

# MIXTURE MODELING AS A WAY FOR OPTIMIZATION OF WORT IN BEER PRODUCTION

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## KEYWORDS

malt, mixture modelling, optimization, biological value, wort

## ABSTRACT

Different malt types used in beer production are responsible not only for beer taste and aroma, but also for its biological value. In our previous research the main brewing and biological (phenolic compounds and antioxidant capacity) characteristics of 20 malt types, which are used in the brewing industry in Bulgaria, were studied. The aim of the present work is the modelling of a three-component mixture of malts (Pilsner, Caramel Munich type 2 and Vienna), in order to obtain wort with increased biological value.

The method for mixtures modelling was used, as the target functions were wort phenolic compounds, determined by Folin–Ciocalteu method and modified Glories method, and wort antioxidant potential, determined by DPPH radical scavenging assay and ferric reducing antioxidant power - FRAP. The proportions of the three malts were determined after ANOVA of the results obtained, in order to guarantee the maximum biological value of the wort.

The model obtained was optimized by applying constraints within the model in order to minimize the phenolic compounds content and maximize the antioxidant potential of wort produced. However, the optimization was carried out also to ensure that the wort produced showed good brewing characteristics. The obtained mixture had the following composition – 60% Pilsner, 20% Caramel Munnich type 2, and 20% Vienna malt.

In the present study, a mathematical-statistical approach was applied for modelling and optimization of the composition of malt mixture in order to produce wort with increased biological value and good brewing characteristics.

## INTRODUCTION

Malt is the main raw material in beer production. It provides not only the necessary amount of starch, which is converted to wort extract during mashing but also the color, antioxidant and polyphenolic profile of wort produced (Eaton, 2017; Kunze, 2019; Carvalho et al., 2014; Vanderhaegen et al., 2006). Various authors showed that 80% of polyphenols in finished beer

originated from malt, while the other 20% were from hops (Carvalho et al., 2014; De Keukeleire, 2000; Quifer-Rada et al., 2015). Barley malt is also the main contributor to the antioxidant properties of beers (up to 95%) due to its melanoidin and polyphenol content (Carvalho et al., 2014; Čechovská et al., 2012; Shopska et al. 2021). The antioxidant profile of malt depends on the raw material composition and the technological regimes of steeping, germination and kilning used for its production. Therefore, different malts have unique antioxidant and phenolic profiles (Shopska et al. 2021; Leitao et al, 2012; Holtekjølen et al., 2006; Madhujith et al., 2006; Lu et al., 2007).

In recent years, because of consumers' demand on healthy food and beverages, one of the main tasks in brewing is the production of wort with both good brewing characteristics and increased biological potential. Usually the process begins with the selection of malts or malt mixtures. More often this process is based on the brewer's experience or on making adjustments in already known malt blends. The brewers judge the quality of the resulting mixture solely by its brewing characteristics (Shopska et al., 2022a; Shopska et al., 2022b). However, the selection of proper malt mixture for producing healthy beverages has to include also evaluation of its antioxidant potential and phenolic content. The best approach for the selection of malt mixture is mixture design (Shopska et al., 2022a; Shopska et al., 2022b; Myers et al., 2016; Cornell, 2002).

In mixture design, the mixture composition is modeled by changing the proportions of the mixture components and studying their effect on the target functions. The total amount of the components of mixture (i.e malt types) gives 100%. It is important to note that the target function depends only on the proportion of the individual component in the mixture and not on the amount of the mixture. Both the main brewing characteristics and those that determine the biological profile of the wort can be selected as target functions. As a result of the modeling, mathematical dependencies are obtained, which give the relationship between the content of the individual components in the mixture and the target functions (Shopska et al., 2022a; Shopska et al., 2022b). Various methods for modeling mixtures can be used - Simplex-Lattice designs, Simplex-Centroid designs, Constrained mixture designs. These methods can be used for exploring a factor space of different

sizes, which determines the accuracy of the obtained models. The method Constrained mixture designs allows to perform constraints in the factor space, which can be used in constraining some of the malts in the mixture (Shopska et al., 2022b; Myers et al., 2016; Cornell, 2002). The knowledge of malt characteristics is crucial for the correct modeling of malt mixtures. Malts are divided into three main groups - basic, special and functional. The basic malts are with highest content in malt mixtures and are with highest enzyme activity and good brewing characteristics. The special and functional malts gives specific color, higher antioxidant and phenolic profile of the beverages produced, as well as some specific characteristics like smoky aroma, acid flavor, etc. (Carvalho et al., 2014; Shopska et al. 2021). Some of special malts also have good brewing characteristics, which decrease with the increase in malt color (Carvalho et al., 2014; Shopska et al. 2021; Shopska et al., 2022a). Therefore, it is necessary to ensure the correct proportions of the different malt types to ensure a mixture with suitable brewing and biological characteristics (Shopska et al. 2021; Shopska et al., 2022a; Shopska et al., 2022b).

In our previous research (Shopska et al. 2021) twenty malt types were investigated in terms of their brewing and biological characteristics. Malts were divided by their antioxidant potential (determined by 5 different methods) to two main groups. The first group included malts with low antioxidant activity such as: Pilsner, Pale ale, Vienna, Munich, Munich dark, Wheat, Rye, Caramel pils, Acidulated and Smoked malt. All other malts (Melanoidin, Red X, Caramel amber, Caramel hell, Black, Special X, Special Wheat, Caramel Munich 1, Caramel Munich 2, Chocolate) fell into the second group of malts with higher antioxidant activity (Shopska et al. 2021).

On the basis of the research conducted three malt types were selected—Pilsner (**P**), Vienna (**V**) and Caramel Munich Type 2 (**CM2**), that have good phenolic profile and adequate antioxidant capacity. The aim of the present work was to model the composition of a three-component mixture of the selected malts using mixture modeling methods.

## MATERIALS AND METHODS

### Malt

We used Pilsner (**P**), Vienna (**V**) and Caramel Munich Type 2 (**CM2**), produced by BestMalz, Germany.

### Mixture Design

Randomized simplex centroid design was used to model the malt mixtures. The plan was a lattice, which consisted of 7 different mixtures (Table 1) in 9 runs. Additionally, in the design centre of the, experiments were conducted to establish the statistical parameters. The design was run in a single block. The order of the experiments has been fully randomized. This had provided protection against the effects of lurking

variables. Since the selected model type was special cubic, the design was intended to fit a model with all first and second-order terms and some third-order terms. The main parameters of the wort (wort extract), as well as the parameters characterizing its biological value (DPPH, FRAP, phenolic compounds), were used as a target functions.

## Wort Characteristics

### Mashing Method

Mashing was conducted according to the Congress mash method of the European Brewery Convention (Analytica EBC, 2019), as described in (Shopska et al. 2021).

Table 1: Randomized simplex centroid design for mixture modelling and optimization

No	Block	Pilsner	CM2	Vienna	Pilsner	CM2	Vienna
	-	-	-	-	g	g	g
1	1	0	0.5	0.5	0	25	25
2	1	1	0	0	50	0	0
3	1	0	1	0	0	50	0
4	1	0.33	0.33	0.33	16.66	16.66	16.66
5	1	0.5	0	0.5	25	0	25
6	1	0	0	1	0	0	1
7	1	0.5	0.5	0	25	25	0
8	1	0.33	0.33	0.33	16.66	16.66	16.66
9	1	0.33	0.33	0.33	16.66	16.66	16.66

### Wort Analysis

Wort extract was determined according to the methods of the European Brewery Convention (Analytica EBC, 2019). Wort extract was measured by the means of Anton Paar DMA 35 density meter (Anton Paar, Graz, Austria).

### Extraction and Determination of Phenolic Compounds

The wort obtained according was diluted with methanol in a proper ratio and filtered, according (Shopska et al. 2021; Shopska et al., 2022a). The methanolic extracts were used for analysing phenolic compounds concentration and antioxidant activity of wort.

*Content of Total Phenolic Compounds (TPC) with Folin-Ciocalteu (FC) Reagent.* The total phenolic compounds content was determined according to (Dvořáková et al., 2008) with some modifications detailed in (Shopska et al. 2021). The absorbance (A) was recorded at 765 nm against a blank prepared using Shimadzu UV-VIS1800 spectrophotometer (Kyoto, Japan). The results were calculated by a calibration curve and were presented as mg Gallic acid equivalent (GAE)/L wort:

$$TPC = \frac{(A_{765} + 0.0083)K_p}{0.0098}, \text{ mg GAE/L} \quad (1)$$

where:  $A_{765}$ —absorbance of the sample of 765 nm,  $K_p$ —dilution coefficient

*Content of Phenolic Compounds by the Glories Method.* The content of total phenols, phenolic acids and flavonoids was determined by a modified Glories method (Shopska et al. 2021; Shopska et al., 2022a; Mazza et al., 1999). The absorbance was measured against a blank prepared with distilled water at three wavelengths—280 (TPC), 320 (phenolic acids content (PA)) and 360 nm (flavonoids (F)). The results were calculated by calibration curves and were presented as GAE/L for TPC, caffeic acid equivalent/L (CAE/L) for PA, and Quercetin equivalent (QE/L) for F, respectively:

$$\text{TPC} = 391.88A_{280}K_p, \text{ mg GAE/L} \quad (2)$$

$$\text{PA} = 210.83A_{320}K_p, \text{ mg CAE/L} \quad (3)$$

$$\text{F} = 321.94A_{360}K_p, \text{ mg QE/L} \quad (4)$$

where:  $A_{280}$ —absorbance of the sample of 280 nm;  $A_{320}$ —absorbance of the sample of 320 nm;  $A_{360}$ —absorbance of the sample of 360 nm;  $K_p$ —dilution coefficient.

*Antioxidant Activity (AOA) of Wort.* AOA against the DPPH (2,2'-Diphenyl-1-picrylhydrazyl) Radical

The wort AOA was measured by the DPPH method (8, 13, 20) using 0.25 mL of methanol extract (working sample) or methanol (control). The percentage inhibition activity was determined by:

$$I = 100 \frac{A_1 - A_2}{A_1}, \% \quad (5)$$

where: I—percentage inhibition;  $A_1$ —the absorbance of the control at 517 nm;  $A_2$ —the absorption of the working sample at 517 nm;

A standard curve, measuring AOA of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) against DDPH radical was obtained:

$$\text{AOA} = \frac{(I + 0.6711)K_p}{0.341}, \mu\text{M TE/L} \quad (6)$$

where: I—percentage inhibition;  $K_p$ —dilution coefficient

*AOA by the FRAP (Ferric Reducing Ability of Plasma) Method.* The FRAP analysis was performed according to the method, described by Benzie and Strain, 1996 with the some modifications detailed in (Shopska et al. 2021; Shopska et al., 2022a). The absorption was read at 593 nm against a blank prepared with methanol. The standard curve, measuring AOA of Trolox was used:

$$\text{AOA} = \frac{(A_{593} + 0.0235)K_p}{0.0024}, \mu\text{M TE/L} \quad (7)$$

where:  $A_{593}$ —absorbance of the sample of 593 nm;  $K_p$ —dilution coefficient

*Modeling, optimization and statistical analysis*

The statistical data processing was performed with the help of Statgraphics Centurion XV, Trial version with the help of algorithms set in the program itself.

## RESULTS AND DISCUSSION

Before commenting on the results, we will give a brief description of the objective functions investigated in the present work. The wort extract is formed in the process of mashing, lautering and wort boiling. In the course of fermentation, it undergoes a number of changes, as a

result of which the sensory profile of the beer is formed. Phenolic compounds are part of the wort extract and are relevant both for its biological value and for the beer stability during its storage. Antioxidant potential is a complex concept that determines the biological value of a given component/set of components. Since different types of components have different chemistry to action, it was adopted to determine the antioxidant capacity by at least two different methods, in this case the inhibition against the DPPH radical and the Ferric Reducing Ability of Plasma were chosen.

The results for mixture modeling are shown in Table 2. First of all, we will comment on the results obtained at each of the points of the simplex lattice. The results at the vertices of simplex (Variants 2, 3 and 6) were similar with our previous studies (Shopska et al. 2021; Shopska et al., 2022a). Pilsner and Vienna malt had similar enzyme activity (22), which resulted in similar wort extract. Caramel Munich 2 malt showed a lower extract due to its higher temperature of kilning. The TPC of Caramel Munich 2 malt was between 2.5 and 5 times higher (depending on the method used for their determination) than the one of Vienna and Pilsner malts. This was due to the kilning regime that promoted the release of malt phenolic compounds from their bound form (Carvalho et al., 2014; Shopska et al. 2021; Shopska et al., 2022a). The AOA of Caramel Munich 2 malt, measured by DPPH and FRAP methods was also the highest. This was due higher content of phenolic compounds and Maillard reaction products, which determined antioxidant capacity (Carvalho et al., 2014). The points on the individual sides of the simplex lattice (Variants 1, 5 and 7) were also very interesting. The results for AOA and TPC of Variants 1 and 7 could be considered as an average of the results for the antioxidant activity and total phenolic compounds of the basic (Vienna or Pilsner) and the special (Caramel Munich 2) types of malt. Therefore, it can be hypothesized that the increase in the proportion of special malt in the mixture should lead to an increase in both mixture TPC and AOA. The extract of the mixtures between base and special malt was similar to the extract of base malt types (Variants 2 and 6). The combination of the two basic malt types (variant 5) led to an increase in the mixture extract, TPC and AOA compared to the base malt itself. The indicators of the mixtures at the central points of the simplex lattice (variants 4, 8 and 9) cannot be determined by simple dependencies, but mathematical models should be developed. The observed differences between  $\text{TPC}_{\text{FC}}$  and  $\text{TPC}_{\text{FG}}$  are due to the fact that the modified Glories method is based on the characteristic absorption of the benzene cycles of the majority of phenols at 280 nm and is less influenced by the oxidative status of the analyzed molecules.

The results in Table 2 was subjected to statistical analysis, and mathematical dependencies were established for the influence of the individual malts in the mixture on different target function (equation 8 to equations 14). The response curves for each of the

parameters are presented in Figure 1 to Figure 7. Table 3 and Table 4 present the data for the models for extract, TPC, determined by FC method, and AOA, as these functions will be used for mixture optimization. The data from the mathematical models (eq. 8 to 14) showed that all three malts affected positively target functions in the linear part of the models. The special malt - Caramel

Munich 2 had the strongest influence on the phenolic content and AOA, which determined the biological value of wort. The results confirmed our previous investigations about the strongest effect of special malts on the wort biological potential (Shopska et al. 2021; Shopska et al., 2022a). The equation 8 showed that all the three malts have a positive effect on the wort extract.

Table 2: Simplex centroid design and experimental results

№	Pilsner	CM2	Vienna	Extract	TPC <sub>FC</sub>	DPPH	FRAP	TPC <sub>G</sub>	PA	F
				°P	mg/L	μmol TROLOX/L	mg/L			
1	0	0.5	0.5	8.29	623.78	1119.81	1328.43	779.84	177.1	93.36
2	1	0	0	8.22	251.33	17.36	281.25	291.56	37.11	19.96
3	0	1	0	5.67	1039.39	1964.67	2234.72	1301.4	316.25	167.41
4	0.33	0.33	0.33	8.31	582.96	932.52	1045.49	644.64	129.66	67.67
5	0.5	0	0.5	8.56	407.30	265.47	379.03	351.91	46.8	24.47
6	0	0	1	8.31	281.28	177.41	519.1	525.12	133.88	159.39
7	0.5	0.5	0	8.24	655.41	989.89	1185.76	719.1	162.34	86.92
8	0.33	0.33	0.33	8.33	601.41	935.63	1055.67	640.11	128.72	63.21
9	0.33	0.33	0.33	8.28	589.32	922.12	1033.36	638.20	127.63	66.36

According to Table 3, the model in its entirety (together with third-order interactions) could be considered as adequate. Table 3 shows the results of fitting different models to the data in Extract. The mean model consists of only a constant. The linear model consists of first-order terms for each of the components. The quadratic

model adds cross-products between pairs of components. The special cubic model adds terms involving products of three components. Each model is shown with a P-value which tests whether that model is statistically significant when compared to the mean square for the term below.

$$\text{Extract} = 8.22 * P + 5.67 * CM2 + 8.31 * V + 5.18 * P * CM2 + 1.18 * P * V + 5.212 * CM2 * V - 10.15 * P * CM2 * V \quad (8)$$

$$\text{TPC}_{FC} = 251.33 * P + 1039.39 * CM2 + 281.28 * V + 40.2 * P * CM2 + 563.98 * P * V - 146.22 * CM2 * V + 218.058 * P * CM2 * V \quad (9)$$

$$\text{DPPH} = 17.36 * P + 1964.67 * CM2 + 177.41 * V - 4.5 * P * CM2 + 672.34 * P * V + 195.08 * CM2 * V + 3154.35 * P * CM2 * V \quad (10)$$

$$\text{FRAP} = 281.25 * P + 2234.72 * CM2 + 519.1 * V - 288.9 * P * CM2 - 84.58 * P * V - 193.92 * CM2 * V + 2614.83 * P * CM2 * V \quad (11)$$

$$\text{TPC}_G = 291.56 * P + 1301.04 * CM2 + 525.12 * V - 308.8 * P * CM2 - 225.76 * P * V - 532.96 * CM2 * V + 1548.38 * P * CM2 * V \quad (12)$$

$$\text{PA} = 37.11 * P + 316.25 * CM2 + 133.88 * V - 57.36 * P * CM2 - 154.78 * P * V - 191.86 * CM2 * V + 327.663 * P * CM2 * V \quad (13)$$

$$\text{F} = 19.96 * P + 167.41 * CM2 + 159.39 * V - 27.06 * P * CM2 - 260.82 * P * V - 280.16 * CM2 * V + 410.37 * P * CM2 * V \quad (14)$$

where: "Extract", °P – target function;  $b_{ijk}$  – regression equation coefficients (i=Pilsner malt; j= Caramel Munich Type 2 malt; k=Vienna malt); P, CM2, V – proportion of the malt in the mixture.

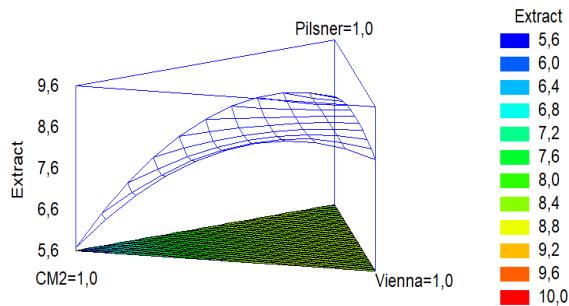


Figure 1: Response Surface for Wort Extract

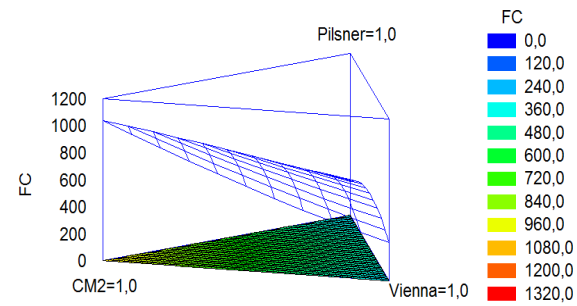


Figure 2: Response surface for TPC determined by FC

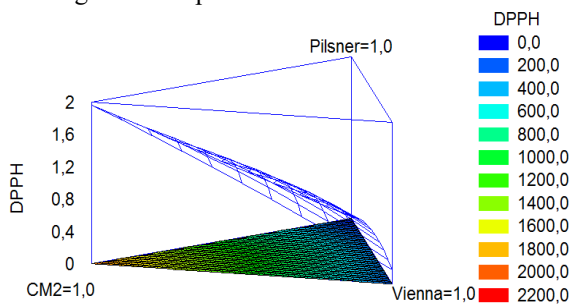


Figure 3: Response Surface for AOA Determined by DPPH

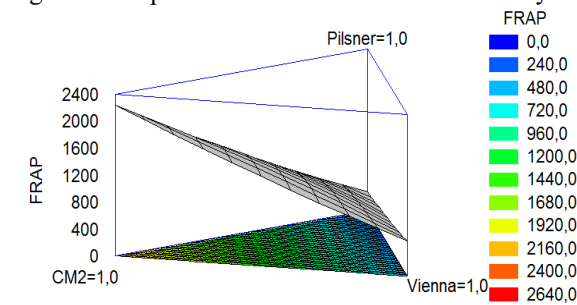


Figure 4: Response Surface for AOA Determined by FRAP

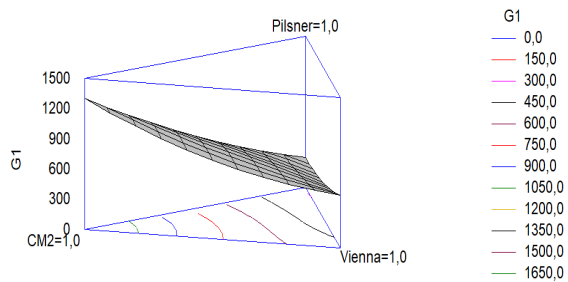


Figure 5: Response Surface for TPC Determined by Glorie method

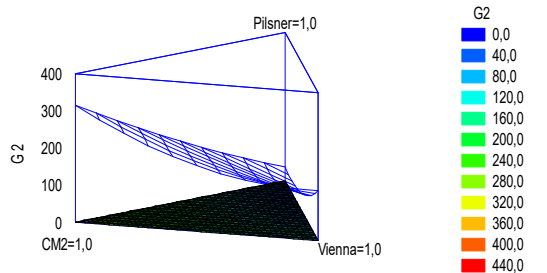


Figure 6: Response Surface for Phenolic Acids Determined by Glorie method

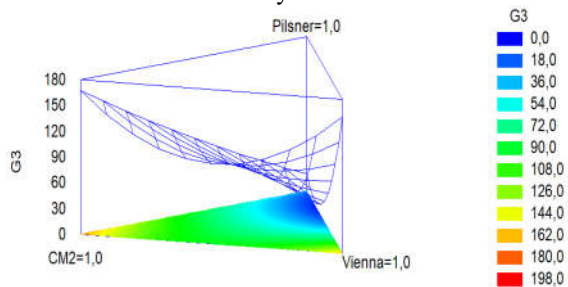


Figure 7: Response Surface for Flavanoids Determined by Glorie method

In the models for TPC and AOA some of the combinations between malts had negative effects on the target functions. More often it was the combination between the two base malts, because they had a relatively low phenolic content. However, a low phenolic content affected positively colloidal stability of beer produced. The data in Table 3 show that the linear model for TPC determined by FC had the highest degree of adequacy. At the same time, however, the third-order model described the experimental results with the highest accuracy. The results for AOA, measured by DPPH and FRAP were analogous. This, in turn, confirmed the observations for a correlation between the content of phenolic compounds and antioxidant activity (Carvalho et al., 2014; Shopska et al. 2021; Shopska et al., 2022a).

The results for TPC, measured by modified Glories method, phenolic acids and flavonoid phenolic compounds replicated the results already discussed. These results and the corresponding models will not be included in the optimization procedure, as they have similar trends to those already described, which will lead to a distortion in the result of the optimization procedure.

The following target functions were selected for the optimization – TPC, determined by FC and AOA, determined by DPPH and FRAP. The extract was excluded from the optimization procedure because it was a results from the enzyme activity of malts and has a relatively increasing tendency. The optimization should be done under certain conditions, namely: reduction of the TPC in wort and maximization of AOA determined by DPPH and FRAP. The concentration of TPC had to be minimal because phenolic compounds affected beer colloidal stability (Eaton, 2017; Kunze. 2019). The AOA had to be maximal if we wanted to have wort with high biological value. It could be achieved if we used more special malts in the mixtures or more malts with higher degree of heat treatment. The results of multi target optimization are presented in Table 5 and Figure 8a. According to the data in Table 5 the mixture should contain 99.98% Caramel Munich 2 malt, which would ensure the highest biological value of the wort. However, this malt mixture was not suitable from brewer's point of view. According to the manufacturer Caramel Munich 2 malt has to be used up to 50% in malt mixtures (BestMalt Catalog). Therefore, the following constraints had to be introduced in the optimization process: the amount of Caramel Munich 2 malt must be up to 50% of the mixture composition; the amount of TPC, measured by FC should be minimized; AOA, determined by DPPH and FRAP should be maximal. The results from second optimization with constraints are presented in Table 6 and Figure 8b.

The optimized malt mixture contained 60 % Pilsen malt, 20% Vienna malt, and 20% Caramel Munich 2 malt. The mixture TPC and AOA were significantly lower than the previous optimized variant. However, it is important to note that after wort boiling, the values of the investigated parameters increased significantly as follows: TPC=700.31 mg/l, DPPH=1692.28  $\mu\text{M TE/L}$  and FRAP=1522.92  $\mu\text{M TE/L}$  (23). This can be explained with the additional amounts of melanoidins which were formed during wort boiling and their effect on the AOA.

The optimized malt mixture was used for the production of different functional beverages (Tomova et al., 2021; Tomova et al, 2022; Trendafilova et al., 2021). Wort was produced in semi-industrial conditions and was inoculated with probiotic yeast strain *Saccharomyces cerevisiae* var. *boulardii* Y1 (Tomova et al., 2021; Tomova et al, 2022) or probiotic lactic acid bacteria *Lactocaseibacillus rhamnosus* (former *Lactobacillus casei* ssp. *rhamnosus*) LBRC11 (Trendafilova et al., 2021). In the beverages with probiotic yeast strain were added grapefruit, tangerine (Tomova et al., 2021), and lemon (Tomova et al., 2022) essential oils. In the beverage with probiotic lactic acid bacteria was added mint essential oil (Trendafilova et al., 2021). Data show that combination of wort with essential oils and probiotic strains led to the production of beverages with good organoleptic profile and significant biological and

functional value (Tomova et al., 2021; Tomova et al., 2022; Trendafilova et al., 2021).

Table 3: Estimated Full Model Effects

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value	Model	SE	R-Squared	Adj. R-Squared
<b>EXTRACT, %</b>									
Mean	441.671	1	441.671	-	-	Linear	0.722744	65.77	48.65
Linear	4.0146	2	2.0073	3.84	0.1172	Quadratic	0.294364	98.58	91.48
Quadratic	2.00278	3	0.667595	7.70	0.2539	Special Cubic	-	100.00	0.00
Special Cubic	0.0866503	1	0.0866503	-	-	-	-	-	-
Error	-1.26954E-13	0	0	-	-	-	-	-	-
Total	447.775	7	-	-	-	-	-	-	-
<b>TPC<sub>FC</sub>, mg/L</b>									
Mean	2,10811E6	1	2.10811E6	-	-	Linear	65.3946	96.10	94.15
Linear	421805.0	2	210902	49.32	0.0015	Quadratic	6.32628	99.99	99.95
Quadratic	17065.8	3	5688.6	142.14	0.0607	Special Cubic	-	100.00	0.00
Special Cubic	40.0218	1	40.0218	-	-	-	-	-	-
Error	3.00432E-10	0	0	-	-	-	-	-	-
Total	2.54702E6	7	-	-	-	-	-	-	-
<b>DPPH, μM TE/L</b>									
Mean	4.26993E6	1	4.26993E6	-	-	Linear	107.97	98.33	97.50
Linear	2.74907E6	2	1.37453E6	117.91	0.0003	Quadratic	91.5169	99.70	98.20
Quadratic	38254.9	3	12751.6	1.52	0.5150	Special Cubic	-	100.00	0.00
Special Cubic	8375.34	1	8375.34	-	-	-	-	-	-
Error	3.87445E-10	0	0	-	-	-	-	-	-
Total	7.06563E6	7	-	-	-	-	-	-	-
<b>FRAP, μM TE/L</b>									
Mean	6.94766E6	1	6.94766E6	-	-	Linear	42.5065	99.74	99.61
Linear	2.79512E6	2	1.39756E6	773.50	0.0000	Quadratic	75.8636	99.79	98.77
Quadratic	1471.93	3	490.642	0.09	0.9583	Special Cubic	-	100.00	0.00
Special Cubic	5755.28	1	5755.28	-	-	-	-	-	-
Error	-6.17547E-10	0	0	-	-	-	-	-	-
Total	9.75001E6	7	-	-	-	-	-	-	-

Table 4: ANOVA

Source	Sum of Squares	Df	Mean Square
Extract			
Special Cubic Model	6.10403	6	1.01734
Total error	0.0	0	
Total (corr.)	6.10403	6	
R-squared = 100.0 %			
TPC <sub>FC</sub>			
Special Cubic Model	438911.0	6	73151.8
Total error	0.0	0	
Total (corr.)	438911.0	6	
R-squared = 100.0 %			
DPPH			
Special Cubic Model	2.7957E6	6	465950.1
Total error	2.47383E-10	0	
Total (corr.)	2.7957E6	6	
R-squared = 100.0 %			
FRAP			
Special Cubic Model	2.80235E6	6	467058.1
Total error	0.0	0	
Total (corr.)	2.80235E6	6	
R-squared = 100.0 %			

Table 5: Multi target optimization – first step

Factor	Low	High	Optimum	Optimum, g	DPPH	FC	FRAP
Pilsner	0,0	1.0	0.00000857643	0.1	1964.41	1039.25	2234.41
CM2	0,0	1.0	0.99984	49.8			
Vienna	0,0	1.0	0.00015124	0.01			

Table 6: Multi target optimization – second step

Factor	Low	High	Optimum	Optimum, g	DPPH	FC	FRAP
Pilsner	0,0	1.0	0.6	30	602.47	486.82	745.20
CM2	0,0	1.0	0.2	10			
Vienna	0,0	1.0	0.2	10			

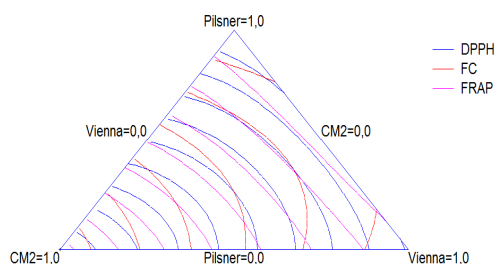


Figure 8a. Multi-target optimization without constrains

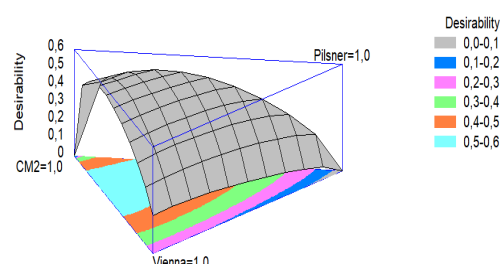


Figure 8b. Multi-target optimization with constrains

## CONCLUSIONS

The aim of the present work was to develop a mixture of malts (Pilsner, Vienna and Caramel Munich 2) for the production of wort with increased biological value, which could be used for functional beverages. Two mixtures were developed and the choice between them was made on the combination between brewing characteristics and biological value of wort. Therefore, a mixture of 60% Pilsner malt, 20% Vienna malt and 20% Caramel Munich 2 malt was selected and used for the production of different types functional beverages with probiotic yeast or lactic acid bacteria strains and with or without essential oils additions. The mixture obtained guaranteed the production of wort with optimized phenolic content and maximum AOA values determined by DPPH and FRAP.

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