5.28 ± 0.01	41.21 ± 0.1	
Day 28	$Y_{\text{Control}}$	4.20 ± 0.01	119 ± 0.1	58.4 ± 0.1	33.72 ± 0.1	0.36 ± 0.01	8.12 ± 0.3
	$Y_{\text{thistle-0.25\%}}$	4.28 ± 0.02	116 ± 0.1	48.3 ± 0.1	45.17 ± 0.1	1.46 ± 0.02	12.67 ± 0.1
	$Y_{\text{thistle-0.50\%}}$	4.26 ± 0.03	114 ± 0.1	45.6 ± 0.1	45.41 ± 0.3	1.12 ± 0.01	19.03 ± 0.2
	$Y_{\text{thistle-0.75\%}}$	4.29 ± 0.03	110 ± 0.1	48.4 ± 0.1	45.24 ± 0.1	0.96 ± 0.01	10.52 ± 0.1
	$Y_{\text{thistle-1\%}}$	4.25 ± 0.02	102 ± 0.3	50.6 ± 0.1	41.35 ± 0.1	1.92 ± 0.02	12.04 ± 0.2
	$Y_{\text{hawthorn-0.25\%}}$	4.32 ± 0.01	105 ± 0.3	53.6 ± 0.1	37.77 ± 0.2	3.82 ± 0.01	19.03 ± 0.2
	$Y_{\text{hawthorn-0.50\%}}$	4.31 ± 0.01	101 ± 0.1	55.6 ± 0.1	37.73 ± 0.1	4.46 ± 0.01	23.74 ± 0.1
	$Y_{\text{hawthorn-0.75\%}}$	4.30 ± 0.01	107 ± 0.2	56.0 ± 0.1	38.15 ± 0.1	4.68 ± 0.01	30.76 ± 0.1
	$Y_{\text{hawthorn-1\%}}$	4.29 ± 0.02	107 ± 0.2	54.0 ± 0.1	35.43 ± 0.2	5.12 ± 0.01	33.49 ± 0.2
	$Y_{\text{sage-0.25\%}}$	4.35 ± 0.02	105 ± 0.1	55.8 ± 0.2	36.80 ± 0.2	2.72 ± 0.01	29.03 ± 0.1
	$Y_{\text{sage-0.50\%}}$	4.33 ± 0.02	108 ± 0.1	54.0 ± 0.1	37.56 ± 0.3	3.36 ± 0.03	22.84 ± 0.1
	$Y_{\text{sage-0.75\%}}$	4.34 ± 0.01	110 ± 0.2	54.4 ± 0.3	35.47 ± 0.2	3.40 ± 0.01	20.52 ± 0.1
	$Y_{\text{sage-1\%}}$	4.28 ± 0.03	116 ± 0.2	55.0 ± 0.1	39.32 ± 0.1	4.17 ± 0.02	20.32 ± 0.3
	$Y_{\text{marjoram-0.25\%}}$	4.30 ± 0.03	107 ± 0.1	51.8 ± 0.1	38.33 ± 0.1	3.42 ± 0.01	33.12 ± 0.1
	$Y_{\text{marjoram-0.50\%}}$	4.29 ± 0.02	110 ± 0.1	54.0 ± 0.2	39.04 ± 0.1	4.16 ± 0.02	40.50 ± 0.1
	$Y_{\text{marjoram-0.75\%}}$	4.30 ± 0.01	105 ± 0.1	55.0 ± 0.1	35.74 ± 0.2	5.40 ± 0.01	38.53 ± 0.1
$Y_{\text{marjoram-1\%}}$	4.30 ± 0.02	106 ± 0.2	56.8 ± 0.1	35.74 ± 0.1	6.30 ± 0.01	42.09 ± 0.2	

samples. The best results were obtained for the 0.5% thistle (*Silybum marianum* L.) addition.

The factor that influencing these parameters, as have been shown by Barkallah et al. and other researchers, is the ability of proteins to retain water and milk fat cells in the structure of yogurt [10, 36–39].

The results for total polyphenol content in the analyzed period (0–28 days) varied in yogurt depending on the type of herb extract that was used. The best results were obtained for the yogurt with marjoram extracts addition; in this case, the TPC level reaching up to 6.30 mg GAE/g during storage.

The yogurt samples with extracts of hawthorn (*Crataegus monogyna*) and sage (*Salvia officinalis* L.) were also rich in polyphenols, and the obtained results show values of 5.12 mg GAE/g and 4.17 mg GAE/g. The total polyphenol content in yogurt samples increases during storage. The total content of polyphenols in the analyzed samples is variable, and the correlation ratio ( $r^2 = 0.139$  and  $r^2 = 0.091$ ) indicates a scattered distribution of the data. The best results were obtained for samples with marjoram (*Origanum vulgare* L.) extracts, which is also noted in the samples with sage (*Salvia officinalis* L.) extracts.

The results of the antioxidant activity correlate with the total content of polyphenols. High antioxidant activity is noted in yogurts with the addition of marjoram extracts both on the first day and on the last day of storage. Antioxidant activity was preserved during storage and even increased in some samples. It can be appreciated that lactic acid and herbal extracts affect the stability of the product over time. According by Muniandy et al., the inclusion of herb extracts prior to bacterial fermentation significantly increased the ( $p < 0.05$ ) antioxidant activity compared to milk alone [12]. This could be attributed to the high content of flavonoids in herb extracts. Similar studies describe that the antioxidant activity of yogurts was enhanced by the presence of natural extracts [3, 14, 16, 22, 23, 25, 29].

The obtained values of colour parameters according to the CIE colour scale are presented in Table 2, for all analysed yogurt samples. The incorporation of the herbs aqueous extracts did not change the colour parameters of the samples. In a similar study, performed by Chouchouli et al., it was shown that the fortification of yogurt with grape seed extract did not affect the colour of the yogurts, and changes were not visually detected [6]. The same aspect was observed

TABLE 2: Colour parameters of the yogurt with different herb extracts.

Storage day	Sample	Colour parameters					
		$L^*$	$a^*$	$b^*$	$C_{ab}^*$	$h_{ab}^*$	$\Delta E^*$
Day 1	$Y_{Control}$	84.91	-6.89	12.61	14.37	118.65	—
	$Y_{thistle-0.25\%}$	83.85	-6.67	11.55	13.34	120.01	1.52
	$Y_{thistle-0.50\%}$	78.8	-6.29	10.87	12.56	120.06	6.38
	$Y_{thistle-0.75\%}$	78.74	-6.21	10.8	12.46	119.90	6.47
	$Y_{thistle-1\%}$	74.27	-5.83	9.97	11.55	120.32	11.01
	$Y_{hawthorn-0.25\%}$	78.59	-6.08	10.71	12.32	119.58	6.65
	$Y_{hawthorn-0.50\%}$	77.99	-6.09	10.88	12.47	119.24	7.18
	$Y_{hawthorn-0.75\%}$	77.47	-5.99	10.84	12.3849	118.9243	7.70
	$Y_{hawthorn-1\%}$	75.23	-5.65	10.61	12.02059	118.036	9.96
	$Y_{sage-0.25\%}$	85.1	-6.77	12.3	14.04005	118.8287	0.38
	$Y_{sage-0.50\%}$	84.56	-6.27	13.01	14.44206	115.7311	0.82
	$Y_{sage-0.75\%}$	80.99	-5.82	12.78	14.04282	114.4845	4.07
	$Y_{sage-1\%}$	84.62	-6	13.57	14.83728	113.8527	1.34
	$Y_{marjoram-0.25\%}$	75.43	-5.47	10.46	11.80392	117.6071	9.82
	$Y_{marjoram-0.50\%}$	75.32	-5.14	10.77	11.93367	115.5129	9.92
	$Y_{marjoram-0.75\%}$	74.78	-4.86	10.99	12.01664	113.856	10.46
$Y_{marjoram-1\%}$	76.06	-4.92	11.34	12.36131	113.4542	9.16	
Day 28	$Y_{Control}$	97.97	-6.04	9.6	11.34	122.18	—
	$Y_{thistle-0.25\%}$	86.02	-7.26	11.92	13.96	121.34	12.23
	$Y_{thistle-0.50\%}$	85.34	-7.21	11.81	13.84	121.40	12.88
	$Y_{thistle-0.75\%}$	82.52	-6.75	11.68	13.49	120.02	15.61
	$Y_{thistle-1\%}$	86.35	-7.25	12.19	14.18	120.74	11.97
	$Y_{hawthorn-0.25\%}$	81.09	-6.58	11.25	13.03	120.32	16.97
	$Y_{hawthorn-0.50\%}$	78.71	-6.27	11.23	12.86	119.18	19.33
	$Y_{hawthorn-0.75\%}$	83.87	-6.79	12.28	14.0322	118.9396	14.37
	$Y_{hawthorn-1\%}$	83.66	-6.67	12.33	14.01848	118.4115	14.58
	$Y_{sage-0.25\%}$	85.52	-6.95	12.23	14.06682	119.6085	12.76
	$Y_{sage-0.50\%}$	82.49	-6.44	12.08	13.68941	118.0626	15.68
	$Y_{sage-0.75\%}$	84.13	-6.3	12.62	14.10512	116.5287	14.17
	$Y_{sage-1\%}$	85.17	-6.43	13.09	14.584	116.1609	13.27
	$Y_{marjoram-0.25\%}$	86.21	-6.61	12.48	14.12241	117.9078	12.12
	$Y_{marjoram-0.50\%}$	85.52	-6.3	12.68	14.15883	116.4203	12.83
	$Y_{marjoram-0.75\%}$	83.88	-6.03	12.78	14.13115	115.2594	14.44
$Y_{marjoram-1\%}$	80.44	-5.43	12.45	13.58261	113.5642	17.77	

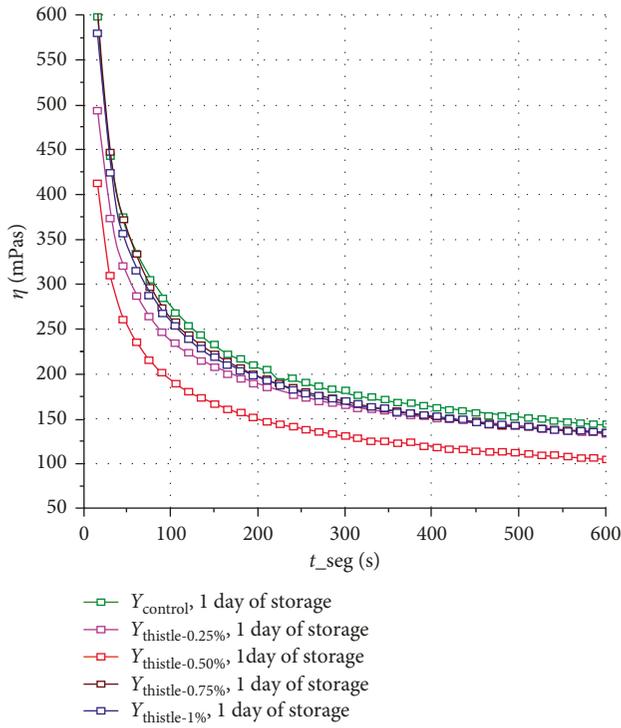
by Caleja et al. in the case of chamomile decoction addition in yogurt [3].

**3.2. Evaluation of the Rheological Properties.** Figures 1–4 show the evolution of samples' viscosity as a function of time at a constant shear rate of  $100 \text{ s}^{-1}$ , for each storage period. This analysis showed the thixotropic characteristics of yogurt samples, which showed a reduction of viscosity in time, in accordance with the data presented in the literature. Several studies confirm that the yogurt is a thixotropic fluid, for example, Mathias et al. [40], Sidor et al. [35], and Sanz et al. [41]. In day 1 of storage, all fortified samples, independent of the amount of herb extract added, showed lower viscosity values than the control sample. However, the graphical representation showed higher viscosity values of fortified samples in the 28th day of storage comparing with the control sample. Sidor et al., evaluating the effect of sea buckthorn powder addition in yogurt, also observed that increasing the dose promoted the formation of a more viscous product [35].

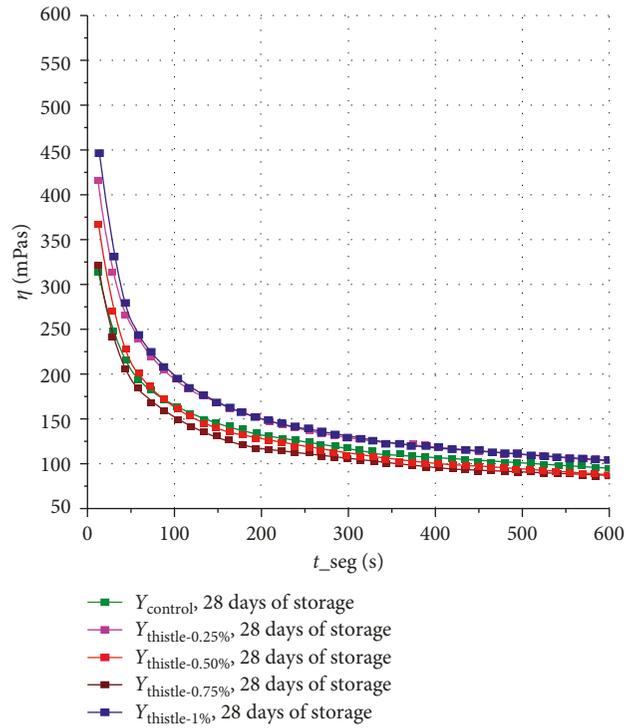
The mechanical spectra obtained for the analyzed samples are shown in Figures 5–8. It can be observed that the form of the spectra was typical for weak gels, in both monitored storage times, with consistency module values ( $G'$ ) slightly higher than those of firmness module values ( $G''$ ). Same trend regarding the viscoelastic properties of yogurt was observed by Sanz et al., when studying the effect of yogurt enrichment with fibers obtained from the non-edible part of asparagus shoots [41]. Also, the incorporation of all types of herb extracts produced an increase in the value of both modules in comparison with the control sample, which can be observed, especially, in the 28th day of storage.

**3.3. Statistical Analysis.** The principal component analysis (PCA) was used to illustrate the relationship between the physicochemical characteristics, the polyphenols content, and the antioxidant activity in yogurt samples. The obtained results for PCA are shown in Figures 9 and 10.

The addition of 1% hawthorn extract, 0.75% marjoram extract, and 0.75% sage has a significant positive influence on

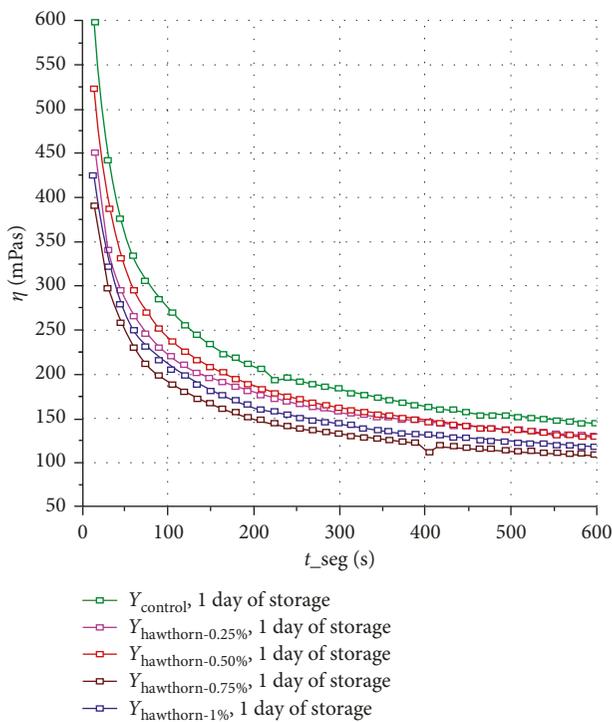


(a)

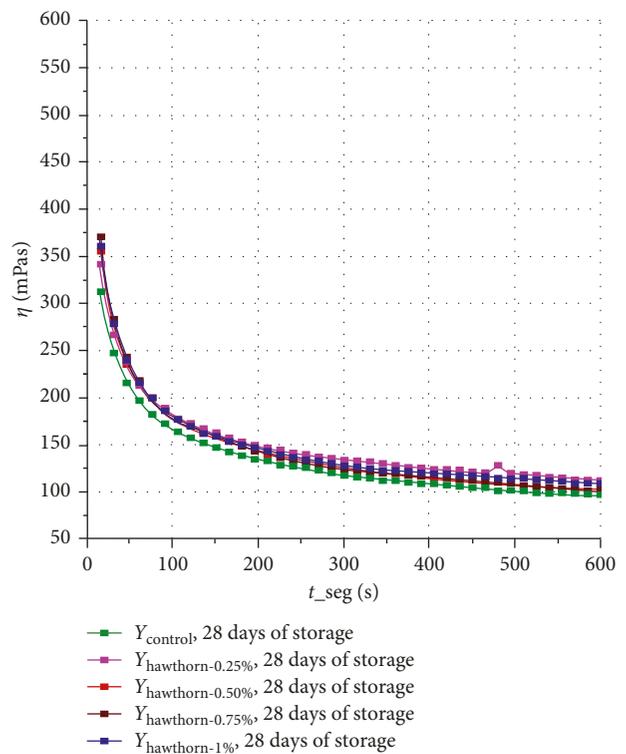


(b)

FIGURE 1: Curve of viscosity versus time of testing thixotropy of yogurt with thistle (*Silybum marianum* L.) extract: (a) 1 day of storage; (b) after 28 days of storage.

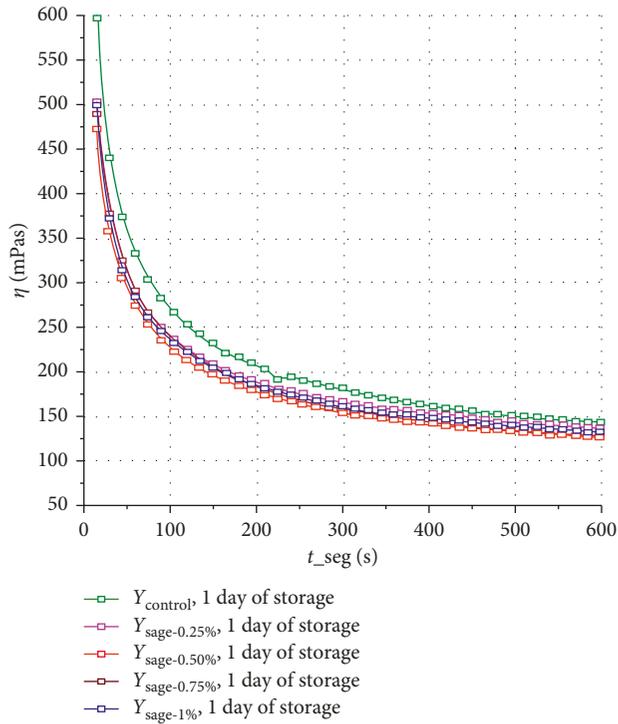


(a)

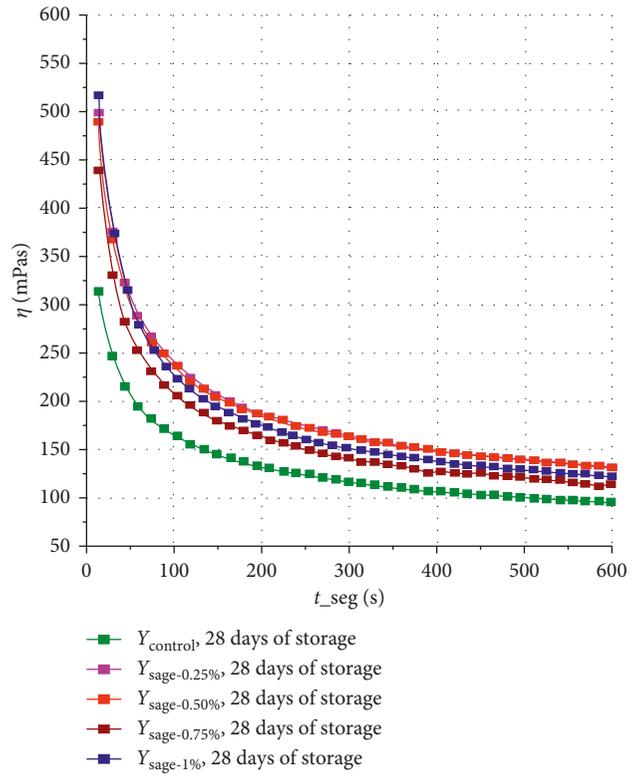


(b)

FIGURE 2: Curve of viscosity versus time of testing thixotropy of yogurt with hawthorn (*Crataegus monogyna*) extract: (a) 1 day of storage; (b) after 28 days of storage.

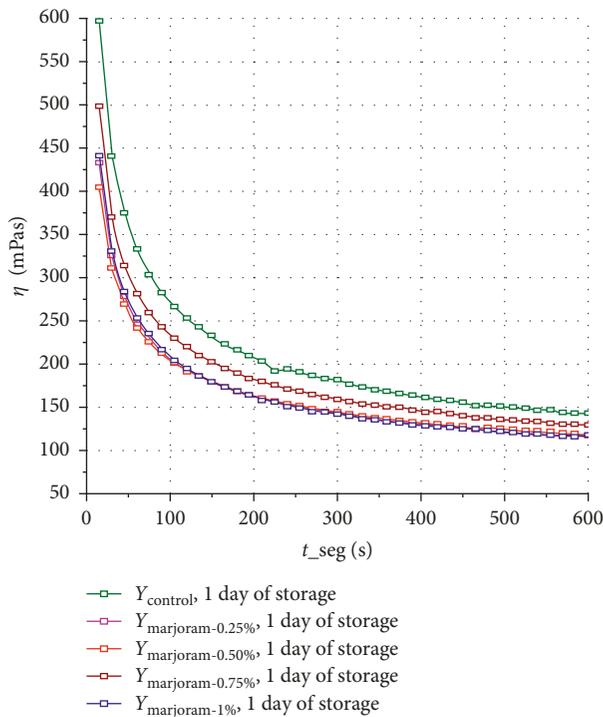


(a)

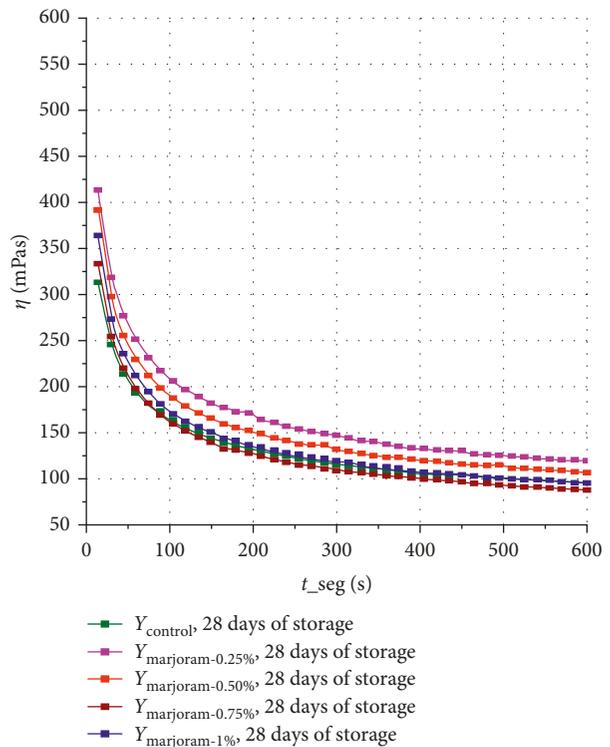


(b)

FIGURE 3: Curve of viscosity versus time of testing thixotropy of yogurt with sage (*Salvia officinalis* L.) extract: (a) 1 day of storage; (b) after 28 days of storage.



(a)



(b)

FIGURE 4: Curve of viscosity versus time of testing thixotropy of yogurt with marjoram (*Origanum vulgare* L.) extract: (a) 1 day of storage; (b) after 28 days of storage.

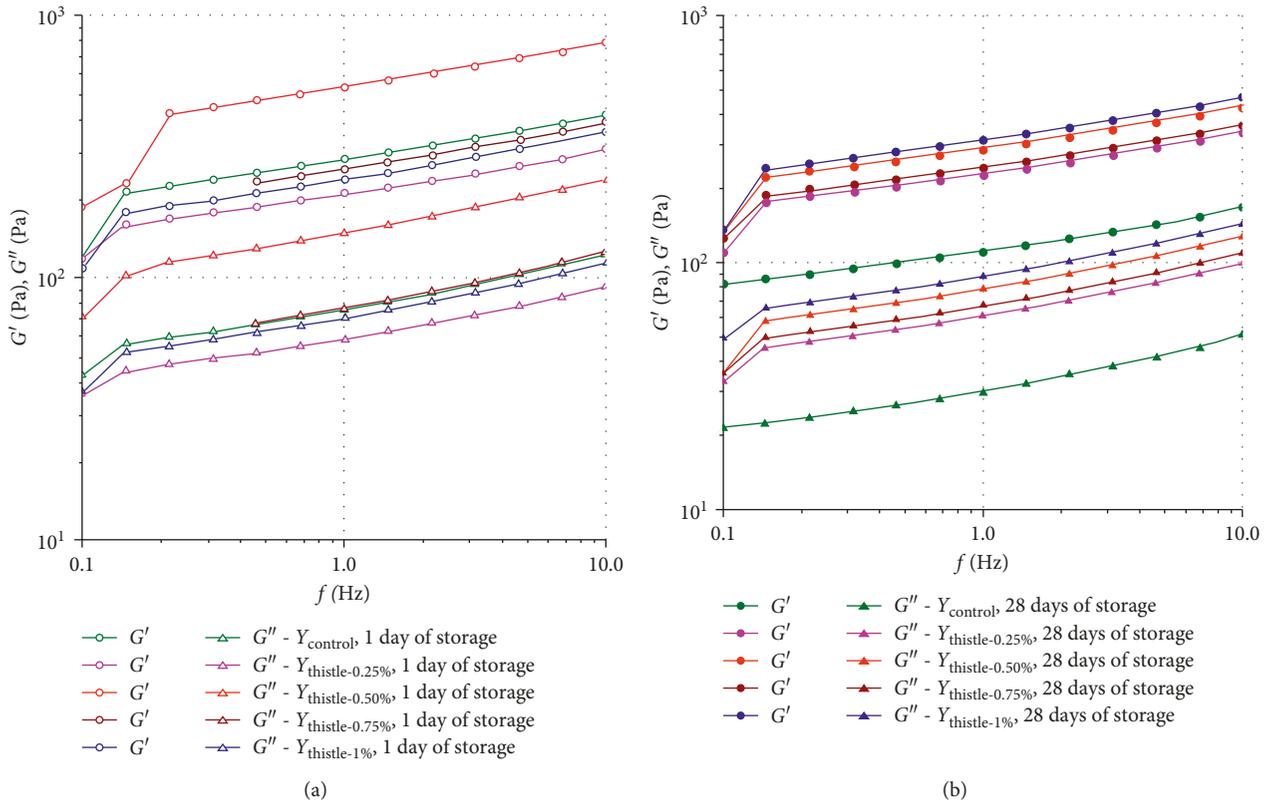


FIGURE 5: Viscoelastic properties of yogurt with thistle (*Silybum marianum* L.) extract: (a) 1 day of storage; (b) after 28 days of storage.

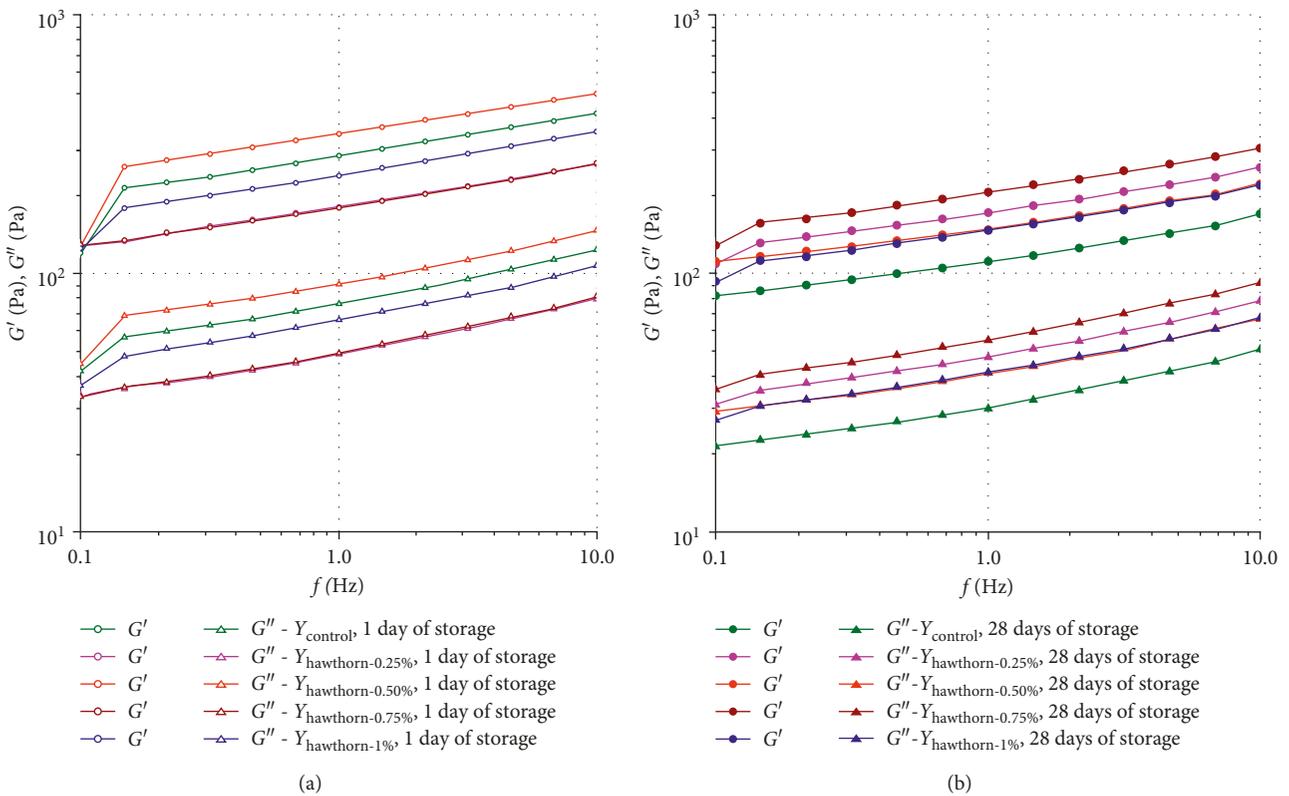


FIGURE 6: Viscoelastic properties of yogurt with hawthorn (*Crataegus monogyna*) extract: (a) 1 day of storage; (b) after 28 days of storage.

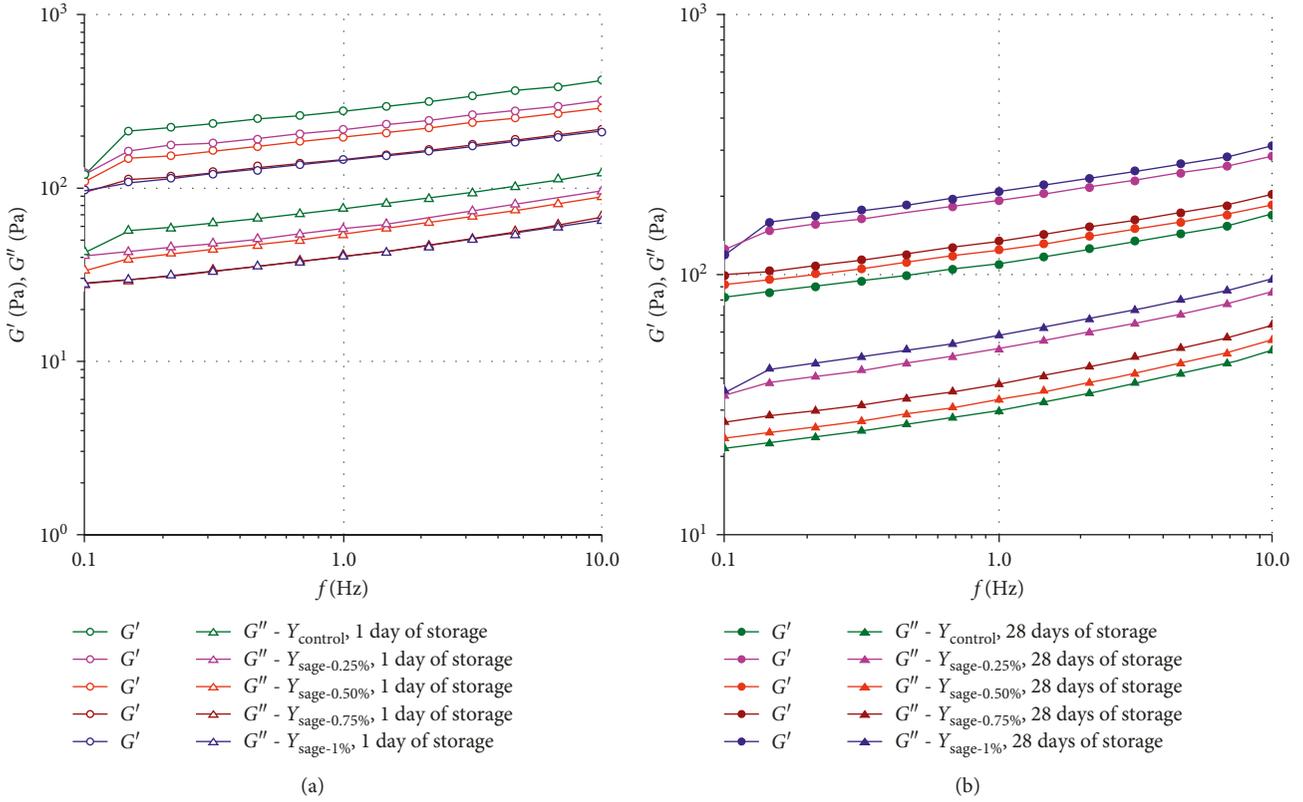


FIGURE 7: Viscoelastic properties of yogurt with sage (*Salvia officinalis* L.) extract: (a) 1 day of storage; (b) after 28 days of storage.

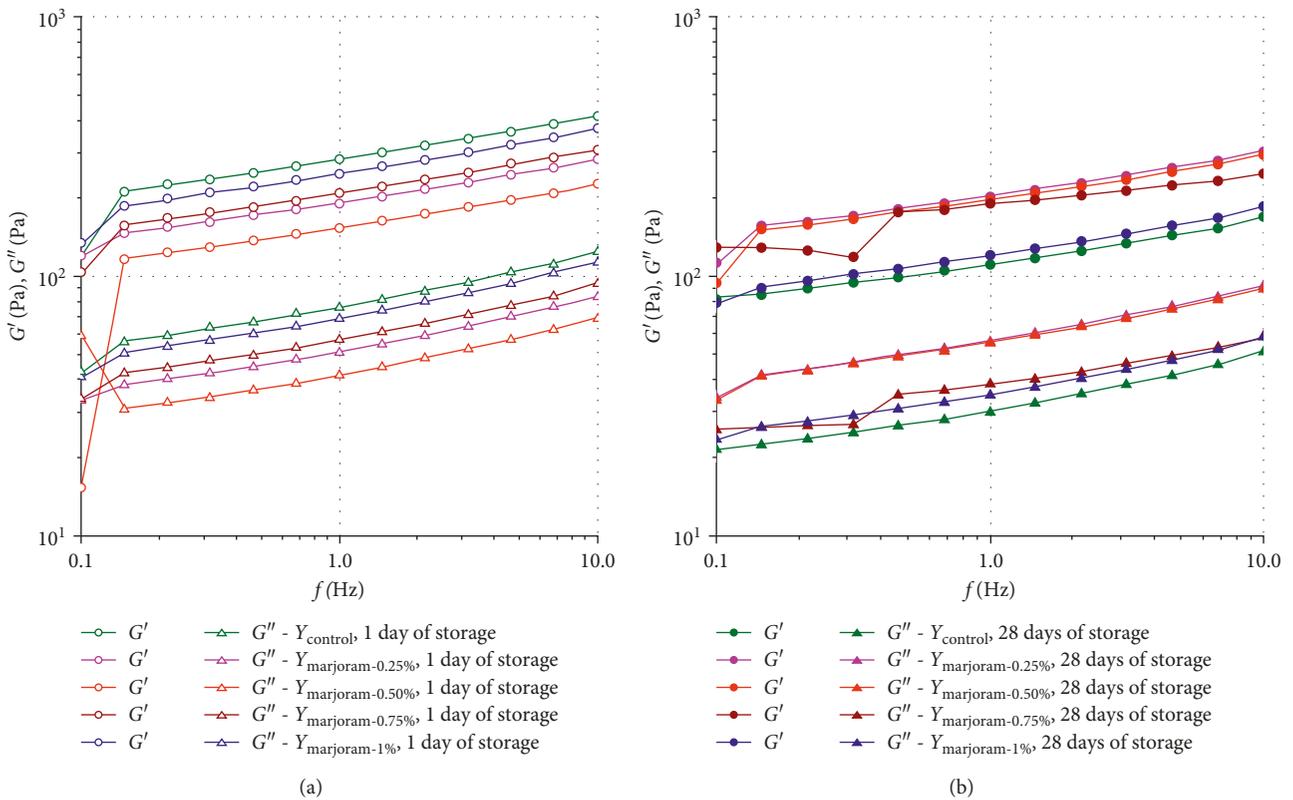


FIGURE 8: Viscoelastic properties of yogurt with marjoram (*Origanum vulgare* L.) extract: (a) 1 day of storage; (b) after 28 days of storage.

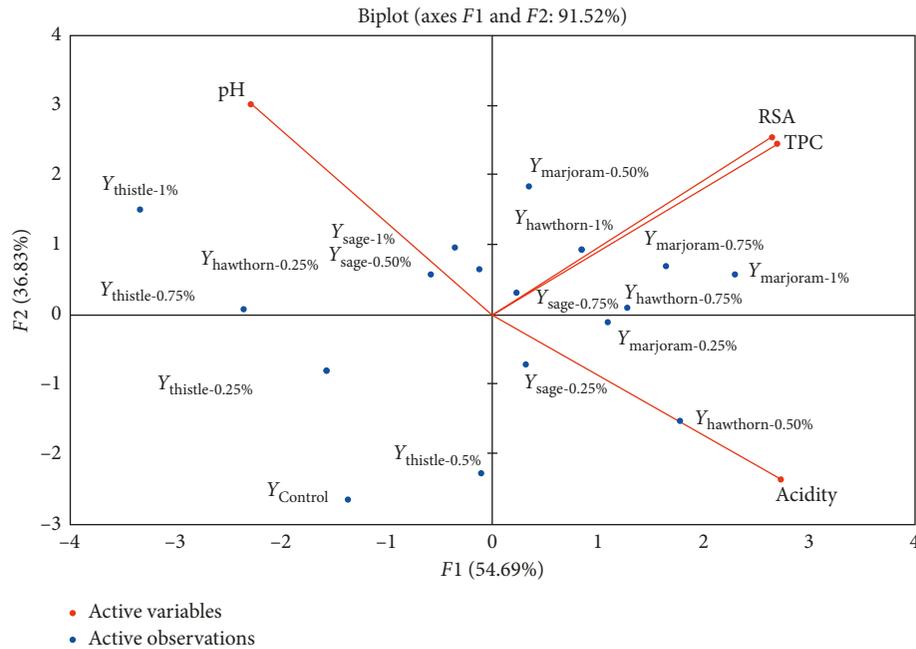


FIGURE 9: PCA loadings of the yogurt characteristics after 1 day of storage.

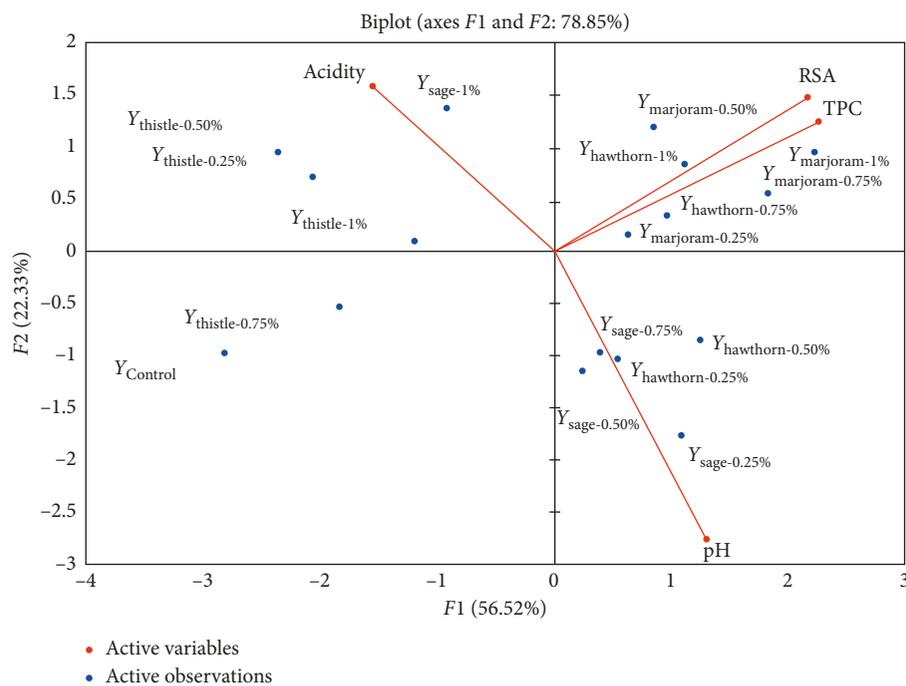


FIGURE 10: PCA loadings of the yogurt characteristics after 28 days of storage.

the antioxidant character of yogurt samples. The acidity of yogurt after a day of manufacture is lower compared to the pH value. The addition of 0.5% hawthorn extract influences the decrease of yogurt acidity.

The addition of sage extract in yogurt does not influence the analyzed physicochemical characteristics. This aspect is distinguished by the distribution of values in separate dials and compared to the control sample (without the addition of plant extracts).

The PCA chart at 28 days after yogurt production indicates an increase in acidity in yogurt samples and a decrease in pH value during storage. The values of the antioxidant activity and the polyphenols content remain high, especially, in the yogurt samples with the addition of marjoram extract. The addition of the hawthorn extract in high amount (0.75/1%) favors the growth of the antioxidant character. These results are in agreement with those obtained by Shyamala which obtained a stability in time of the yogurt

samples when different levels of coriander (*Coriandrum sativum*) and spinach (*Spinacia oleracea*) extracts were added [42].

#### 4. Conclusions

This manuscript demonstrates that extracts from different herbs can be incorporated successfully into a fermented dairy product such as yogurt.

So far, the aqueous extracts obtained from the four plants which are considered in this study, respectively, thistle (*Silybum marianum* L.), hawthorn (*Crataegus monogyna*), sage (*Salvia officinalis* L.), marjoram (*Origanum vulgare* L.), have not been used in the manufacture of yogurt. The final results show that the physicochemical and rheological properties of the yoghurt with herb extracts addition were improved compared to the control sample after 28 days of storage.

Syneresis and water holding capacity was improved due to the thistle extract (*Silybum marianum* L.) addition. The best results in terms of antioxidant properties were obtained when marjoram extract (*Origanum vulgare* L.) was incorporated. The highest rheological values were obtained for the samples with thistle extract (*Silybum marianum* L.) addition. According to the data obtained, the best quality in terms of the physicochemical and rheological properties was in the case of the sample with 0.5% thistle extract (*Silybum marianum* L.) addition, while from the point of view of the nutritional value, the best quality was in the case of the sample with 1% marjoram extract (*Origanum vulgare* L.) addition.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

#### Acknowledgments

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