Journal of Applied Biological Sciences
Uygulamalı Biyoloji Bilimleri Dergisi
E-ISSN: 2146-0108 12 (2): 19-24, 2018

Formulation and Nutritional Evaluation of A Healthy New Diet Soup Powder Supplemented with Pınar Melkior (*Lactarius piperatus*) Mushroom

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Received: 4 May 2018
Accepted: 8 August 2018

Abstract
The mushroom soup is cooked and consumed among the people. However, because of the adding white flour into the mushroom soup, protein and fiber content is decreasing and carbohydrate ratio is increasing. Pinar melkior (*Lactarius piperatus*) mushroom has been specially selected for our research into diet soup formulation due to its high antioxidant, protein and fibrous content. In this study; instead of white flour, a dietary soup formulation was prepared using pectin, locust bean gum polysaccharide derivatives produced produced from by products of fruits that are not digestible in human metabolism and endemic Pinar melcior mushroom.
For this reason, instead of white flour, we prepared a diet soup formulation using endemic Pınar melcior which is known as a medicinal herb, using polymer carbohydrate derivatives which are difficult to digest and obtained from by products of the organic fruit sector. The amount of moisture, amount of ash, amount of protein, antimicrobial activity has been tested to analyze the chemical properties of prepared soup. Also, the antioxidant activity of the soup, (with total reduction activity method, with cuprac method, cupric ions (Cu^{2+}) reducing capacity, with 2,2-diphenyl-1-picryl-hydrazyl free radical (DPPH•) scavenging, superoxide anion radical scavenging) was determined. The test results suggest that the prepared formulation will provide a wide range of use in the food industry due to its long shelf life due to the content of both mushroom and polymeric carbohydrates and its intestinal system is hardly digested due to its fibrous structure.
Keywords: Pınar melkior (*Lactarius piperatus*), Diet soup, Antioxidant activity, Antimicrobial activity

INTRODUCTION

It is the common point of civilizations with the accumulation of many cultures that the Turkish cuisine has different climates. Soup comes as the pioneer of indispensable dishes of Turkish cuisine. Soup is a watery food that is easy to digest, formed by mixing many nutritious foods. In traditional soups, mostly white wheat flour is used. Obesity, cardiovascular diseases, diabetes and some types of cancer are becoming more common with the use of white wheat flour too much. These diseases are directly related to dietary habits[1].

Mushrooms are known throughout the world as healthy foods and fibrous nutrients. It contains herbal proteins, chitin, essential amino acids, vitamins, minerals and low fat in a considerable amount of its composition[2, 3]. In the dry matter of an edible mushroom; 56.8% carbohydrate, 25.0% protein, 5.7% fat and 12.5% ash[4, 5]. However, mushrooms are not only a source of nutrients, but also have medicinal properties due to their bioactive compounds. [6-8]. Because mushrooms are used as dietary nutrients and because of their high antioxidant activity and fibrous structure, they are found in healthy foods.

Almost all organisms have antioxidant defense and repair systems to protect against oxidative damage[9]. These systems are particularly inadequate to completely prevent damage in uncontrolled oxidative conditionsOxidative damage causes many health disorders, including chronic diseases such as diabetes, rheumatoid arthritis, cancer and ulcerative colitis. The presence of phytochemicals in addition to vitamins and provitamins in fruits and vegetables has been recently considered of crucial importance in the prevention of cancer, cardiovascular diseases, diabetes, etc.[7, 10, 11]. Antioxidant activity of any food item is an important criteria of food that attract consumers as its involvement in reducing in the incidence of degenerative diseases[12].

In many epidemiological studies; soup intake has a negative correlation with body mass index and fat intake [13, 14].The results show that soup intake reduces the risk of obesity.Soup intake is recommended for the improvement of obesity [15]. Leptin; a peptide hormone secreted by white adipose tissue that moves to the hypothalamus to support weight loss, which increases energy consumption by reducing appetite and food intake[16-18].However, obese people have higher leptin levels [19, 20].

Pectin is one of the most abundant fibers found on the primary cell wall and middle lamella of higher plants. Pectin is a heteropolysaccharide present in the plant cell wall and middle lamella, consisting essentially of three copolymers, homogalacturonane, (1-4) linked, a-D-galacturonic acid and methyl ester; rhamnogalacturonan-I, (1-2) repeat-linked, a-L-rhamnose- (1-4) a-D-galacturonic acid disaccharide and rhamogalacturan-II. In addition, some pectins have small components of xyoglucuronan, apiogalacturonan, galacturonogalacturonan, galactogalacturonan and arabinogalacturonan[21, 22]. It may have wider applications such as short chain pectin, drug delivery systems, plant protection, prebiotic, heavy toxic metal chelation, anti-cancer agent, radioactive detoxification, anti-obesity, anticoagulant, cholesterol-lowering agent and these pectin functions may be affected by structures and molecular weights closely related[23, 24].

Locust bean gum is a galactomannan consisting of β-(1→4) linked mannopyranosyl backbone attached to α-(l → 6) linked D-galactopyranosyl side chains[25]. The galactomannans are ground endosperm or extracted polysaccharides thereof, obtained from the seeds of Leguminosae.

Since pectin and locust bean gum are natural polysaccharide derivatives, it would be interesting to use them in soup formulations. For this reason, in this study; it was planned to make a diet soup formulation and investigate the shelf

life by using Pınar melkior (*Lactarius piperatus*) mushroom mushroom which has pectin and locust bean gum polysaccharides, which are not digested in human metabolism and has high fiber and antioxidative content.

MATERIALS AND METHODS

Material

In this study, Pınar melkisi (Lactarius piperatus) mushroom was collected from İzmir-Karagöl region and diagnosed by botanists in Atatürk University, Faculty of Science, Department of Biology.The purchased mushrooms were stored at -20 °C until used.

As material in production; Peppermint or carob (powdered granule) was used as an ingredient, Pınar melkisi mushroom, onion, olive oil, fresh milk, pure water, some spices red pepper, black pepper, and thyme, salt.

Preparation of Pınar melkisi Mushroom Diet Soup

First, the onions were finely chopped and cooked in olive oil. Flour was added to the onion and cooked a little. Small chopped mushrooms are added on it. Pınar melkisi (Lactariuspiperatus) mushrooms were cooked and then a 1: 3 milk / water mixture was added. After mixing the soup, salt, pepper, red pepper and thyme were added.

The same formulation was also prepared using pectin and goat horn instead of flour.

Prepared soups were dried in a lyophilizer at -60 °C for 92 hours.

Some Chemical Analyzes of Soups Prepared in Different Formulations

Dry Matter and Assay Ash

The method of AOAC [26] was applied to determine the dry matter content of the saddles prepared in different formulations. In a sensitive balance of 0.1 mg, porcelain crucibles were taken intact and 3,5-4 g of lyophilized soup samples were weighed and dried at 105° C for 4 hours. It was then cooled in a desiccator and weighed on a precision scale. The same samples were burned in the ash furnace at 900 ° C for 2 hours for raw ash storage. After burning, it was weighed in a desiccator and then the process was repeated.

As a result of the analysis, the following formulas were used for the calculation of percentages of dry matter and ash:

$$Crude\ Ash\ (\%) = \frac{[Tare(g) + Crude\ Ash\ ] - [Tare(g)]}{Sample\ Quantity(g)} * 100$$

$$Dry\ Matter\ (\%) = \frac{[Tare(g) + Dry\ matter\ (g)] - [Tare(g)]}{Sample\ Quantity\ (g)} * 100$$

Antioxidant Capacity Assays

10 g of the lyophilized soup samples were taken for the determination of antioxidant capacity and 30 mL of distilled water and ethanol were mixed in the magnetic stirrer for 1 day. The soup extracts were then filtered to remove the supernatant alcohol in the evaporator and the water samples were removed on the lyophilizer, and then the soup samples were stored at -20°C for use in antioxidant assays.

Copper (II) Reducing (CUPRAC) Antioxidant Capacity

CUPRAC antioxidant capacity of prepared soup extracts Apaket al.[27]. For this purpose; 1 mL of 0.01 M CuCl2 solution, 1 mL of 7,5x10-3 M neocuprin ethanol solution, 1 mL of ammonium acetate buffer solution were added to the test tubes. 10, 30, 50 µg / mL soup extracts were added to the final volume of 4 mL with distilled water. The stirred test tube contents were incubated for 30 minutes at room temperature. BHA and α-tocopherol were used as standards. Obtained color change the spectrophotometric determination of the absorbance measurement at 450 nm.

Determination of total reducing capacity

In this assay, soup extracts were transferred to 10, 30, 50 µg / mL test tubes and 1 mL of pure water was added. 2.5 mL of 0.2 M pH 6.6 phosphate buffer, 2.5 mL of K3Fe (CN)6 solution was added and incubated for 20 minutes in a water bath adjusted to 50 ° C. After the incubation, 2.5 mL of 10% TCA was added. Take 2.5 mL of the supernatant of the solution and add 2.5 mL of purified water, 0,1 mL of 0.5 mL of FeCl3. The absorbance change of the reaction medium was read spectroscopically at absorbance 700 nm. In the blind experiment, pure water was used instead of the sample.

DPPH Free Radicals Removing Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is a stable and long-lasting nitrogen radical as opposed to other radicals in the organism. The displacement of one of the free electrons creates a dark violet-colored solution [28].

DPPH radical sweeping effect was made according to the method proposed by Blois [29]. 10, 30, 50 µg / mL soup extracts and the final volume was supplemented with 3 mL of ethanol. 1 mL of 1 mM DPPH solution was added to all tubes and the mixture was incubated at room temperature for 30 minutes in the dark. Subsequently, the absorbance against the ethanol-derived fraction was measured spectroscopically at absorbance 517 nm. In the blind experiment, ethyl alcohol was used instead of the sample. DPPH free radical scavenging activity was calculated according to the following formula:

$$DPPHScavenging\ effect(\%) = \left[\left(Ao - \frac{A1}{Ao}\right) * 100\right]$$

Ao: Absorbance of the control reaction

A1: Absorbance of sample reaction

Super Oxide Anion Radicals Scanvenging Activity

Superoxide anion scavening activity Liu et al.[30] measured according to the proposed meta. The test tubes were added separately from 10, 30, 50 µg / mL soup extracts respectively. 0.05 M 100 mL 1.33 * 10-5 M Riboflavin, 4.46 * 10-5 M Metionine and 8.15 * 10-8 M NBT were added to the phosphate buffer at pH 7.8. The final volume is added as 2.5 mL from the prepared mixture. It is stimulated with 20 W fluorescent light for 40 minutes at room temperature. As a control, the solution was used without sample. Absorbance change was measured spectroscopically at 562 nm [31].

The percentage of superoxide anion removal activity was calculated according to the following formula:

$$\%\ İnhibition = 100 - \left[\frac{Ao - A1}{Ao}\right] * 100$$

Ao: Absorbance of the control reaction

A1: Absorbance of sample reaction

Assayof Antimicrobial Activity

Antimicrobial activity was determined immediately after waiting for 1 month at all temperatures and 1 month at +5 °C. For this purpose disc method was used[32]. AcinetobactercalcoaceticusandPediococcusacidilacibacteria were used to determine the antimicrobial activities of the prepared mushroom soup samples

The microorganisms to be used for the determination of antimicrobial activity were incubated in Nutrient Broth medium at 34 °C for 24 hours. These bacteria grown on sterile petri dish were planted with drigaski. Discs of 6 mm in diameter were formed from the middle of these sowing petries. Soup extracts were formed into the formed disks and

the prepared petrel was incubated at 34 °C for 24 hours. At the end of the period, the diameters of the inhibition fields around the discs were measured.

RESULT AND DISCUSSION

Total Dry Matter and Ash

Diet soup samples are prepared using flour, pectin and locust beam gum and lyophilized with Pınar Melkiormushroom soup were dried at 105 ° C and the resultingdry matter % and ash% results are given in Table 1.

Table 1. Dry matter and ash results of prepared soup

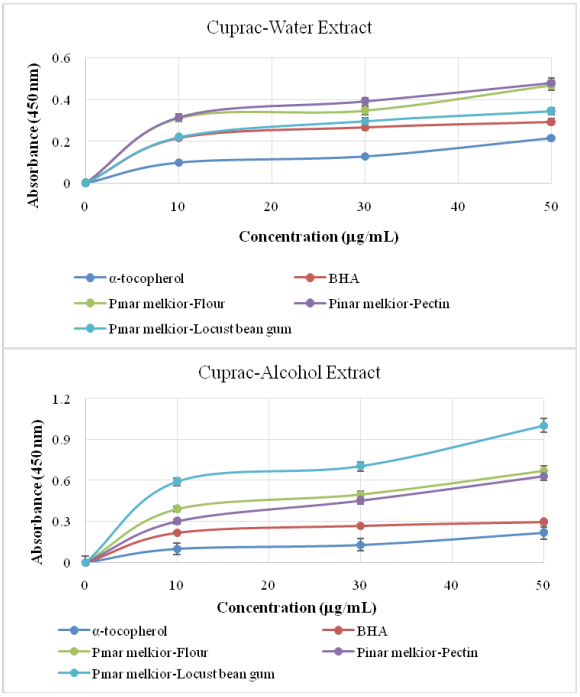
Soup	Total Dry Matter (%)	Ash (%)
Flour-Pınar Melkior Soup	90,9	1,37
Pectin-Pınar Melkior Soup	95,12	1,55
Locust beam gum-Pınar Melkior Soup	94,87	1,32

The highest amounts of ash in the prepared soup were respectively determined as pectin-Pınar Melkior Soup >Flour-Pınar Melkior Soup > Locust beam gum-Pınar Melkior Soup. The amount of minerals is high because of the ash values of the soup prepared with pectin compared to others. This result shows that pectin-Pınar Melkior soup has a high nutrient and fiber content [33].

Antioxidant Capacity Determination Results

Copper (II) Reductive Antioxidant Capacity (CUPRAC) Results

The antioxidant results of copper (II) reduction antioxidant activity (CUPRAC) of the Pınar Melkior mushroom diet soup prepared with flour, pectin and locust beam gum are given in Figure 1.



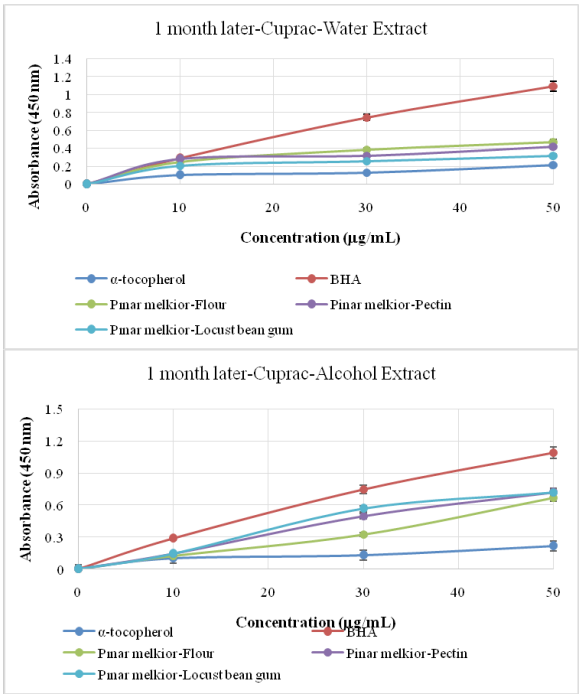


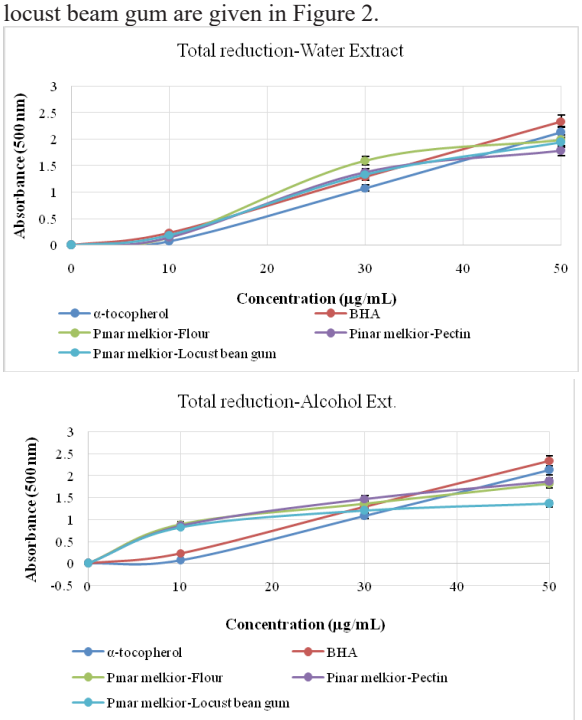
Figure 1. Copper reduction capacity graphs of soup **1:** Water extract **2:** Alcohol extract, **3:** 1 month later- water extract **4:** 1 month later- alcohol extract

The highest copper reduction activity by the CUPRAC method was observed in alcohol extracts. The highest activity in alcohol extracts is respectively locust beam gum-Pınar Melkior Soup> Flour-Pınar Melkior soup> Pectin-Pinar Melkiorsoup> BHA> alpha-tocopherol.

The antioxidant capacity of the alcohol extract is higher in antioxidant results made 1 month after the preparation of the lyophilized soup. The highest activity was observed at a concentration of 50 µg / mL[34, 35].

Total Reduction Capacity Results

The results for the total reduction antioxidant capacity of the Pınar Melkior diet soup prepared with flour, pectin and locust beam gum are given in Figure 2.



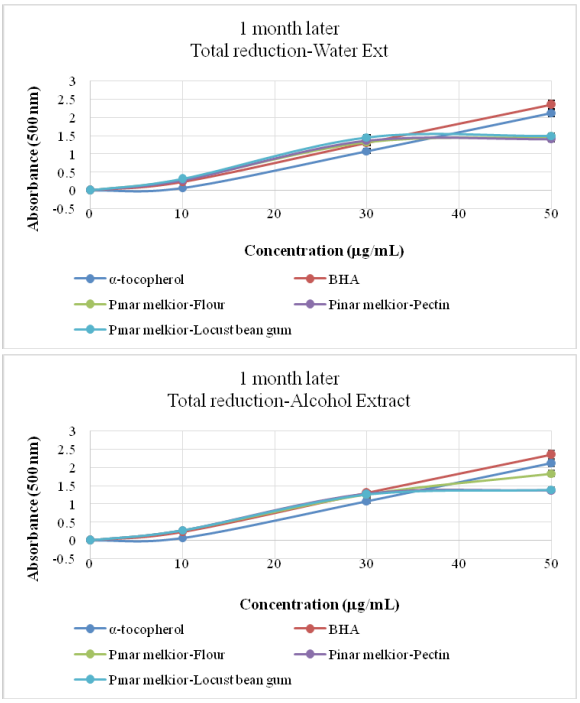


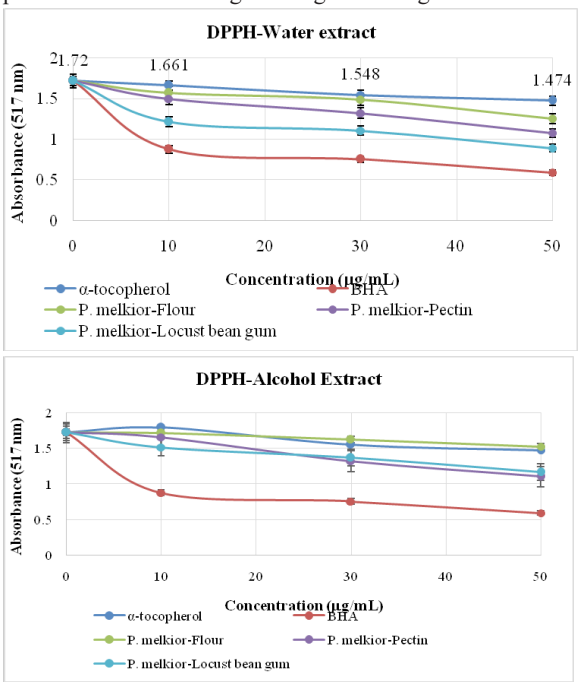
Figure 2. Total reduction graphs of soups 1: Water extracts 2: Alcohol extracts, 3: 1 month later-Water extracts 4: 1 month later-Alcohol extracts

As a result of total reduction capacity was found to be highest in the concentration of 50 µg / mL of water extract, BHA> α-tocopherol>Locust bean gum-Pinar Melkior soup> pectin-Pınar Melkior soup; respectively.

After 1 month of the lyophilized soups, total reduction activity was determined in order of antioxidant by BHA> α-tocopherol> Pectin-Pınar Melkior soup> Flour-Pınar Melkior soup >locust bean gum-Pınar Melkior soup in alcohol extract at a concentration of 50 µg / mL[36].

DPPH Radical Sweeping Effect Results

The results obtained for the DPPH radical sweeping effect of the Pınar Melkior diet soup prepared with flour, pectin and locust beam gum are given in Figure 3.



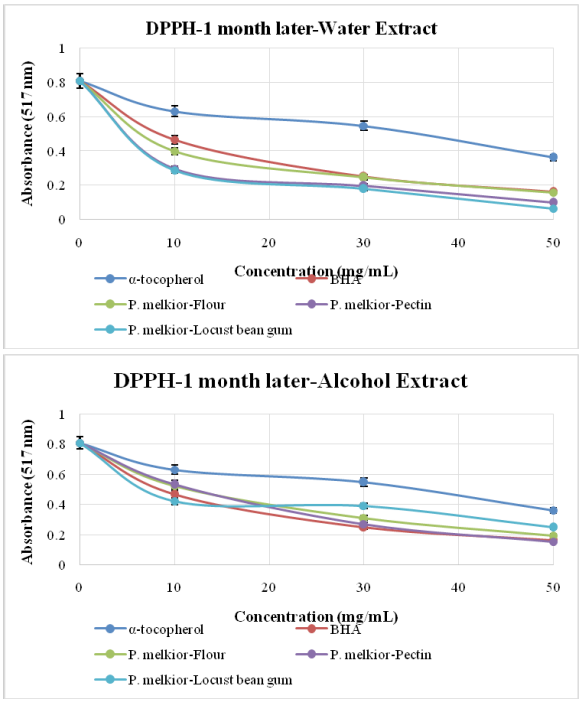


Figure 3.DPPH radical sweeping graphs of soups 1: Water extracts 2: Alcohol extracts, 3: 1 month later-Water extracts 4: 1 month later-Alcohol extracts

As a result of the DPPH radical sweeping effect, the highest activity was obtained in the alcohol extract at a concentration of 10 µg / mL, α-tocopherol> Flour-Pınar Melkior soup> Pectin-Pinar Melkior soup> Locust beam gum-Pınar Melkior soup > BHA, respectively.

One month later, as a result of the DPPH radical sweeping effect, the highest activity was obtained in the alcohol extract at a concentration of 10 µg / mL, α-tocopherol> Pectin-Pinar Melkior soup> Flour-Pınar Melkior soup> BHA> Locust bean gum-Pinar Melkior soup, respectively (Demir et al., 2014).

Superoxide Anion Removal Activity Results

The results obtained for the Superoxide anion removal activity of the Pınar Melkior diet soup prepared with flour, pectin and locust beam gum are given in Figure 4.

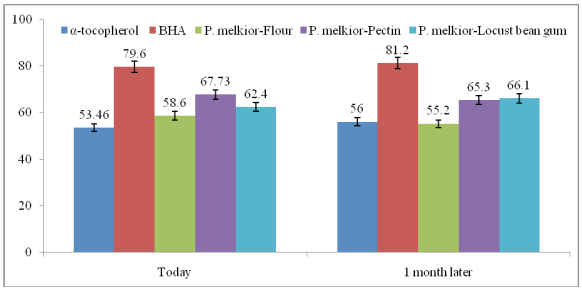


Figure4.Liyofilize çorbaların süper oksitanyon giderme aktivitesi (1: Water extracts 2: Alcohol extracts, 3: 1 month later-Water extracts 4: 1 month later-Alcohol extracts)

As a result of superoxide anion removal activity, the highest activity was determined in 67% of the soup formulation prepared with pectin after BHA. BHA> Pectin-Pınar Melkior soup> Locust bean gum-Pınar Melkior soup> Flour-Pinar Melkior soup> α-tocopherol activity determined, respectively. BHA> Locust bean gum-Pinar Melkior soup>Pectin-Pınar Melkior soup> α-tocopherol>Flour-Pınar Melkior soup was determined as antioxidant after 1 month of lyophilized soups (Demir et al., 2014).

Results of Antimicrobial Activity Analysis

The results of antimicrobial activity using the disk diffusion method are given in Table 2.

Table 2. Results of antimicrobial activity of soups

Bacteria	Control	Flour		Locust bean gum		Pectin	
	Diameter (mm)	Diameter (mm)		Diameter (mm)		Diameter (mm)	
		Water	Alcohol	Water	Alcohol	Water	Alcohol
Acinetobacter calcoaceticus	0 mm	19 mm	12 mm	22 mm	13 mm	22 mm	15 mm
Pediococcus acidilactici	0 mm	12 mm	11,5 mm	13 mm	12 mm	15 mm	17 mm
Acinetobacter calcoaceticus (1 month later)	0 mm	18 mm	12 mm	22 mm	13 mm	21 mm	14 mm
Pediococcus acidilactici (1 month later)	0 mm	11 mm	11 mm	13 mm	12 mm	14 mm	16 mm

Acinetobacter calcoaceticus and Pediococcus acidilactici used in this study were tested against test microorganisms when the antimicrobial activities of different additives were compared, it was seen that the most effective result was soup prepared with pectin, locust bean gum and flour, respectively.

CONCLUSION

The long shelf life of the diet soup formulation, which is the result of the test, is based on both fungal and polymeric carbohydrate content. It is also thought that the intestinal system can also solve the digestive problems due to the high fiber structure of the soup. For this reason, diet soup will find a wide use in the food industry.

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