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The sensoryanalysis and antioxidant activity of the honey marmalade

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Abstract
It has been proven that low-quality food products and snacks can adversely affect the health of consumers. The honey and other bee products, which are functional food products, have gained value in recent years with the importance given to beekeeping. The bee products can scavenge free radicals with the phenolic compounds they contain.They are also known to be natural sources of antioxidants that can combat oxidative stress. The antioxidant activity of honey is enhanced by adding phenolic compounds, sugars, proteins, carotenes, amino acids, Maillard reaction products and organic acids.
In this study, the honey was used as the main ingredient, and the honey marmalade consists of vanilla, propolis, pollen and royal jelly. The ash, moisture, % antioxidant activity, protein-carbohydrate-fat, refractive index, pH and acidity analysis of the obtained nutritive product were performed. In addition, the sensory analysis wasevaluated on 15peoplefor the parameters such as the appearance, clarity, odor, taste and general acceptance. As a result, thanks to the bioactive components contained in the functional product obtained, a new product that is both delicious and high in nutritional value to consumers.

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I. INTRODUCTION

Most people who have recently adopted a healthy lifestyle tend to use natural products as supplements and complementary medicine or alternative treatments (Zaid et al., 2021). Honey is a natural product that has been used as a complementary medicine since ancient times (Lan Nguyen et al., 2019). The honey has been widely used since ancient history, as it has therapeutic values due to the presence of high nutritional and bioactive components (Dzugan et al., 2018; Zaid et al., 2021).

The biologically active compounds in the honey are investigated into two groups as antibacterial and antioxidants. The polyphenol compounds (phenolic acids and flavonoids), vitamin C, vitamin E and enzymes (for example, catalase, peroxidase) are responsible for the antioxidant activity of honey. According to the Honey Communiqué, reference analysis values of flower honey should be within the limit values given in the explanation (the moisture: 20%, sucrose: 5 g/100 g, fructose + glucose: 60 g/100 g, fructose/glucose: 0.9-1.4, the amount of water-insoluble 0.1 g/100 g, the free acidity: 50 meq/kg, the electrical conductivity: 0.8 mS/cm, the diastase amount: 8, HMF: 40 mg/kg, proline: 300 mg/kg).

The propolis is a resinous and sticky natural complex matrix produced by honey bees from mucilage and sap collected from different regional and botanical sources (plant leaves, flower buds, bark, etc.) (Abbasi et al., 2018). The pollen, which is found in the heads of the male reproductive organs of plants, is one of the important food sources of honey bees. The royal jelly, known as superfood, is the food that is secreted from the upper jaw and pharyngeal salivary glands of 5-15 days old worker bees, and that the queen bees are fed for life (Braakhuis, 2019; Abbasi et al., 2018; Nguyen et al., 2019; Ramsay et al., 2019). The vanilla powder could be a different food additive for inclusion in functional foods due to its distinctive flavor. Besides usage of vanilla as a food additive, it also has complementary medicinal applications like alleviation of fever, spasms, gastrointestinal irritations. Hundreds of chemical compounds are found in vanilla extract; however, the main contributor is the vanillin which possess antioxidant activity (Singletary, 2020). Figure 1 shows the molecular structure of vanillin. For food products to be accepted by consumers, they must meet, among other requirements, their organoleptic properties, which are mainly influenced by flavor.The taste is one of the most important factors in attracting consumers and maximizing food quality.

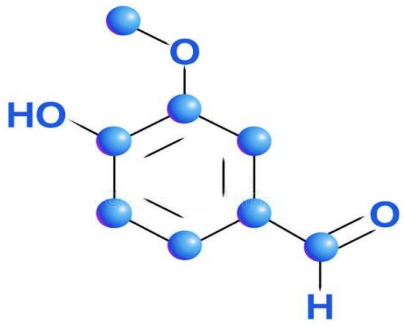


Figure 1. The molecular structure of vanillin

In this study, it was aimed to obtain an untested product by using propolis, pollen, royal jelly and vanilla, with honey as the main ingredient. The ash, moisture, % antioxidant activity, protein-carbohydrate-fat, refractive index, pH and the acidity analysis of the formulated nutrient product obtained. In addition, sensory tests of the new formulation on 15 people were characterized according to the appearance, clarity, odor, taste and general acceptance. The use of royal jelly, pollen, honey and propolis, which are bee products, in different forms and used as a medicine in the treatment of human diseases is called Apitherapy in the medical language. Thanks to the bioactive component contents of the products used, it is aimed to present the new product to the consumer in the functional food category.

II. EXPERIMENTAL PROCEDURE

2.1.Materials

The functional product was obtained by mixing different amounts of honey, propolis, pollen, royal jelly and vanilla.The A1 sample contains on average 94% honey, 2% vanilla, 1% bee pollen, 2% royal jelly, 1% propolis. The A2 sample contains an average of 91% honey, 5% vanilla, 1% bee pollen, 2% royal jelly, 1% propolis. The flower honey was used as a honey sample. The marmalade was mixed until it became homogeneous under laboratory conditions. The final products were stored at +4 ^{o}C until further analysis. In the preparation of the product material, raw material support was received from a private commercial company.

2.2.Methods:

The Moisture Analysis: The prepared honey mixture (5 g) was weighed, and it was mixed by adding 100 mL of distilled water. After the solution was filtered through a folded filter paper with a certain mass, it was read in a refractometer at 25 ^{o}C and the moisture content was determined by adding a coefficient of 0.002 to the measured results (Soylu et al., 2020).

The Protein Analysis: Total protein content was determined by the Kjeldahl method (AOAC, 2000), with some modifications. The homogenized sample (1 g) was placed in the Kjeldahl flasks, 12 g K_2SO_4, 0.3 g $CuSO_4.5H_2O$, were added on it. The concentrated H_2SO_4 solution (25 mL) was added slowly, the Kjeldahl flask prepared by discarding the boiling stone or glass bead was placed in the incineration set. Incineration was carried out for 15 minutes at 200–250 ºC and for 30–45 minutes at 350–380 ºC.After burning all samples, Kjeldahl flasks were cooled and distillation was performed. Distillation was carried out by adding 100 mL of 35% NaOH solution and distillate was collected in an Erlenmayer flask which contained of 4% H_3BO_4 solution (25 mL) and 2-3 drops of indicator (methyl red and bromocresol green (5:1)) were added to the solution. The distillation process continued until 150 mL of distillate was collected for 5 minutes. Titration was done with 0.1 N HCl and total protein content was calculated with the following equation (1):

$$\%\,TP = \frac{(V_t - V_0) \times N \times 14 \times 6.25 \times 100}{w \times 1000} \quad (1)$$

Where; N: normality of HCl, w: mass of the sample (g), V_t: volume, in mL, of HCl used in the sample titration, V_0: volume, in mL, of HCl used in the blank titration, 6.25: protein conversion factor, 14: molar mass of nitrogen. The total protein content was expressed as g protein /100 g dry matter (dm).

Crude Fat Analysis: The crude fat content of samples was determined according to the Soxhlet method as described in AOAC (2000). Samples were weighed onto Whatman filter paper (No.1) at 4 g and placed in the Soxhlet apparatus. Petroleum ether was used as the extraction solvent. Samples were extracted in Soxhlet extractor at a rate of five drops per second by condensation for about 4 h. Crude fat was determined as the

weight of fats in the extract after removal of the solvent by using Rotary evaporator at 60 ºC. Total fat content was expressed as g fat/100 g dm.

The Ash Content Determination: The porcelain crucibleswere kept in the muffle furnace for about 2 hours, then cooled in a desiccator. For ash determination, 2 g of sample was weighed and transferred to crucibles. The pre-burning process was carried out until there was no smoke in the burner flame. The samples were burned in the muffle furnace at 525 ± 10 ^{o}C until they turned white or ash color and reached constant weight. After 30 minutes in the desiccator, it was weighed immediately and the ash amount was calculated after removing the tare of the crucibles(Soylu et al., 2020).

pH Analysis: The distilled water (12.5 mL) was added to the sample (5 g) and mixed in the homogenizer until homogenized. The pH meter device was immersed in the homogenized sample and the pH value was measured at 20±2 ^{o}C (Soylu et al., 2020).

The Acidity Determination: The sample (5 g) was weighed and 37.5 mL distilled water was added to it. The flask was then closed and mixed thoroughly. After adding 5 drops of phenolphthalein indicator to the solution, it was titrated with standard sodium hydroxide solution to the equivalence point and the result was calculated as meq/kg (Soylu et al., 2020).

The Determination of %Antioxidant Activity: The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams et al. (1995). The samples were reacted with the stable DPPH radical in methanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute methanol and 0.3 mL of DPPH radical solution. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The samples were read [Absorbance (Abs)] at 517 nm after 120 min of reaction using a UV-VIS spectrophotometer. The mixture of methanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing methanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to Mensor et al., 2001). The following formula (2) was used for calculations;

$$AA\% = 100 - \frac{(Abs_{sample} - Abs_{blank}) \, x \, 100}{Abs_{control}} \quad (2)$$

The Sensory Analysis: In this study, two new formulations were developed using the honey, propolis, bee pollen, vanilla and royal jelly, and named as A1 and A2 samples. For each sample, a sensory analysis test was performed on 15 people. In the sensory analysis, the appearance, clarity, odor, taste, and general parameters of the samples were scored based on the five points: (5) very good;(4) good;(3) neither good nor poor;(2) poor;(1) very poor.

III. RESULTS AND DISCUSSION

The honey contains more than 180 substances, including many minor components such as carbohydrates (predominantly fructose and glucose), protein, enzymes, amino acids, lipids, water, vitamins, minerals, volatile chemicals, phenolic acids and phytochemicals (flavonoids). It is a supersaturated sugar solution.

The moisture content is very important for honey because high moisture content increases the value of water activity and leads to yeast growth which leads to fermentation during storage. If there is less than 17.1% moisture in honey, there will be no increase in microbial growth. If this ratio is between 17.1-20.0%, the product exhibits a stable structure, and if it is above 20.0%, osmophilic yeasts begin to grow rapidly. The texture, stability and shelf life of honey are related to its free acidity, pH and water activity. The pH value is one of the important quality parameters of honey, and the pH value affects the shelf life, stability and textural structure of honey. In addition, the pH value in honey is dependent on the ionized acids and minerals in its content, and it affects the growth of microorganisms and properties such as enzymatic reactions. The pH value in the honey content specified in the Turkish Standards Institute Honey Standard has been reported as 3.40-6.10.According to the Turkish Standards Institute Honey Standard, the highest ash content in honey was reported as 0.60% in flower honey and 1.20% in secretory honey.The main composition standards of flower honey and analysis results of the obtained products are given in Table 1 and Table 2, respectively (Damico et al., 2021).

Table 1. The main composition standards of flower honey.

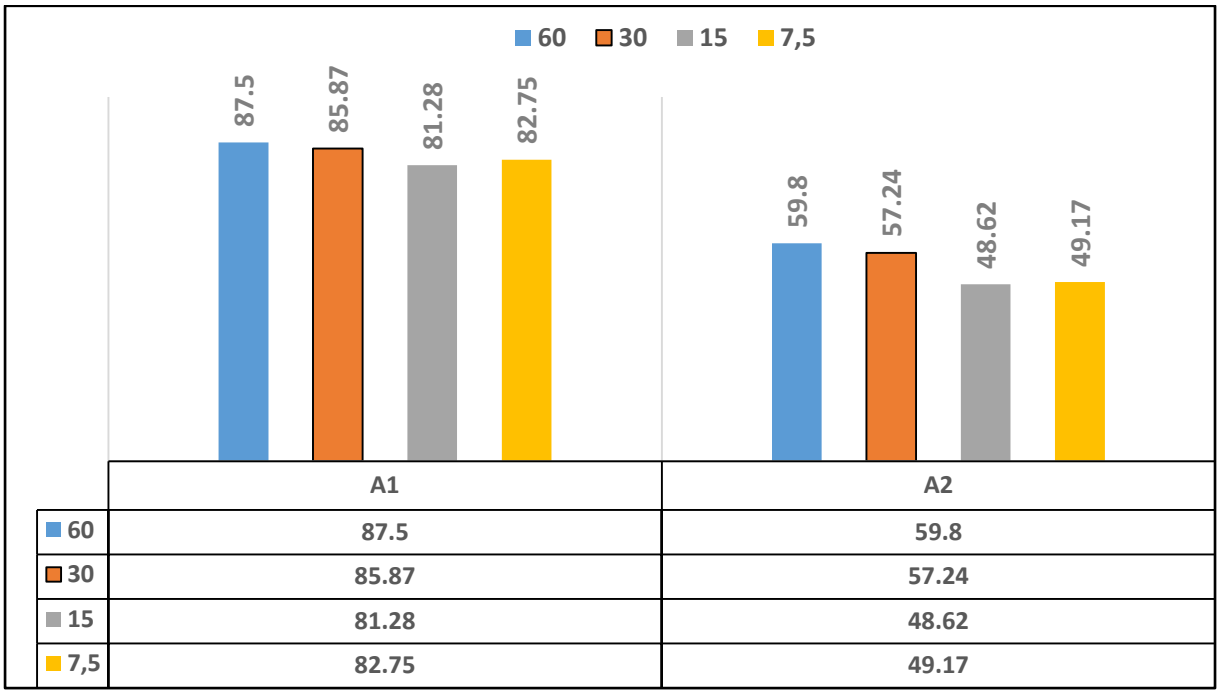
PROPERTIES	THE VALUES
MOISTURE CONTENT	20.0
FRUCTOSE AND GLUCOSE (G/100 G)	60
SUCROSE (G/100 G)	5.0
WATER-INSOLUBLE CONTENT (G/100 G)	<0.1
ELECTRICAL CONDUCTIVITY (MS/CM)	0.8
FREE ACID (MEQ/KG)	50.0
DIASTASE ACTIVITY (SCHADE SCALE)	8.0
HYDROXY METHYL FURFURAL (HMF, MG/KG)	40.0

Table 2. Analysis results of the obtained the final products

ANALYSIS TYPE	A1	A2
MOISTURE CONTENT%	14.8	14.2
THE PROTEIN CONTENT%	1.346	1.599
CARBOHYDRATE%	97.54	97.07
CRUDE FAT %	1.11	1.33
ASH CONTENT	0.003	0.002
PH	3.75	3.65
ACIDITY	73 meg/kg	73 meg/kg
REFRACTIVE INDEX	1.4998	1.5012

%Antioxidant Activity Analysis

The antioxidant activity analysis was performed according to the DPPH method. The obtained results are shown in Figure 2. DPPH solution with different concentration values was prepared using 60 µM stock solution and a total of 4 serial solutions were used with 50% dilution method. The % antioxidant activity values of A1 and A2 samples showed very effective results. The antioxidant activity of the final products was considerably improved by adding pollen, royal jelly and propolis. The % antioxidant activity of the A1 sample is higher than the A2 sample. This is due to the higher amount of honey in its content. According to the analysis results, as the DPPH solution concentration decreased, the % antioxidant activity values also decreased.



	A1	A2
60	87.5	59.8
30	85.87	57.24
15	81.28	48.62
7,5	82.75	49.17

Figure 2.%Antioxidant activity of A1 and A2 samples.

The Sensory Analysis

In this study, it was obtained to A1 and A2 final products by changing of theirs' contents. The sensory analysis test of the obtained A1 and A2 samples was performed with a scoring system from 1 to 5. In the sensory testing, the parameters of the appearance, clarity, odor, taste, and general to the samples were scored. The scaling is as follows;(5: very good, 4: good, 3: neither good nor poor, 2: poor, 1: very poor). According to the sensory analysis sample A1 showed higher general acceptability compared to the sample A2. The evaluations obtained according to the results and spider diagram of the sensory analysis are given in Table 3 and Figure 3, respectively.

Table 3. %The sensory analysis results of A1 and A2 samples.

PARAMETERS	VERY GOOD	GOOD	NEITHER GOOD NOR POOR	POOR	VERY POOR
A1					
APPEARANCE	26.66	53.33	13.33	6.66	-
CLARITY	46.66	40	6.66	6.66	-
ODOR	53.3	33.33	6.66	6.66	-
TASTE	60	13.33	20	6.66	
GENERAL	40	40	13.33	6.66	-
A2					
APPEARANCE	33.33	46.66	20	-	-
CLARITY	26.66	66.66	6.66	-	-
ODOR	33.33	40	26.66	-	-
TASTE	40	20	40	-	
GENERAL	26.66	60	13.33	-	-

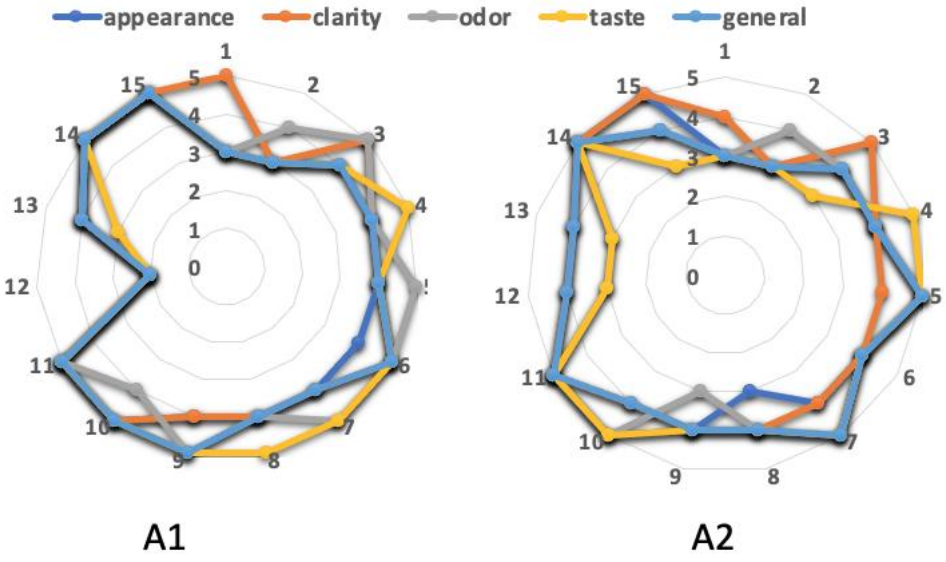


Figure 3. Spider diagram of the sensory analysis

IV. CONCLUSIONS

As a result, the moisture andash content, acidity, refractive index and brix value, pH and % antioxidant activity analyses of the functional product obtained from a mixture of honey, propolis, royal jelly and vanilla were carried out and the nutritional values were determined. The compliance of the analysis results with the values reported in the Turkish Food Codex Honey Communiqué was interpreted. The moisture content and the free acidity value of the product were found to be much higher than the reported standards. This difference may be due to the compounds such as royal jelly, pollen and propolisadded to the functional product. The antioxidant capacity of the final products is considerably improved.

REFERENCES

[1]. Abbasi, A.J., Mohammadi, F., Bayat, M., Megersa Gema, S., Ghadirian, H., Seifi, H., Bayat, H., Bahram, N., 2018. Applications of Propolis in Dentistry: A review. Ethiop J Health Sci., 28(4):509.
[2]. Andargie, M., Vinas, M., Rathgeb, A., Möller, E., Karlovsky, P., 2021. Lignans of Sesame (Sesamum indicum L.): A Comprehensive Review. Molecules, 26, 883.
[3]. AOAC (2000). Official methods of analysis of the association of analytical chemists. (17th ed.) Arlington, Virginia, USA.
[4]. Braakhuis, A. 2019. Evidence on the Health Benefits of Supplemental Propolis, Nutrients, 11, 2705.
[5]. Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebenson Wiss Technol, 28:25-30.
[6]. Damico ME., Rueppell O., Shaffer Z., Han B., Raymann K. 2021. High royal jelly production does not impact the gut microbiome of honey bees. Anim Microbiome,. 13;3(1):60. doi: 10.1186/s42523-021-00124-1.
[7]. Dzugan, M., Tomczyk, M., Sowa, P., Grabek-Lejko, D., 2018. Antioxidant Activity as Biomarker of Honey Variety, Molecules, 23, 2069.
[8]. Lan Nguyen, H.T., Panyoyai, N., Kasapis, S., Pang, E., Mantri, N. 2019. Honey and Its Role in Relieving Multiple Facets of Atherosclerosis, Nutrients, 11, 167.
[9]. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, et al.. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res, 15:127-130
[10]. Przybyłek, I., Karpinski, T.M. 2019. Antibacterial Properties of Propolis, Molecules , 24, 2047.
[11]. Ramsay, EI., Rao, S., Madathil, L., Hegde, S.K., Baliga-Rao, M.P., George, T., Baliga, MS., 2019. Honey in oral health and care: A mini review, Journal of Oral Biosciences 61, 32–36.
[12]. Singletary, K. W. (2020). Vanilla: potential health benefits. *Nutrition Today*, *55*(4), 186-196.
[13]. Soylu, P., Bayram, B., Dağdaş B., 2020. Production of Functional Food from a Mixture of Honey, Propolis, Royal Jelly, Yarrow (Achillea millefolium) and Echinacea (Echinacea paradoxa), Investigation of the Physicochemical and Biochemical Properties of the Product.Hayvancılık Araştırma Dergisi Journal of Bahri Dagdas Animal Research 9 (1):25-38.
[14]. Zaid, S.S.M., Ruslee, S. S., Mokhtar, M.H, 2021. Protective Roles of Honey in Reproductive Health. A Review. Molecules. 26, 3322.

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