ARAŞTIRMA MAKALESİ   RESEARCH ARTICLE

Determination of physicochemical and sensory properties of beverages produced using protein and prebiotic sources

Protein ve prebiyotik kaynaklar kullanılarak üretilen içeceklerin fizikokimyasal ve duyusal özelliklerinin belirlenmesi

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ARTICLE INFO	ABSTRACT
	The aim of this study was to determine the physicochemical and sensory properties of protein and prebiotic containing beverages that can be consumed by individuals from all age groups. The study was designed by using the Response Surface Method, and chocolate whey protein isolate (WPI), pea protein isolate (PPI) and inulin were used as independent variables. The maximum and minimum ranges of these variables were determined as 5.0-10.0 g/100 mL for WPI, 2.0-6.0 g/100 mL for PPI and 3.0-7.0 g/100 mL for inulin. As a result of the experimental design, the optimum production conditions were determined as 9.66 g WPI, 2.00 g PPI and 5.00 g inulin. Under these production conditions, the optimum physicochemical responses were determined as follows: the pH value was 7.02, the titration acidity was 0.02, the water-soluble dry matter content was 15.38, the L* value was 46.87, the a* value was 5.79, and the b* value was 8.54. Sensory responses were determined as follows: the taste value was 6.16, the odour value was 6.71, the aftertaste value was 6.50, and the overall acceptability value was 6.56. Under these conditions, the desirability value of the optimised beverage was found to be 97%. With this study, a new functional beverage formulation with high sensory acceptability was developed.
	ÖZET
	Bu çalışmanın amacı, her yaş grubundan bireylerin tüketebileceği protein ve prebiyotik içeren içeceklerin fizikokimyasal ve duyusal özelliklerinin belirlenmesidir. Çalışma, Tepki Yüzey Yöntemi (TYY) kullanılarak tasarlanmış ve bağımsız değişken olarak çikolatalı peynir altı suyu protein izolatı (WPI), bezelye protein izolatı (PPI) ve inülin kullanılmıştır. Bu değişkenlerin maksimum ve minimum aralıkları WPI için 5.0-10.0 g/100 mL, PPI için 2.0-6.0 g/100 mL ve inülin için 3.0-7.0 g/100 mL olarak belirlenmiştir. Deneysel tasarım sonucunda, optimum üretim koşulları 9.66 g WPI, 2.00 g PPI ve 5.00 g inülin olarak belirlenmiştir. Bu üretim koşullarında, optimum fizikokimyasal yanıtlar şu şekilde belirlenmiştir: pH değeri 7.02, titrasyon asitliği 0.02, suda çözünür kuru madde içeriği 15.38, L* değeri 46.87, a* değeri 5.79 ve b* değeri 8.54. Duyusal yanıtlar şu şekilde belirlenmiştir: tat değeri 6.16, koku değeri 6.71, ağızda kalan tat değeri 6.50 ve genel kabul edilebilirlik değeri 6.56. Bu koşullar altında optimize edilmiş içeceğin arzu edilebilirlik değerinin %97 olduğu tespit edilmiştir. Bu çalışma ile, duyusal kabul edilebilirliği yüksek yeni bir fonksiyonel içecek formülasyonu geliştirilmiştir.
Cite/Atıf	

INTRODUCTION

In recent years, the rapid increase in lifestyle-related diseases (especially diabetes, obesity, cancer and cardiovascular diseases) and the rise in health care spending have significantly increased people's tendency towards healthy lifestyles (Plasek et al., 2020; Gayathry & John, 2021; Topolska et al., 2021; Carvalho et al., 2023). Nowadays, individuals consider food not only to meet their basic nutritional needs but also as an important tool in disease prevention and management (Demirbag et al., 2023; Pérez-Marroquín et al., 2023). In this context, functional products attract more attention of consumers and the variety of these products is rapidly increasing (Yılmaz Ersan & Topçuoğlu, 2019; Topolska et al., 2021; Oba & Yılmaz, 2022; Wanyonyi et al., 2025). Functional foods contain various components such as protein, carbohydrate, fatty acids, dietary fibre, vitamins, minerals, bioactive compounds, probiotics and prebiotics (Birch & Bonwick, 2019; Çiftçi & Öncül, 2022; Obayomi et al., 2024). Especially functional beverages are among the effective and suitable products for the incorporation of these ingredients into formulations. In addition, it stands out as one of the most dynamic and preferred categories among functional foods due to properties such as meeting the demands of consumers with different lifestyles, appealing to individuals from all age groups, providing ease of transport and thirst-quenching (Gayathry & John, 2021; Pérez-Marroquín et al., 2023; Ersus et al., 2024).

Proteins, which are a component of functional products, are vital components for the daily maintenance of biological functions of cells, tissues and organs and are among the basic building blocks of a healthy diet (Munekata et al., 2021; Tjahyo et al., 2024). One of the most widely used protein sources in functional beverages is whey proteins (Ahern et al., 2023). Although these proteins are a valuable by-product of the dairy industry, they pose a significant environmental threat when not properly utilised (Quant et al., 2019; Saubenova et al., 2024). In addition to being low cost and readily available, they have superior functional properties and high nutritional value due to being rich in all essential amino acids (Saubenova et al., 2024).

In recent years, increasing concerns about the sustainability and environmental impacts of protein sources and the widespread adoption of a vegan diet have accelerated the search for alternative protein sources (Mantzourani et al., 2020; Tan et al., 2021; Ahern et al., 2023; Thakur et al., 2023; Hidalgo-Fuentes et al., 2024). Plant proteins are alternatives to animal proteins because they are cheap and sustainable sources (Lu et al., 2019). Plant-based diets provide significant health benefits by reducing the risk of developing chronic diseases such as cardiovascular diseases, diabetes, obesity and cancer (Lu et al., 2019; Sadler et al., 2024; Silva et al., 2024; Yuan et al., 2024). While legumes stand out with their high protein and dietary fibre content, they offer important advantages in terms of nutrition thanks to their low glycaemic index (Tan et al., 2021; King et al., 2024). Pea, one of the most widely consumed legumes, is a healthy and safe protein source due to its easy accessibility, low cost, high nutritional value and low allergenicity (Lu et al., 2019; Boukid et al., 2021). Thanks to these properties, pea proteins significantly increase their potential for use in functional products (Lu et al., 2019; Ahern et al., 2023).

Prebiotics also have an important role in the formulation of functional products and stand out with their health-promoting properties (Oba & Yılmaz, 2022; Demirbag et al., 2023). Prebiotics improve intestinal health by preventing constipation, reduce cancer risk, and support the growth and activity of probiotic bacteria (Oba & Yılmaz, 2022; Demirbag et al., 2023; Obayomi et al., 2024). Inulin is a water-soluble polysaccharide classified as indigestible carbohydrates and has prebiotic properties because it reaches the colon without being digested in the upper gastrointestinal tract and is fermented there. In addition, inulin has the potential to reduce the body's inflammatory state, contribute to weight control, improve gut health, stabilise body weight, and show positive effects on glucose and lipid metabolism (Oba & Yılmaz, 2022; Obayomi et al., 2024).

In line with this information, the aim of this study was to investigate the physicochemical and sensory properties of functional beverages produced using protein sources (WPI and PPI) and prebiotics (inulin), which are considered as health-promoting ingredients. In this study, the effects of different protein and inulin ratios on physicochemical

parameters such as pH, titration acidity, brix, colour and sensory acceptability of beverages were analysed and it was aimed to determine the optimal beverage formulation that can meet consumer expectations.

MATERIALS and METHODS

Raw materials and chemicals
Chocolate WPI (Hardline Nutrition, Türkiye), PPI (Myprotein Co, UK), inulin (Fibrelle, Türkiye), sunflower oil (Komili, Türkiye) and guar gum (Alfasol, Türkiye) were used in beverage production. The chemicals used in the analyses were of analytical grade purity and aqueous solutions were prepared using deionised water.

Experimental design and optimization
This study was designed using the Response Surface Method (RSM), which is an experimental design method. Design Expert 7.0 software (Stat-Ease Inc., USA) was used for RSM. The beverages were designed and optimised by using Central Composite Design (CCD) in RSM. The first step in this method was to determine the independent variables that are thought to affect the dependent variables and their levels (Myers et al., 2016). For this purpose, preliminary experiments were performed and a CCD with linear and quadratic models was used to examine the combined effects of three independent variables (WPI (A), PPI (B) and inulin (C)). As a result of the preliminary trials, the maximum and minimum amount ranges of the independent variables used in the study were determined to be 5.0-10.0 g/100 mL for the variable A, 2.0-6.0 g/100 mL for the variable B and 3.0-7.0 g/100 mL for the variable C. The dependent variables were selected as physicochemical (pH, titratable acidity, water soluble dry matter, colour values (L*, a* and b*)) and sensory (taste, odour, aftertaste and overall acceptability) characteristics.

The production of beverages according to CCD was carried out in 20 experiments ($N = 2^k + 2k + n_0$, N is the number of experiments, k is the number of factors and n_o is the number of replicates) in different combinations of three variables and the production ranges are shown in Table 1.

Production of beverages
Firstly, water was added to the mixture of WPI and PPI in the amounts specified in the experimental design (Table 1) and heat treatment was applied (75°C/30 min). After the heat treatment, homogenisation process was applied at 22000 rpm for 10 min. Then inulin (at the ratios determined in the experimental design), guar gum (0.05%) and oil (1-3%) were added to the mixture and homogenised again at 22000 rpm for 5 min. Finally, the beverages were pasteurised (85°C/10 min) and stored at +4°C for analyses.

Physicochemical analyses
The pH values of the beverages were measured by using a calibrated pH meter (WTW-Inolab pH meter, Oberbayern, Germany) using standard buffer solutions (Çiftçi & Öncül, 2022).

Titratable acidity (TA) was carried out according to the AOAC procedure and results were expressed as % lactic acid (% LA) equivalent (AOAC, 2000).

Water soluble solids (°Brix) were measured at room temperature by using a digital refractometer (Milwaukee MA871, Rocky Mount, NC, USA) (Oba & Yılmaz, 2023).

The CIE L* (lightness), a* (redness/greenness) and b* (yellowness/blueness) colour values of the beverages were measured using a calibrated colour meter (Hunter Lab MiniScan, Reston, Virginia, USA) with white and black plates (Ersus et al., 2024).

Table 1. Experimental design used in the production of beverages

Çizelge 1. İçecek üretiminde uygulanan deneysel tasarım

Independent variables	Code	-1	0	+1
WPI (g/100 mL)	A	5.0	7.5	10.0
PPI (g/100 mL)	B	2.0	4.0	6.0
Inülin (g/100 mL)	C	3.0	5.0	7.0

Run	A	B	C
1	7.5	4.0	3.0
2	7.5	4.0	5.0
3	7.5	4.0	5.0
4	10.0	4.0	5.0
5	7.5	4.0	5.0
6	5.0	6.0	7.0
7	5.0	6.0	3.0
8	5.0	2.0	7.0
9	10.0	2.0	3.0
10	7.5	4.0	5.0
11	7.5	4.0	5.0
12	10.0	2.0	7.0
13	7.5	4.0	5.0
14	5.0	2.0	3.0
15	7.5	6.0	5.0
16	10.0	6.0	3.0
17	5.0	4.0	5.0
18	7.5	2.0	5.0
19	10.0	6.0	7.0
20	7.5	4.0	7.0

Sensory evaluation

The sensory evaluation of the beverages was carried out by 10 trained panellists aged between 25 and 45 years, consisting of faculty members and graduate students from the Faculty of Agriculture at Hatay Mustafa Kemal University. Prior to the study, the panellists were given detailed information about the purpose, processes and expected contributions of the study and their participation was completely voluntary. The evaluation process was conducted in accordance with ethical principles.

The beverages were coded with three-digit numbers and filled into randomly arranged opaque plastic cups. The beverages were evaluated in terms of four attributes (taste, odour, aftertaste and overall acceptability) using a hedonic scale of 1-9 points. For all attributes, the scale was rated as '1 = did not like at all', '5 = liked moderately' and '9 = liked very much' (Lawless & Heymann, 2010).

The optimisation of beverages and statistical evaluation

Design Expert 7.0 software was used for regression analysis, analysis of variance and optimisation of the experimental design. Numerical optimisation was performed to determine the dependent and independent variables of the beverages and the optimum values of these variables. The basis of the method was the desirability function (Myers et al., 2016). In the optimisation, the maximum and minimum value ranges of the independent variables specified in the experimental design were selected. The dependent variables were the minimum and maximum ranges of pH, TA, Brix, colour (L*, a* and b*), taste, odour and aftertaste values, the maximum value of overall acceptability.

The experimental data obtained as a result of the analyses were statistically tested at 95% confidence interval ($p < 0.05$) using SPSS programme (Version 22, IBM, USA). The significance of the differences between the mean analysis results of the beverages with different formulations was evaluated by one-way ANOVA. Moreover, Duncan multiple comparison test was used to determine the difference between the groups and the results were expressed with statistical letters.

RESULTS and DISCUSSIONS

Physicochemical properties of beverages

The physicochemical properties of the beverages were analysed in terms of pH, TA, Brix and colour parameters (L*, a* and b*). The obtained data are presented in Table 2 and 3D RSM model graphics are presented in Figure 1.

Protein beverages may have neutral pH values (Liu et al., 2021). It has also been reported that the water used in the production of beverages can also affect the pH and acidity of beverages (Tireki, 2021). Besides water, positively and negatively charged proteins can interact with the environment at values above or below the isoelectric point and thus cause the pH value to change (Pérez-Ramírez et al., 2021). The pH values of beverages ranged from 6.90 to 7.14 (Table 2). While the effects of WPI and PPI amounts in the formulations on pH values were found to be significant ($p < 0.01$), the effect of changes in inulin amount was found to be insignificant ($p > 0.05$). Increases in the amount of WPI and PPI in the formulation decreased the pH value (Figure 1a). Increasing protein concentrations in the beverage composition causes pH values to decrease from neutral to acidic values (Pérez-Ramírez et al., 2021). Among the beverages in our study, the formulations with the lowest protein content (numbers 8 and 14) showed the highest pH values.

The TA values of the beverages ranged from 0.01 to 0.03 (Table 2). While the effects of WPI and PPI amounts in the formulations on pH values were found to be significant ($p < 0.01$), the effect of changes in inulin amount was found to be insignificant ($p > 0.05$). Increases in the amount of WPI and PPI in the formulation increased the TA value (Figure 1b).

The brix values of the beverages ranged from 13.00% to 20.08% (Table 2) and the effect of independent variables was found to be significant ($p < 0.01$). As the amount of independent variables increased, the brix values increased (Figure 1c) and the highest effect was due to WPI. The brix values of the beverages were similar to the studies in the literature (Oliveira et al., 2018; Vasquez-Orejarena et al., 2018; Gimhani & Liyanage, 2019; Garay et al., 2021).

The colour is one of the important quality parameters affecting the acceptability of a product (Aribah et al., 2020). Chocolate beverages have their own natural colours and these colours are accepted by consumers (Ling et al., 2021). The L* values of the beverages ranged between 44.81 and 63.12 (Table 2) and the effect of the independent variables was found to be significant ($p < 0.01$). While the L value increased with increasing PPI amount, the L value decreased with increasing WPI amount (Figure 1d). Compared to beverages produced by Akin & Ozcan (2021) using similar protein sources, L* values in our study showed lower results. It was found that this difference was due to the use of chocolate WPI and the lowest L* values belonged to the formulations with the highest WPI (numbers 9 and 12). The results obtained in our study were similar to the colour values of cocoa beverages reported by Pérez-Ramírez et al. (2021).

The a* values of the beverages ranged from 4.13 to 5.79 (Table 2) and the effect of WPI and PPI was found to be significant ($p < 0.01$). The effect of changes in the amount of inulin on a* value was found to be insignificant ($p > 0.05$). While the a* value increased with increasing WPI, the a* value decreased with increasing PPI (Figure 1e).

The b* values of the beverages ranged from 7.64 to 10.09 (Table 2). The effect of WPI and PPI was significant ($p < 0.01$). The effect of changes in the amount of inulin on b* values was found to be insignificant ($p > 0.05$). The b* value increased with increasing WPI and PPI (Figure 1f).

Table 2. The physicochemical values of beverages: Experimental results and RSM predictions

Çizelge 2. İçeceklerin fizikokimyasal değerleri: Deneysel sonuçlar ve TYY tahminleri

	pH		TA		Brix		L*		a*		b*	
Run	Experimental data	RSM prediction	Experimental data	RSM prediction	Experimental data	RSM prediction	Experimental data	RSM prediction	Experimental data	RSM prediction	Experimental data	RSM prediction
1	7.02±0.01[de]	7.02	0.03±0.00[hi]	0.02	13.08±0.11[ab]	13.97	53.52±0.09[d]	53.58	5.18±0.02[ghi]	5.12	9.17±0.07[h]	9.05
2	7.01±0.01[cd]	7.02	0.03±0.01[fghi]	0.02	14.16±0.09[d]	14.88	55.12±0.04[gh]	55.06	5.41±0.87[ij]	5.03	8.90±0.03[g]	9.06
3	7.01±0.01[d]	7.02	0.03±0.01[i]	0.02	14.08±0.11[d]	14.88	54.62±0.20[f]	55.06	5.14±0.03[fgh]	5.03	9.46±0.02[j]	9.06
4	6.99±0.01[bc]	6.96	0.03±0.00[ghi]	0.03	17.04±0.09[ij]	16.70	50.00±0.03[c]	49.98	5.54±0.01[jk]	5.56	9.25±0.13[hi]	9.32
5	7.01±0.00[cd]	7.02	0.03±0.00[fghi]	0.02	15.12±0.11[f]	14.88	55.21±0.05[h]	55.06	5.05±0.02[efg]	5.03	9.21±0.04[h]	9.06
6	7.04±0.01[f]	7.02	0.02±0.00[def]	0.02	16.08±0.11[h]	15.76	62.38±0.04[o]	65.43	4.13±0.02[a]	4.11	9.34±0.03[i]	9.55
7	7.03±0.01[ef]	7.02	0.02±0.00[efg]	0.02	13.08±0.11[ab]	13.55	61.50±0.17[n]	62.46	4.35±0.01[ab]	4.30	9.75±0.04[l]	9.53
8	7.14±0.01[g]	7.17	0.01±0.00[a]	0.02	13.00±0.00[a]	13.59	59.76±0.10[m]	57.82	4.54±0.01[bc]	4.72	7.64±0.03[a]	8.06
9	7.05±0.01[f]	7.02	0.02±0.00[bcd]	0.02	14.00±0.11[e]	15.00	44.81±0.01[a]	44.70	5.71±0.01[k]	5.95	8.26±0.02[c]	8.56
10	7.01±0.01[d]	7.02	0.02±0.00[cde]	0.02	15.08±0.11[f]	14.88	54.98±0.03[g]	55.06	5.01±0.02[efg]	5.03	9.17±0.07[h]	9.06
11	7.01±0.01[d]	7.02	0.02±0.00[cde]	0.02	15.00±0.00[f]	14.88	53.83±0.06[e]	55.06	4.88±0.02[def]	5.03	8.65±0.10[e]	9.06
12	7.01±0.01[d]	7.01	0.02±0.00[cde]	0.02	17.48±0.11[k]	17.01	45.23±0.06[b]	47.67	5.79±0.02[k]	5.77	8.44±0.04[d]	8.58
13	7.02±0.01[de]	7.02	0.02±0.00[cde]	0.02	15.84±0.09[g]	14.88	55.57±0.06[i]	55.06	4.89±0.03[def]	5.03	8.75±0.07[f]	9.06
14	7.14±0.01[g]	7.13	0.02±0.00[ab]	0.02	15.08±0.11[f]	14.00	54.47±0.22[f]	54.85	4.80±0.03[cde]	4.90	7.75±0.08[b]	8.04
15	6.99±0.00[b]	6.97	0.02±0.00[efg]	0.03	17.04±0.09[ij]	16.39	58.09±0.02[k]	58.86	4.68±0.03[cd]	4.73	9.56±0.09[k]	9.80
16	6.90±0.01[a]	6.91	0.03±0.00[i]	0.03	17.12±0.11[j]	16.53	53.56±0.11[d]	52.30	5.37±0.02[hij]	5.35	10.09±0.03[n]	10.05
17	7.01±0.01[cd]	7.02	0.02±0.00[abc]	0.02	13.16±0.09[b]	13.50	63.12±0.05[p]	60.14	4.38±0.03[ab]	4.51	8.87±0.03[g]	8.79
18	7.03±0.01[ef]	7.07	0.02±0.00[abc]	0.02	13.88±0.11[c]	14.53	50.09±0.11[c]	51.26	5.77±0.04[k]	5.38	9.15±0.04[h]	8.31
19	6.90±0.01[a]	6.91	0.03±0.00[hi]	0.03	20.08±0.11[l]	21.16	56.84±0.40[j]	55.27	5.06±0.04[efg]	5.17	9.92±0.21[m]	10.07
20	7.01±0.01[bcd]	7.02	0.02±0.00[fghi]	0.02	16.96±0.09[i]	16.08	58.50±0.03[l]	56.55	4.97±0.02[efg]	4.94	9.78±0.04[l]	9.07

Data were calculated as the mean±standard deviation of three measurements (Mean±SD).

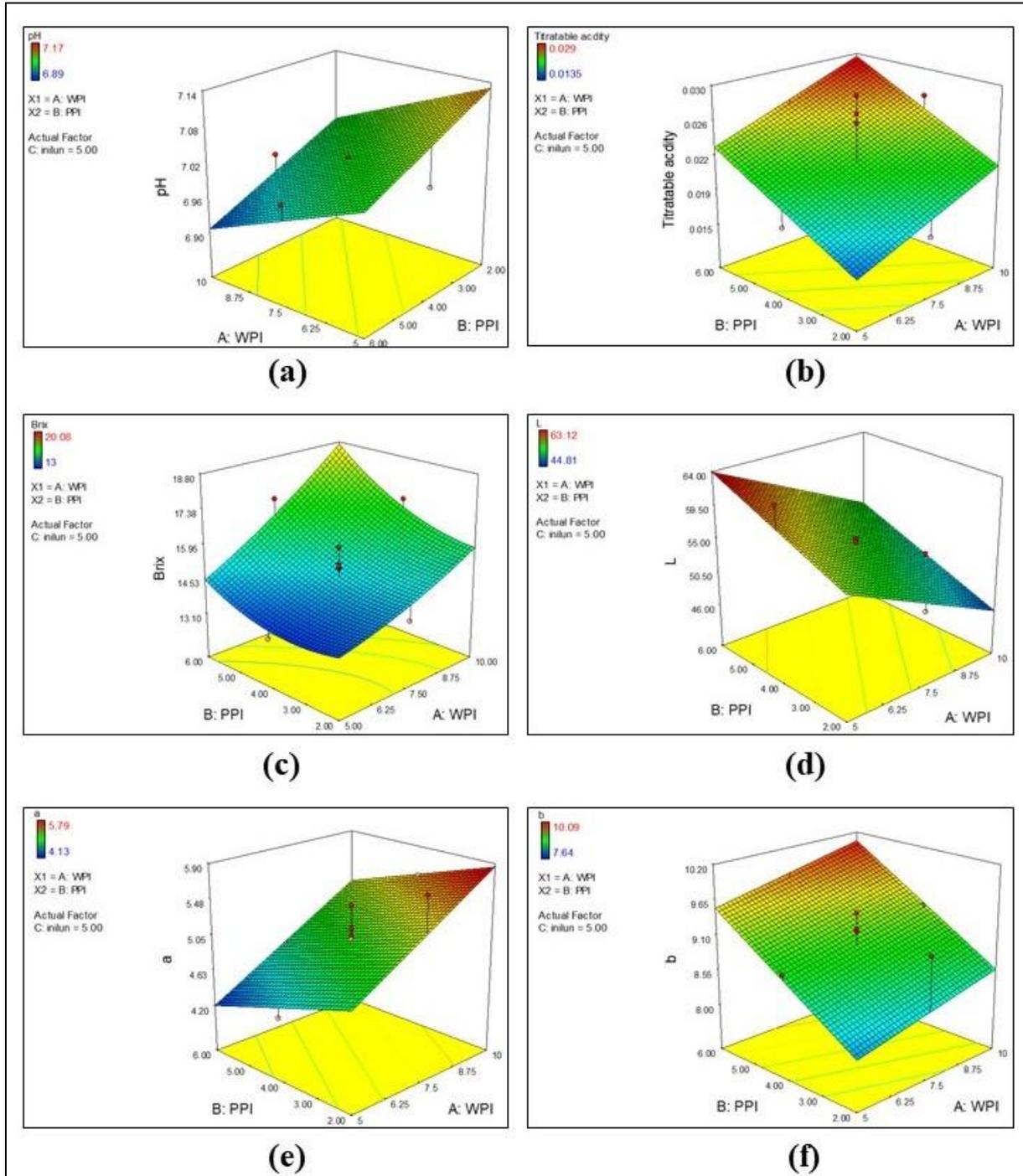Different letters in the same column showed the difference between the means (p<0.05).

Figure 1. 3D RSM model plots of physicochemical values of beverages

Şekil 1. İçeceklerin fizikokimyasal değerlerinin 3D TYY model grafikleri

Sensory evaluation of beverages

Sensory evaluations are very important in determining the overall acceptability of products in product development studies (Lawless & Heymann, 2010; Doğan et al., 2015). It has been reported that beverages should offer an acceptable taste and appearance in addition to the basic ingredients in order to be accepted by consumers (Oltman et al., 2015). The sensory evaluations of the beverages were evaluated according to a 1-9 point scale in terms of taste, odour, aftertaste and overall acceptability. The obtained data are presented in Table 3 and 3D RSM model graphics are presented in Figure 2.
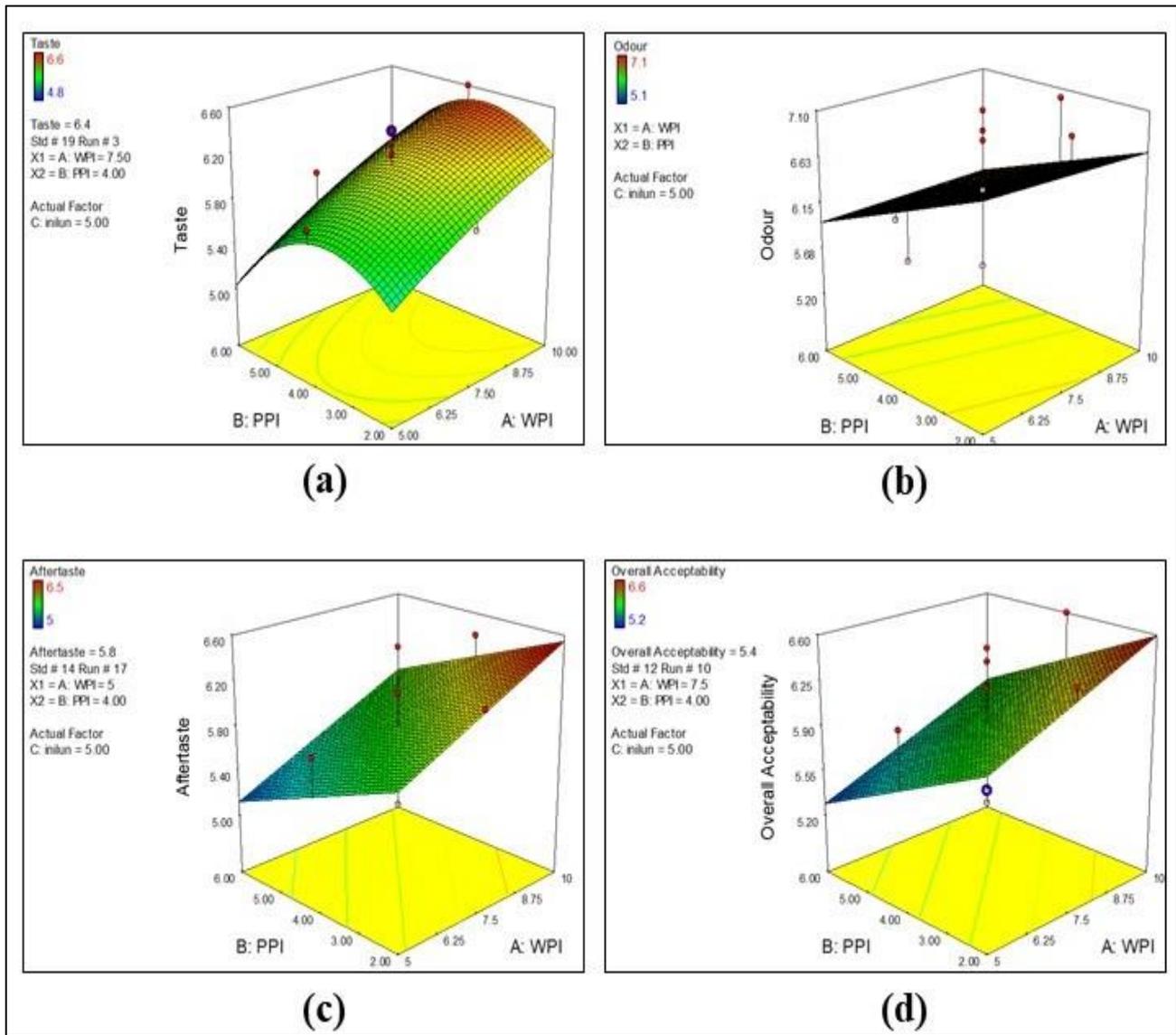
Figure 1. 3D RSM model plots of sensory evaluation of beverages
Şekil 2. İçeceklerin duyusal değerlendirmelerinin 3D TYY model grafikleri

The taste scores of the beverages ranged from 4.80 to 6.60 (Table 3) and the effect of WPI and PPI was found to be significant ($p<0.01$). The effect of changes in the amount of inulin on the taste values was found to be insignificant ($p>0.05$). As the amount of WPI increased, the taste values of the beverages increased (Figure 2a). As the amount of PPI increased from 2.0% to 4.0%, an increase in taste scores was observed. However, as the amount of PPI increased from 4.0% to 6.0%, the taste scores decreased.

The odour scores of the beverages ranged between 5.10 and 7.10 (Table 3) and the effect of the amount of PPI was found to be significant ($p<0.01$). The effect of changes in the amount of WPI and inulin on odour values was found to be insignificant ($p>0.05$). The increase in the amount of PPI caused a decrease in odour scores (Figure 2b).

The aftertaste scores of the beverages ranged from 5.00 to 6.50 (Table 3). The increase in the amount of WPI had a positive effect on aftertaste, while the increase in the amount of PPI had a negative effect (Figure 2c). While the changes in the amounts of WPI and PPI were found to be significant ($p<0.05$), the effect of changes in the amount of inulin on aftertaste values was found to be insignificant ($p>0.05$).

Table 3. Sensory evaluation of beverages: Experimental results and RSM predictions

Çizelge3. İçeceklerin duyusal değerlendirmesi: Deneysel sonuçlar ve TYY tahminleri

	Taste		Odour		Aftertaste		Overall acceptability	
Run	Experimental data	RSM prediction	Experimental data	RSM prediction	Experimental data	RSM prediction	Experimental data	RSM prediction
1	6.20±1.32[a]	6.17	7.00±1.94[cd]	6.56	6.40±1.35[a]	5.80	6.20±1.48[a]	5.96
2	6.20±1.81[a]	6.14	7.10±1.10[d]	6.33	6.50±1.43[a]	5.84	6.50±1.35[a]	5.95
3	6.40±2.01[a]	6.14	6.80±1.23[abcd]	6.33	6.10±1.29[a]	5.84	6.50±1.58[a]	5.95
4	6.60±1.65[a]	6.47	7.00±1.25[cd]	6.14	6.40±1.65[a]	6.21	6.60±0.97[a]	6.24
5	5.70±1.16[a]	6.14	6.90±1.20[bcd]	6.33	6.10±1.10[a]	5.84	6.40±0.97[a]	5.95
6	5.20±2.15[a]	5.36	6.10±1.66[abcd]	5.74	5.10±1.79[a]	5.17	5.30±1.70[a]	5.29
7	4.80±1.93[a]	4.79	6.20±1.69[abcd]	6.20	5.00±1.94[a]	5.09	5.30±1.83[a]	5.31
8	5.60±2.80[a]	5.54	6.40±1.71[abcd]	6.84	6.10±1.97[a]	5.85	6.00±2.31[a]	6.01
9	6.60±1.26[a]	6.50	6.70±1.83[abcd]	6.92	6.40±1.07[a]	6.51	6.50±1.51[a]	6.61
10	5.80±2.15[a]	6.14	6.30±1.83[abcd]	6.33	5.40±1.65[a]	5.84	5.40±1.58[a]	5.95
11	6.00±2.31[a]	6.14	6.40±1.43[abcd]	6.33	6.10±1.20[a]	5.84	6.20±1.32[a]	5.95
12	5.90±1.91[a]	5.97	6.10±2.02[abcd]	6.46	6.40±2.12[a]	6.59	6.50±2.01[a]	6.59
13	6.30±1.77[a]	6.14	5.50±1.84[abcd]	6.33	5.10±2.08[a]	5.84	5.30±2.06[a]	5.95
14	5.40±2.07[a]	5.42	7.10±1.10[d]	7.30	5.10±1.66[a]	5.77	5.40±1.58[a]	6.03
15	5.80±1.69[a]	5.49	5.20±2.57[ab]	5.78	5.10±1.73[a]	5.50	5.20±1.81[a]	5.59
16	5.80±1.81[a]	5.92	5.10±1.37[a]	5.82	5.90±0.99[a]	5.83	5.80±1.14[a]	5.89
17	5.80±1.62[a]	5.69	6.30±1.34[abcd]	6.52	5.80±1.81[a]	5.47	6.10±2.08[a]	5.66
18	5.80±1.69[a]	5.87	7.10±1.37[d]	6.88	6.20±1.93[a]	6.18	6.40±1.84[a]	6.31
19	5.80±1.69[a]	5.84	5.30±1.49[abc]	5.36	5.70±1.42[a]	5.91	5.60±1.58[a]	5.87
20	6.40±1.17[a]	6.19	5.90±1.79[abcd]	6.10	5.90±1.66[a]	5.88	5.70±1.57[a]	5.94

Data were calculated as the mean±standard deviation of 10 measurements (Mean±SD).
Different letters in the same column showed the difference between the means (p<0.05).

The overall acceptability of the beverages ranged from 5.20 to 6.60 (Table 3). While the effect of changes in WPI and PPI were found to be significant (p<0.05), the changes in inulin were found to be insignificant (p>0.05). While increases in WPI increased overall acceptability, increases in PPI decreased overall acceptability (Figure 2d). Although the bean flavour of pea proteins limits its use in foods by affecting consumer acceptance (Trikusuma et al., 2020), it is widely used in product development studies as a protein source due to its high nutritional content (Bäuerle & Kühn, 2022). Similar to this information, an increase in the amount of WPI in beverages led to an increase in the overall acceptability scores, while an increase in the amount of PPI led to a decrease in the overall acceptability scores. The decrease in overall acceptability scores observed with increasing PPI content was found to be compatible with the findings of Bäuerle & Kühn (2022).

In general, it was found that the independent variables affecting the sensory characteristics of the beverages were WPI and PPI. The use of different flavours such as fruit or chocolate has been reported to enhance the sensory profile of beverages (Singh et al., 2020; Garay et al., 2021). A study by Nam et al. (2017) demonstrated that the chocolate flavour was the most preferred option in beverages containing vanilla, fruit, and chocolate-flavoured soy and whey proteins. In conclusion, the incorporation of chocolate-flavoured ingredients has been demonstrated to enhance the sensory acceptability of legume proteins in beverages formulated with protein and prebiotic components.

Statistical evaluation of beverages analyses

In the context of the physicochemical and sensory analyses of the beverages, the results of the statistical analysis of the independent variables obtained by the RSM and the equations derived from the coded factors are presented in Table 4. In the equations derived from the coded factors obtained as a result of the regression analysis performed with the RSM, the variables A, B, and C represents WPI, PPI and inulin, respectively.

A, one of the independent variables, was found to have a significant effect on pH, TA, brix, L*, a*, b*, taste, aftertaste and overall acceptability values (p<0.01). The effect of B was found to be significant on pH, TA, brix, L*, a*, b*, taste, odour, aftertaste and overall acceptability values (p<0.001). The effect of C was found to be significant on brix and L* values (p<0.01).

In the model explaining the effect of the independent variables on the pH value, it was determined that the effect of the variable A and B was found to be significant (p<0.01), while the effect of the variable C was found to be insignificant (p>0.05). When the equations derived from the coded factors explaining the relationships between the independent variables were analysed, it was found that all independent variables had a negative linear effect on the pH value (Table 4). This showed that the pH value decreased with the increase of all three variables.

The effect of the variables A and B on the TA value was found to be significant (p<0.01), while the effect of the variable C was found to be insignificant (p>0.05). When the equations derived from the coded factors explaining the relationships between the independent variables were analysed, it was determined that independent the variables A and B had a positive linear effect and the variable C had a negative linear effect (Table 4). This shows that the TA value increases with the increase of independent variables A and B, while the TA value decreases with the increase of variable C.

In the model explaining the effect of the independent variables on the brix value, it was determined that the effect of all three variables was found to be significant (p<0.01). When the equation derived from the coded factors explaining the relationships between the independent variables was analysed, it was seen that all three variables had positive linear, two-way interaction terms (WPI-TEFE, WPI-Insulin, WPI-Insulin) and quadratic effects (Table 4). It was determined that the brix values of the beverages increased with the increase of these variables and the highest positive linear effect was caused by the WPI variable.

In the model explaining the effect of the independent variables on the L* value, it was determined that the effect of all three variables on the L* value was found to be significant (p<0.01). When the equation derived from the

coded factors explaining the relationships between the independent variables was analysed, it was determined that variable A had a negative linear effect and variables B and C had a positive linear effect (Table 4).

In the analysis of the a* value, it was determined that the model was statistically significant (p<0.001). When the equation derived from the coded factors explaining the relationships between the independent variables was analysed, it was determined that the variable A had a positive linear effect and the variables B and C had a negative linear effect (Table 4). This showed that the a* value increased as variable A increased and decreased as variables B and C increased.

In the analysis of the b* value, it was determined that the model was statistically significant (p<0.001). The effect of the variables A and B on the b* value was found to be significant (p<0.01), while the effect of the variable C was found to be insignificant (p>0.05). When the equation derived from the coded factors explaining the relationships between the independent variables was analysed, it was found that all three variables had a positive linear effect and the b* values of the beverages increased with the increase of these variables (Table 4).

Table 4. Model summaries and analyses of variance (ANOVA) of dependent variables of beverages
Çizelge 4. İçeceklerin bağımlı değişkenlerinin model özetleri ve varyans analizleri (ANOVA)

Response	Model	R^2	Adj. R^2	SD	Final equation in terms of coded factors
pH	Linear ***	0.83	0.80	0.03	$pH = 7.02 - 0.06A - 0.05B - 0.01C$
TA	Linear ***	0.64	0.57	0.01	$TA = 0.02 + 0.003A + 0.004B - 0.001C$
Brix	Quadratic **	0.87	0.75	0.92	$Brix = 14.88 + 1.60A + 0.93B + 1.06C + 0.50A \times B + 0.61A \times C + 0.65B \times C + 0.22A^2 - 0.58B^2 + 0.14C^2$
L*	Linear ***	0.91	0.90	1.60	$L^* = 55.06 - 5.08A + 3.80B + 1.49C$
a*	Linear ***	0.88	0.85	0.18	$a^* = 5.03 + 0.53A - 0.30B - 0.09C$
b*	Linear ***	0.73	0.68	0.38	$b^* = 9.06 + 0.26A + 0.74B + 0.01C$
Taste	Quadratic **	0.83	0.69	0.26	$Taste = 6.14 + 0.39A - 0.19B + 0.01C + 0.01A \times B - 0.16A \times C + 0.11B \times C - 0.06A^2 - 0.46B^2 + 0.04C^2$
Odour	Linear *	0.48	0.38	0.52	$Odour = 6.33 - 0.19A - 0.55B - 0.23C$
Aftertaste	Linear *	0.49	0.39	0.41	$Aftertaste = 5.84 + 0.37A - 0.34B + 0.04C$
Overall acceptability	Linear *	0.44	0.34	0.41	$Overall\ Acceptability = 5.95 + 0.29A - 0.36B - 0.01C$

***p<0.001, **p<0.01, *p<0.05; SD, standard deviation.

In the analysis of the taste value, it was determined that the quadratic model was significant (p<0.01). When the equation derived from the coded factors explaining the relationships between the independent variables was analysed, it was found that the variables A and C had a positive linear effect and the variable B had a negative linear effect (Table 4). In addition, negative (WPI-Inulin) and positive (WPI-PPI and PPI-Inulin) two-way interaction terms, positive and negative quadratic effects of this variables were found in the model (p<0.01). These results showed that the variables had both a linear and a quadratic effect on taste values.

In the analysis of the the odour value, it was determined that the model was significant (p<0.05). The effect of the variables B on the odour value was found to be significant (p<0.01), while the effect of the variable A and C was found to be insignificant (p>0.05). When the equations derived from the coded factors explaining the relationships between the independent variables were analysed, it was determined that all three variables had a negative linear effect (Table 4).

In the analysis of the the aftertaste value, it was determined that the model was significant ($p<0.05$). The effect of the variables A and B on the odour value was found to be significant ($p<0.05$), while the effect of the variable C was found to be insignificant ($p>0.05$). When the equation derived from the coded factors explaining the relationships between the independent variables was analysed, it was determined that variables A and C had a positive linear effect and variable B had a negative linear effect (Table 4). This showed that the aftertaste value increased as variables A and C increased and decreased as variable B increased.

In the analysis of the the overall acceptability value, it was determined that the model was significant ($p<0.05$). The effect of the variables A and B on the overall acceptability value was found to be significant ($p<0.05$), while the effect of the variable C was found to be insignificant ($p>0.05$). When the equation derived from the coded factors explaining the relationships between the independent variables was analysed, it was determined that variables A had a positive linear effect and variable B and C had a negative linear effect (Table 4).

Optimisation of beverages

In the optimisation of the beverages, the optimum production conditions were determined based on the value ranges defined for the independent variables specified in the experimental design. The optimal conditions were found to be 9.66 g/100 mL WPI, 2.00 g/100 mL PPI, and 5.00 g/100 mL inulin. Under these production conditions, the pH value was 7.02, the TA value was 0.02, the brix value was 15.38, the L* value was 46.87, the a* value was 5.79, the b* value was 8.54, the taste value was 6.16, the odour value was 6.71, the aftertaste value was 6.50 and the overall acceptability value was 6.56. It was determined that the desirability value of the optimised beverage under these conditions was 97% (Figure 3).
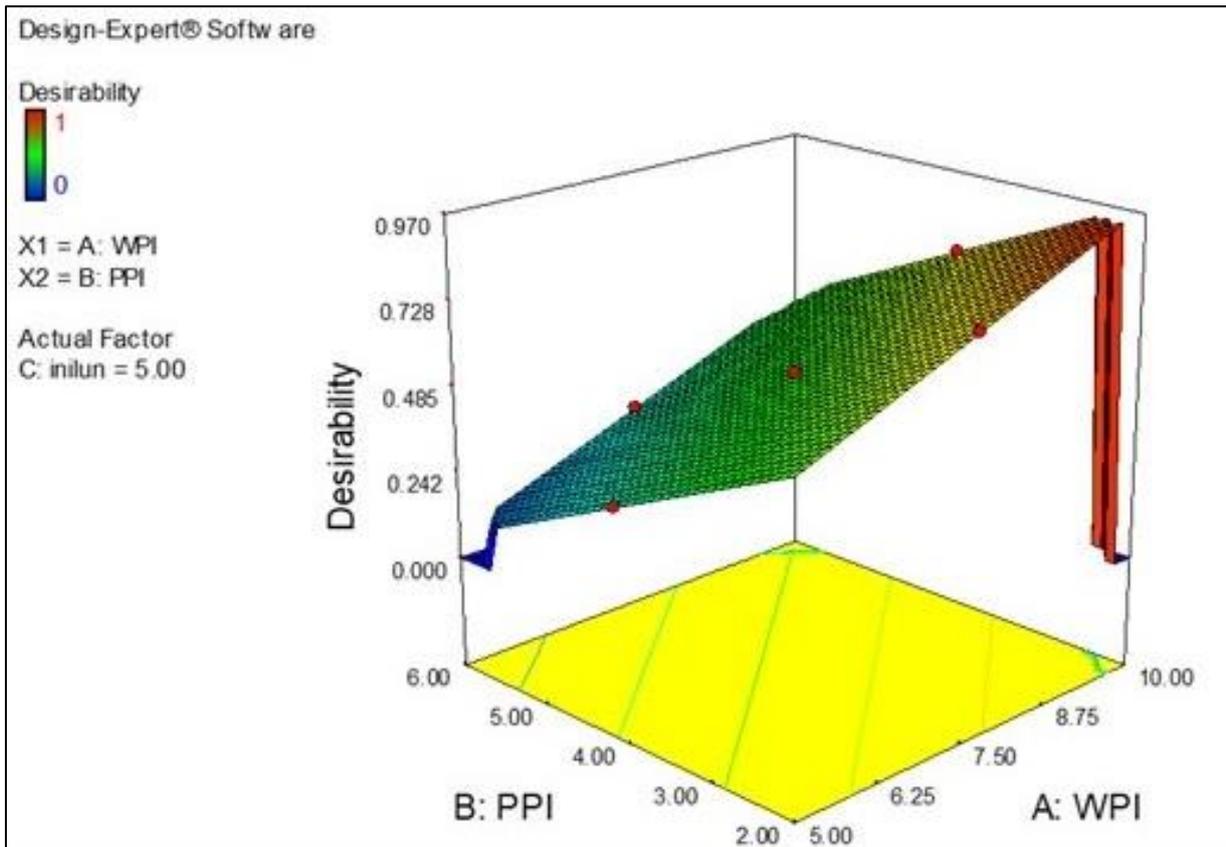

Figure 2. 3D RSM graph of the effect of independent variables on the desirability value

Şekil 3. Bağımsız değişkenlerin arzu edilebilirlik değerine etkisinin 3D TYY grafiği

Due to increasing consumer awareness in recent years, food and beverages are consumed not only to satisfy hunger, but also for health protection and control. This trend increases consumer demand for products with functional properties that offer immunological benefits. Due to the increasing interest in functional foods with health-protective and healing properties, the functional beverage sector has also been growing day by day. Functional beverages are widely consumed worldwide and constitute a rapidly growing sub-segment of the functional food sector. In this study, the development of beverages containing protein and prebiotics offers a remarkable innovation when evaluated in terms of functional food sector. The chocolate whey protein isolate and pea protein isolate used in the formulation appeal to a wide consumer group for both animal and plant protein sources. In addition, the prebiotic effect of inulin provides an important contribution to functional beverages with its digestive health supporting properties. As a result of the evaluation of the physicochemical and sensory properties of the beverages produced in the study, positive results were obtained in terms of consumer acceptance. Moreover, the ingredients used in the formulation are widely available in the food industry, allowing the scale-up of production, supporting its commercial viability. In this context, it is thought that this study will contribute to the development of innovative products with high commercial potential in the field of functional beverages.

In future studies, it may be recommended to carry out more comprehensive research on processing and fortification technologies in order to develop products rich in nutrient content and suitable for the taste of consumers. High-pressure homogenisation, ultrasonic processes and non-thermal innovative technologies can potentially be used in the development of new beverages with preserved and enriched nutrient content and reduced production costs.

ACKNOWLEDGEMENT

STATEMENT OF CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

STATEMENT OF ETHICS CONSENT

Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects.

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