Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences

Tome 72, No 12, 2019

AGRICULTURAL SCIENCES

Food science and technology

OPTIMIZATION OF THE MIXED MELON-BERRY JUICE COMPOSITION USING SIMPLEX CENTROID EXPERIMENTAL DESIGN

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(*Submitted by Academician A. Atanassov on April 9, 2019*)

Abstract

Combinations of different berry juices (bilberry, strawberry, and raspberry juice) for obtaining blended berry juices with high antioxidant activity were studied using simplex centroid design. The target functions for optimization were total polyphenols (TPP), free radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and total monomeric anthocyanins (TMA). The highest contents of total polyphenols and total anthocyanins and antioxidant capacity (DPPH and FRAP assay) values were observed for the bilberry juice. The optimal area of combinations of freshly pressed juices in the berry juice formulas with high antioxidant activity was obtained. In a second series of experiments berry juices based on the definite optimal area were used for polyphenol enrichment of melon juice. Consistent with the results obtained from the simplex centroid design, the highest total polyphenolic content and antioxidant capacity values were observed for the mixed melon-based juice containing 50% bilberry, followed by the mixtures with 25% bilberry and 25% raspberry or strawberry. Besides antioxidant capacity enhancement, the addition of berry juice blends improves sensory (flavour and colour) quality of the mixed melon-based juices.

Key words: melon, berry, antioxidant capacity, total polyphenols, simplex centroid design

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DOI:10.7546/CRABS.2019.12.16

1. Introduction. A great number of studies have shown an inverse correlation between increased fruit and vegetable intake and reduced risk of chronic disorders like cardiovascular diseases. Due to the wide spectrum of secondary plant metabolites such as polyphenols in plant derived foods an inhibition in low density lipoprotein oxidation can be suggested, and therefore they can be considered responsible for the beneficial effects on human health [1, 2].

Several human intervention studies performed on fruit juices intake and suggest an improved antioxidant status following fruit juice consumption [3–5]. Furthermore, there is a strong consumer demand for natural products, that are minimally processed and with a "clean" label (no additives) [6]. Mixed fruit beverages have a series of advantages, combining different flavours and great variety of bioactive compounds [7].

Melon, particularly cantaloupe, plays a significant role in the human diet, being source of vitamins (B_1, B_2, PP, A) and minerals (potassium, calcium, sodium, chlorine, iron) [8]. Melon intake has been recommended for exhaustion and anemia, as well as in atherosclerosis and other cardiovascular diseases. It also enhances the action of antibiotics, reducing their toxicity [9]. Melons have strong specific flavour and low acidity, which leads to complications and difficulties within the production and storage of melon juices. Solution to this problem could be mixing melon juice with other fruit juices with high acidity, which will improve the flavour properties and increase the shelf-life of the final "fresh-like" products [10].

Therefore, the present study evaluated the potential for development of new functional beverages based on melon juice. Single-fruit or blended berry juices were added for polyphenol enrichment. The composition of the berry (bilberry, strawberry and raspberry) juice blends was optimized using simplex centroid experimental design.

2. Materials and methods. ***2.1. Chemicals.*** For analytical purposes the following reagents were used: DPPH [2,2-diphenyl-1-picrylhydrazyl] and Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); TPTZ [2,4,6-tripyridyl-s-triazine] and gallic acid monohydrate (Fluka, Buchs, Switzerland); Folin-Ciocalteau's reagent (Merck, Darmstadt, Germany). All other reagents and solvents were of analytical grade.

2.2. Plant materials. Frozen strawberry (*Fragaria×ananassa* Duch., cv. Siabelle), raspberry (*Rubus idaeus* L., cv. Bulgarski rubin) and bilberry (*Vaccinium myrtillus* L.) fruits, all harvested in 2014, were provided by Cima 99 Ltd. (Striama, Bulgaria) and stored at $-18\,^{\circ}$C upon usage. Fresh cantaloupe melon (*Cucumis melo* L. var. *cantalupensis*) fruits were obtained from a local market in Plovdiv in 2015. Fruits were processed according to the flow diagram shown in Fig. 1.

2.3. Experimental design. A three-component simplex centroid design was used. The mixture components consisted of bilberry juice (X_1), strawberry juice (X_2) and raspberry juice (X_3). Component proportions were expressed as

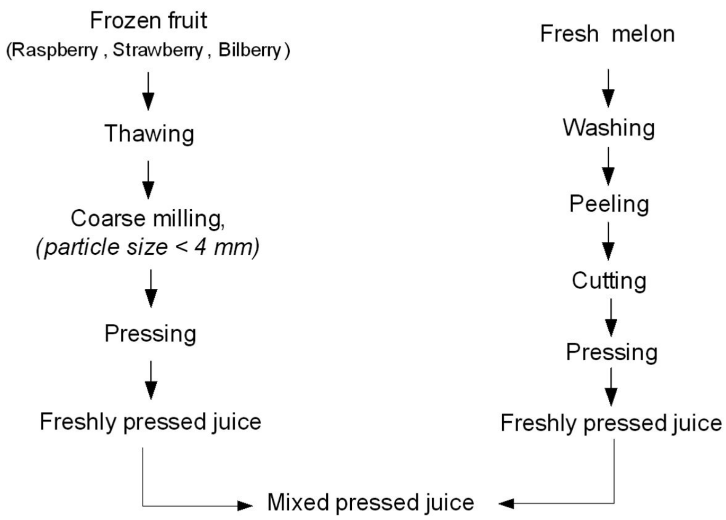


Fig. 1. Process flowchart for obtaining of the freshly pressed juices

fractions of the mixture with a sum ($X_1 + X_2 + X_3$) of one (100%). The seven points were three single ingredient treatments, three two-ingredient mixtures and one three ingredient mixture. Obtained freshly pressed juices were blended as follows: 1 – 100% bilberry juice (X_1); 2 – 100% strawberry juice (X_2); 3 – 100% raspberry juice (X_3); blend 1 – X_1:X_3 = 1:1; blend 2 – X_1:X_2 = 1:1; blend 3 – X_2:X_3 = 1:1; blend 4 – X_1:X_2:X_3 = 1:1:1 (Fig. 2a).

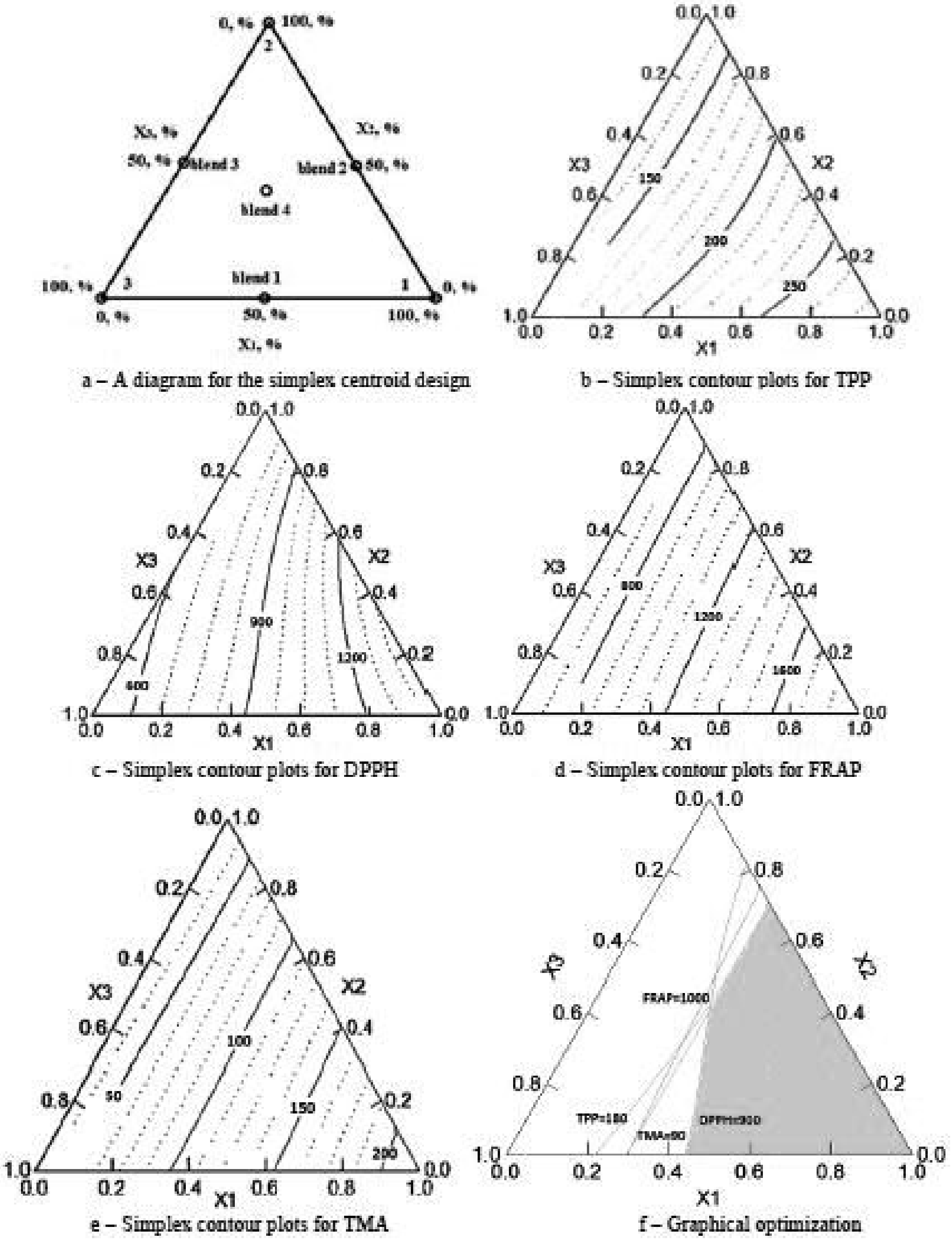
Single-fruit or blended berry juices were added to melon juice (Table 2).

2.4. Sample preparation. An aliquot (5 g) of juice sample was transferred into 50 mL volumetric flask using 40 mL of acidified (0.1% HCl) methanol. After extraction for 24 h at 10 °C, the flask was filled up to the mark with acidified methanol and filtered through a paper filter. Extraction was performed in triplicate.

2.5. Analytical methods. All measurements were performed with a Helios Omega UV-Vis spectrophotometer equipped with VISION*lite* software (all from Thermo Fisher Scientific, Madison, WI, USA) using 1 cm path length cuvettes.

2.5.1. Determination of total polyphenols. The content of total polyphenols (TPP) was determined by the method of SINGLETON and ROSSI [11] modified as follows: appropriately diluted sample extract (0.1 mL) was mixed with 0.5 mL of FC-reagent (diluted with distilled water 1:4, v/v) and 1.5 mL of sodium carbonate solution (7.5%, w/v) and the volume was made up to 10 mL with distilled water; the mixture was incubated for 2 h at room temperature before the absorbance was measured at 750 nm. The results were presented as mg gallic acid equivalents (GAE) per 100 g of juice.

2.5.2. Determination of total monomeric anthocyanins. The amount of total monomeric anthocyanins (TMA) was determined by the pH-differential method [12].



a – A diagram for the simplex centroid design

b – Simplex contour plots for TPP

c – Simplex contour plots for DPPH

d – Simplex contour plots for FRAP

e – Simplex contour plots for TMA

f – Graphical optimization

Fig. 2. Experimental design and graphical optimization for the freshly pressed juices in the blended berry juices with high antioxidant activity

The sample extract was diluted in parallel with buffer pH 1.0 (0.025 M potassium chloride) and buffer pH 4.5 (0.4 M sodium acetate). After 1 h of incubation at room temperature, the absorbance was measured at 520 and 700 nm. Results were calculated using a molar extinction coefficient of 26900 L/(mol cm) and molecular weight of 449.2 g/mol [13], and expressed as equivalents of cyanidin 3-glucoside (CGE) in mg per 100 g of juice.

2.5.3. Determination of total antioxidant capacity. The total antioxidant capacity was determined by the DPPH (free radical scavenging activity) and FRAP (ferric reducing antioxidant power) assay. Trolox, a water-soluble vitamin E analogue, was used as a reference in both assays and the antioxidant capacity was expressed as µmol Trolox equivalents (TE) per 100 g of juice.

DPPH assay was based on the method of BRAND-WILLIAMS et al. [14] modified as follows: 2250 µL of a DPPH methanolic solution (6×10^{-5} M) were mixed with 250 µL of sample extract (diluted with distilled water 1:3, v/v); absorbance at 515 nm was measured after 15 min of reaction in a cap-sealed cuvette kept in dark at room temperature.

FRAP assay was performed according to BENZIE and STRAIN [15] with some modifications. The FRAP reagent was prepared by mixing 2.5 mL of a TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L), 2.5 mL of a $FeCl_3$ water solution (20 mmol/L) and 25 mL of an acetate buffer (0.3 mol/L, pH 3.6). In the assay, 2250 µL of FRAP reagent and 250 µL of sample extract (diluted with distilled water 1:3, v/v) were mixed in a cuvette and absorbance at 593 nm was measured after 4 min of reaction.

2.6. Consumer acceptance test. A consumer panel ($n = 15$) evaluated overall acceptability and six other sensory attributes (aroma, flavour, texture, sweetness, acidity, and aftertaste) of four mixed juices (MJ1, MJ2, MJ3, MJ4). Participants evaluated samples using environmentally-controlled partitioned booths illuminated with white incandescent light in the sensory lab at the Institute of Food Preservation and Quality, Plovdiv, Bulgaria. Experiments were replicated twice in a randomized block design. A nine-point hedonic scale (dislike extremely – like extremely) was used for samples evaluation.

2.7. Statistical analysis. The results reported in the present study are the mean values of at least three analytical determinations and the coefficients of variation, expressed as the percentage ratios between the standard deviations and the mean values, were found to be $< 5\%$ in all cases. The means were compared using one-way ANOVA, performed with Microsoft Excel, and Tukey's test at a 95% confidence level.

Scheffe's canonical special cubic equation for three components was fitted to data collected at each experimental point using backward stepwise multiple regression analysis as described in [16]. This canonical model differs from full polynomial models in that it does not contain a constant term (intercept equal to zero). Variables in the regression models, which represent two-ingredient or three-ingredient interaction terms, were referred to as “non-linear” terms. The postulated canonical special cubic equation was:

$$(1)\quad Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3,$$

where Y is a predictive dependent variable (total polyphenols – TPP, free radical scavenging activity – DPPH, ferric reducing antioxidant power – FRAP, total monomeric anthocyanins – TMA); β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, and β_{123} are the corresponding parameter estimates for each linear and cross-product term produced for the prediction models for bilberry juice, strawberry juice, and raspberry juice, respectively. An analysis of variance was performed on the data and response surfaces were generated for each response using predictive models. The fitted model for TPP, DPPH, FRAP, and TMA was used to optimize the blended berry juices.

The terms in the canonical mixture polynomial have simple interpretations which can be found in specialized texts [17]. The usual way to summarize mixture proportions is via triangular (ternary) graphs. One can add a fourth dimension to the triangle, perpendicular to the first three, to plot the value for the dependent variable or it can be indicated, as is more usual, in a two-dimensional plot where the contour of constant height is graphed on a triangle.

3. Results and discussion. Mixing different fruit juices is an intriguing possibility for obtaining new beverages with high antioxidant activity [18]. As a first step, in the present study simplex centroid experimental design was applied for the antioxidant capacity optimization of a three component berry (bilberry, strawberry and raspberry) juice system. The efficiency of this approach has already been demonstrated [19] for polyphenolic content enhancement in an enzyme-assisted extraction process.

The highest contents of total polyphenols and total anthocyanins and antioxidant capacity values were observed for the bilberry juice (Table 1, variant 1). Increasing the proportions of strawberry and/or raspberry juice, the phytochemical parameters decreased, reaching 54% and 56% lower average contents of total polyphenols and total anthocyanins, respectively, for the ternary berry juice blends (bilberry juice/strawberry juice/raspberry juice = 1:1:1).

The two assays used represent different mechanisms of evaluating antioxidant capacity. While the DPPH assay measures the ability of plant extracts to scavenge free radicals, the FRAP assay quantifies the total concentration of redox-active compounds [20]. In general, the changes of the total antioxidant capacity (Table 1, Fig. 2) correspond to the results obtained for the total polyphenols. Interestingly, despite the similar total polyphenolic contents of the ternary blend and blend 3 (strawberry juice/raspberry juice = 1:1), significantly higher antioxidant capacity values were observed for the ternary berry juice blends both for the DPPH and FRAP assay.

Using the simplex method and related with it procedures of modelling and optimization after treatment of the results equations for the components concen-

Table 1

Berry juice variants and results of the simplex centroid design

Berry juice variant[a]	TPP[b] (mg GAE/100 g)	DPPH[b] (µmol TE/100 g)	FRAP[b] (µmol TE/100 g)	TMA[b] (mg CGE/100 g)
1	281 ± 13a	1364 ± 61a	1914 ± 86a	218 ± 10a
2	123 ± 6b	584 ± 26b	593 ± 27b	22 ± 1b
3	136 ± 6b	490 ± 22c	569 ± 26b	33 ± 1c
Blend 1	216 ± 10c	1267 ± 57d	1352 ± 61c	132 ± 6d
Blend 2	230 ± 10c	953 ± 43e	1277 ± 57c	128 ± 6d
Blend 3	129 ± 6b	601 ± 27f	587 ± 26b	27 ± 1bc
Blend 4	128 ± 6b	914 ± 41e	1093 ± 49d	97 ± 4e

[a] Juice variants as in Fig. 2a
[b] Means ± standard deviations ($n = 3$). Different letters within a column indicate significant differences (Tukey's test, $P < 0.05$)

Table 2

Overall acceptance and composition of the mixed melon-berry juices and responding contents of total polyphenols (TPP) and antioxidant capacity (DPPH and FRAP) values of the mixed melon-berry juices

Mixed juice variant	Contents (%)				Phytochemical characterisation			Overall acceptance
	Melon juice	Bilberry juice	Raspberry juice	Strawberry juice	TPP[a] (mg GAE/100 g)	DPPH[a] (µmol TE/100 g)	FRAP[a] (µmol TE/100 g)	
MJ1	50	50	–	–	123 ± 6a	605 ± 27a	737 ± 33a	7.5
MJ2	50	25	25	–	93 ± 4b	497 ± 22b	544 ± 26b	7.8
MJ3	50	25	–	25	103 ± 5b	520 ± 23b	522 ± 24b	7.8
MJ4	50	16.67	16.67	16.67	63 ± 3c	358 ± 16c	348 ± 16c	9.0

[a] Means ± standard deviations ($n = 3$). Different letters within a column indicate significant differences (Tukey's test, $P < 0.05$)

tration in the matrix – total polyphenols (TPP), free radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and total monomeric anthocyanins (TMA) have been obtained as follows:

$$\text{TPP} = 280.8X_1 + 123.3X_2 + 136.3X_3 + 53.8X_1X_2 + 84.6X_1X_3 - 4.0X_2X_3 - 247.5X_1X_2X_3, \text{mg GAE/100 g } (R^2 = 0.993), \tag{2}$$

$$\text{DPPH} = 1364.0X_1 + 584.0X_2 + 490.4X_3 + 1170.0X_1X_2 + 103.2X_1X_3 + 255.2X_2X_3 - 1920.3X_1X_2X_3, \text{µmol TE/100 g } (R^2 = 0.995), \tag{3}$$

$$\text{FRAP} = 1913.5X_1 + 593.4X_2 + 569.4X_3 + 392.2X_1X_2 + 142.2X_1X_3 + 24.0X_2X_3 + 27.6X_1X_2X_3, \text{µmol TE/100 g } (R^2 = 0.999), \tag{4}$$

$$\text{TMA} = 217.9X_1 + 22.3X_2 + 32.6X_3 + 47.2X_1X_2 + 10.6X_1X_3 - 3.4X_2X_3 + 6.0X_1X_2X_3, \text{mg CGE/100 g } (R^2 = 0.999). \tag{5}$$

The resulting equations with high accuracy describe the change of the contents of the dependent variable ($R^2 > 0.9$). The two-dimensional contour plots are shown in Fig. 2b–e.

For optimization of the ternary blend obtained from bilberry juice, strawberry juice, and raspberry juice the following limiting conditions have been accepted: total polyphenols > 180 mg GAE/100 g, free radical scavenging activity > 900 µmol TE/100 g, ferric reducing antioxidant power > 1000 µmol TE/100 g, and total monomeric anthocyanins > 90 mg CGE/100 g. Optimization has been carried out by the superposition of the contour plots for predicted TPP, DPPH, FRAP, and TMA content of the blended berry juices with high antioxidant activity. The optimum area for the freshly pressed juices in the blended berry juices is presented in Fig. 2f (the darkened area).

In a second series of experiments berry juices based on the definite optimal area were used for polyphenol enrichment of melon juice (Table 2).

Consumers' evaluation of the four mixed juices (MJ1, MJ2, MJ3, MJ4) is presented monadically using the 9-point hedonic scale (dislike extremely, neither like nor dislike, like extremely). Mean values of overall acceptance are given in Table 2. All test juices were rated high.

Consistent with the results obtained from the simplex centroid design, the highest total polyphenolic content and antioxidant capacity values were observed for the mixed melon-based juice containing 50% bilberry (Table 3, MJ1), followed by the mixtures with 25% bilberry and 25% raspberry or strawberry.

Besides antioxidant capacity enhancement, the addition of berry juice blends improves sensory (flavour and colour) quality of the mixed melon-based juices (data not shown). Moreover, this polyphenol enrichment could be worthwhile, allowing extended microbiological shelf-life of the "fresh-like" products [21].

3. Conclusions. The results obtained demonstrate the efficiency of simplex centroid experimental design for optimization of the composition of berry juice blends with respect to their polyphenolic content and antioxidant capacity. Moreover, the addition of blended berry juices allows development of melon-based functional beverages, both enriched in polyphenols and possessing high sensory acceptance.

Acknowledgements. We are grateful to Cima 99 Ltd. (Striama, Bulgaria) for providing the frozen fruits.

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