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Development of a novel low-calorie lime juice based prebiotic/ high-antioxidant beverage using combined design optimization methodology

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Abstract:	A novel lime-juice based low-calorie functional beverage was developed by using D-optimal combined design optimization. For the preparation of the beverage, the following functional ingredients were used; lime juice, lime peel essential oil (LEO) as a flavoring agent and bioactive component, sucralose as a low-calorie sweetener, inulin/polydextrose (I/P) mixture as prebiotics fibers, pectin as a thickening agent and soluble dietary fiber, lutein as a carotenoid colorant and antioxidant, and peppermint extract (ME) as a flavoring agent and bioactive component. A combined design consisting of one mixture factor (LEO/ME ratio), one numeric factor (lutein concentration), and one categoric factor (presence or absence of prebiotics) was used for optimizing the functional beverage based on the sensory quality. Regression models were adequately fitted to the data of sensory acceptance with a determination coefficient >90%. The sample containing a mixture of prebiotics, 2:3 (v/v) ratio of LEO: ME, and 3 mg/100 ml lutein was selected as the best formulation among the six optimal beverages which was suggested by design expert software. This final optimum sample showed the highest total phenolic (44.22 mg gallic acid equivalents/L) and flavonoid (25.49 mg quercetin equivalents/L) contents, as well as its antioxidant activity (as DPPH• scavenging), was 38.30%.
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Cover letter

Dear Editor in chief

I would like to submit our research paper entitled "**Development of a novel low-calorie lime juice based prebiotic/ high-antioxidant beverage using combined design optimization methodology**" for your consideration to publish in the "***LWT-Food Science and Technolog***". I beg to inform you that this work was supported by the University of Tabriz and this study originated in Tabriz University. I confirm that the article has not published previously and is not under consideration for publication elsewhere. No conflict of interest exists, if accepted, the article will not be published elsewhere in the same form, in any language, without the written consent of the publisher.

Respectfully,

Given epidemiological evidence of the health-protective effects of dietary fiber and the growth rate of nutraceutical beverages worldwide, the development of fiber-enriched beverages is an interesting option to achieve high dietary fiber intake by the average population. Few studies have been conducted on the acceptability of addition of prebiotics to juices and fruit nectars. The development of a drink that is low in calories, high in fiber and rich in antioxidant bioactive compounds, and also has good sensory characteristics seems to be very interesting from health aspect. Submitted article optimizes the sensory properties of a novel low-calorie lime juice-based beverage enriched with inulin/polydextrose prebiotics, lutein, LEO, and ME to investigate its possible technological potential as a functional beverage. For this purpose, the effects of combination of functional ingredients and their mixing level on the sensory quality of beverages were assessed, and the formulation of the beverages was optimized using combined design approach. Finally, the formulation with the highest bioactive compounds content and antioxidant potential was introduced as the final optimal beverage. The manuscript contains 4 tables, 5 figures, and 32 pages (including references, figures and tables), and supplementary material.

Thank you very much for your attention and consideration.

Sincerely yours,

Prof. B. Ghanbarzadeh

University of Tabriz,

Tabriz, Iran

Highlights

- A novel prebiotic low-calorie functional beverage was developed.
- Six optimum formulas with good organoleptic quality were proposed by CD.
- The beverage with the highest bioactive and antioxidant potential was selected.
- It may have a therapeutic potential for diabetic and hypertension patients.

Development of a novel low-calorie lime juice based prebiotic/ high-antioxidant beverage using combined design optimization methodology

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Abstract

A novel lime-juice based low-calorie functional beverage was developed by using D-optimal combined design optimization. For the preparation of the beverage, the following functional ingredients were used; lime juice, lime peel essential oil (LEO) as a flavoring agent and bioactive component, sucralose as a low-calorie sweetener, inulin/polydextrose (I/P) mixture as prebiotics fibers, pectin as a thickening agent and soluble dietary fiber, lutein as a carotenoid colorant and antioxidant, and peppermint extract (ME) as a flavoring agent and bioactive component. A combined design consisting of one mixture factor (LEO/ME ratio), one numeric factor (lutein concentration), and one categoric factor (presence or absence of prebiotics) was used for optimizing the functional beverage based on the sensory quality. Regression models were adequately fitted to the data of sensory acceptance with a determination coefficient >90%. The sample containing a mixture of prebiotics, 2:3 (v/v) ratio of LEO: ME, and 3 mg/100 ml lutein was selected as the best formulation among the six optimal beverages which was suggested by design expert software. This final optimum sample showed the highest total phenolic (44.22 mg gallic acid equivalents/L) and flavonoid (25.49 mg quercetin equivalents/L) contents, as well as its antioxidant activity (as DPPH$^{\bullet}$ scavenging), was 38.30%.

Keywords: High-fiber prebiotic beverage, Lutein, Lime peel essential oil, Peppermint extract, Optimization

1. Introduction

Functional beverages are non-alcoholic drinks that are enriched with nutraceuticals, in addition to the basic nutritional value of the product, to provide multiple health-related benefits. These functional ingredients include amino acids, vitamins, minerals, antioxidants, essential fatty acids, phytonutrients (such as carotenoids, polyphenols, terpenes, and phytosterols), fibers, prebiotics, and probiotics. Consumer interest in functional foodstuffs that not only be highly nutritious and healthy but also be easy to prepare and ingest has led to the growing popularity of fruit-based functional beverages. Unfortunately, most conventional functional beverages are sweetened and contain significant amounts of sugars and hence, consumption of them can potentially increase the risk of diabetes, obesity, and heart disease. Using of non-nutritive-high intensity sweeteners such as sucralose can meet consumer demand for no/low-sugar content products aimed at obesity prevention, weight control, and diabetes management (Edwards, Rossi, Corpe, Butterworth, & Ellis, 2016).

Lime (*C. aurantifolia*) is a good source of nutrients and bioactive compounds which can be useful for the positive regulation of oxidative stress, lipid profiles, and inflammatory cascades. Moreover, due to its abundant flavonoid content, lime juice can potentially exert neuroprotective effects and become general dietary brain food (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014; Kawaii, Tomono, Katase, Ogawa, & Yano, 1999). The highly acidic lime juice has a distinct aroma and flavor and a unique sour taste, and is often used to enhance the flavor and aroma of foods and beverages. Therefore, it can be a suitable matrix for developing new value-added beverages.

Inulin and polydextrose as soluble prebiotic fibers play an important role in promoting health and preventing diseases, including improving digestive health and function, inhibiting the proliferation of harmful microorganisms and improving the growth and activity of beneficial intestinal bacteria, attenuation of postprandial blood glycemic and insulinemic response, reducing calorie intake, and the risk of obesity and type 2 diabetes and related disorders (Dahl & Stewart, 2015; Kasapoğlu, Daşkaya-Dikmen, Yavuz-Düzgün, Karaça, & Özçelik, 2019). These prebiotics can be used in transparent, low glycemic, and sugar-free beverages suitable for people with diabetes (Shahidi & Ambigaipalan, 2016). Peppermint (*Mentha piperita*, from Lamiaceae) extract is one of the most important medicinal and aromatic herbal extracts with distinguished bioactive potential. It is a blood sugar regulator and has shown significant inhibition against key enzymes of type 2 diabetes (α-glucosidase) and hypertension (angiotensin 1-converting enzyme, ACE) (Cam et al., 2020). Lime (*C. aurantifolia*) peel essential oil (LEO) is commercially important citrus essential oils (EOs) (the most important by-product of citrus processing) with high nutraceutical, antioxidant, and sensory characteristics as well as economic importance. The findings suggest that LEO can affect food intake and a diverse array of processes involved in energy expenditure and fuel utilization, all of which suppress weight gain (Asnaashari et al., 2010). LEO is classified as Generally Recognized as Safe (GRAS) according to Food Additive Status List (Dosoky & Setzer, 2018) and is a valuable product for flavoring purposes. Lutein, a natural bioactive colorant and potent antioxidant, is known to play an established role in eye health and a protective role against cardiovascular and chronic diseases, cancer, etc. (Ma, Yuan, Yang, Wang, & Lv, 2020; Yan et al., 2020). Positive effects of lutein on health issues such as age-related macular degeneration (AMD) have been reported at dietary intake levels of 6-14 mg/day (Cheng, Ferruzzi, & Jones, 2019).

Sensory acceptability is a crucial factor when designing newly enriched foodstuffs, and customer acceptance issues are required to be overcome at first. Combined design (CD) is a versatile experimental design technique that can be applied to operate multi-objective optimization design under reduced experimental runs. It has recently been used as one of the most popular methods for optimizing product formulation in the food industry (Icyer et al., 2016). Recently, studies have been conducted on the formulation and evaluation of the properties of new functional fruit-based beverages and sugar-free products (Arruda, Silva, Pereira, Meireles, & Pastore, 2020; Arya & Shakya, 2021; Cassani, Tomadoni, Moreira, & Agüero, 2018; N. Liu, Nguyen, Wismer, & Temelli, 2018; Salinas, Garvin, Ibarz, & Ibarz, 2019). The chemical compounds of cold-pressed LEO and their antioxidant effects as well as their contribution to sensory properties have been investigated (Lin, Chuang, Chen, & Yang, 2019; S. Liu, Li, & Ho, 2022). The development of a drink that is low in calories, high in fiber and rich in antioxidant bioactive compounds, and also has good sensory characteristics seems to be very interesting from health aspect. The objectives of current research were to develop a low-calorie functional beverage enriched with inulin/polydextrose prebiotics, lutein, LEO, and ME with high consumer acceptance. For this purpose, the effects of functional ingredients and their using level on the sensory quality were modeled, and the formulation of the beverages was optimized using CD approach. Finally, the beverage with the highest bioactive compounds content in terms of the total phenolic and flavonoid contents and antioxidant potential was selected as the final optimum formulation.

2. Material and methods

2.1. Material

The lime (*C. aurantifolia*) concentrate (~45 ºBrix), lime (*C. aurantifolia*) peel essential oil (cold-pressed), and peppermint (*Mentha piperita L.*) extract was provided from Takdaneh Co. (Tabriz, Iran), all stored in a dark container at 4 °C until use. Inulin (92.52% pure) and polydextrose (95.5% pure) were obtained from Pyson Co., Ltd. (Shaanxi, China). High-methoxyl (HM) pectin (galacturonic acid, ≥ 74.0% on a dry basis) and all other chemicals were supplied from Sigma-Aldrich (Germany), and all were of analytical grade.

2.2. Preparation of the beverages

Protocol for the preparation of 1 L beverages in a laboratory scale is shown in **Fig. S1**. Final product contains 10% w/v of "reconstituted lime juice with 8.3 °Brix". The concentration of sucralose was equivalent in sweetness to 10% w/v sucrose based on a previous study (Wee, Tan, & Forde, 2018). LEO, ME, and lutein were added at concentration ranges determined based on the sensory analysis (data not shown); and depending on the CD points **(Table 1)**. A beverage must contain 6 g or more of dietary fiber per serving (20% of the daily reference value (DRV)) to make a "high in fiber" claim (FDA, 2012). The DRV for fiber is 30 g per day based on a 2500-calorie diet. Considering the purity of inulin and polydextrose, beverages were formulated with 2.85 and 2.78% w/v inulin and polydextrose, respectively, to meet the recommendation of providing 6 g inulin and polydextrose fibers in serving sizes of 240 ml (FDA, 2012). All sample preparations were carried out in triplicate.

2.3. Determination of physicochemical properties

2.3.1. Extraction of phytochemicals

The antioxidant capacity, total flavonoid content (TFC), and total phenolic content (TPC) were assessed on an extract of antioxidants from beverage specimens, all as described previously (Cassani, Gerbino, del Rosario Moreira, & Gómez-Zavaglia, 2018; Cassani, Tomadoni, Viacava, Ponce, & Moreira, 2016). 2 ml of each sample was homogenized with 10 ml ethanol (80% v/v). The homogenate was centrifuged at 13500 g for 15 min at 4 °C. The supernatant was collected and filtered through a Whatman #1 filter paper. The ethanolic extract was stored at −20 °C for analysis. All assays were done in triplicate.

2.3.2. Total phenolic content (TPC)

Sample to water (1:20) of the extracts (200 μl) was added to 1 ml of the Folin–Ciocalteu reagent (FCR) (diluted 1:10). After 3 min of incubation at 20 °C, 800 µL of 7.5% Na_2CO_3 solution was added followed by the incubation of reaction mixture at the same temperature for 2 hours. The absorbance at 765 nm was determined by a UV-Vis double-beam spectrophotometer (Shimadzu UV-1700, Japan), and the TPC was calculated using gallic acid as standard. The calibration curve of the gallic acid was created at 10-200 mg/L, and the TPC was reported as mg gallic acid equivalents (GAE)/L of the specimen.

2.3.3. Total flavonoid content (TFC)

In brief, the ethanolic extract (0.2 ml) was mixed with deionized H_2O (1.28 ml) and $NaNO_2$ (0.06 ml, 5%). After 5 min at 20 °C, $AlCl_3$ (60 µl, 100 g/L) was incorporated, and 6 min later, NaOH (0.4 ml, 40 g/L) was added under the same conditions. The mixtures were stirred and the absorbance was measured at 510 nm using a UV-Vis double-beam spectrophotometer (Shimadzu UV-1700, Japan). The TFC was calculated based on the calibration curve of quercetin (10-200 mg/L), and the results were expressed as mg quercetin equivalents (QE)/L of the sample.

2.3.4. Antioxidant capacity by the DPPH$^\bullet$ scavenging assay

100 µL of ethanol was mixed with 3.9 ml of ethanolic DPPH$^\bullet$ solution (39.43 mg/L) (blank) to determine the initial absorbance of the DPPH$^\bullet$ solution. Then, 100 µL of ethanolic extract was added to 3.9 ml DPPH$^\bullet$ ethanolic solution (39.43 mg/L). The mixture was shaken immediately and incubated at 20 °C in the dark. After one hour, the decrease in absorbance at 517 nm was measured using a UV-Vis double-beam spectrophotometer (Shimadzu UV-1700, Japan). The DPPH$^\bullet$ scavenging activity was expressed as the inhibition percentage of the DPPH$^\bullet$ using the following equation:

$$Radical\ scavenging\ activity\ (\%) = \frac{A_0 - A_s}{A_0} \times 100 \qquad (1)$$

where A_0 and A_s correspond to the absorbance of the control blank and sample, respectively.

2.3.5. Ascorbic acid content (AA)

The ascorbic acid content of samples was quantified using the iodine titration method as described by taking 0.88 mg AA, equivalent to 1 ml of iodine solution (preparation details are given in the **supplementary material**) (Aghajanzadeh, Kashaninejad, & Ziaiifar, 2016). 20

ml of the samples were mixed with 150 ml of distilled water and were titrated with iodine solution in the presence of 1% starch solution as an indicator up to the solutions reaching a fixed dark-blue color. All measurements were run in triplicate. AA content was estimated using **Eq. 2**:

$$mg\ ascorbic\ acid\ /\ 100\ mg\ sample\ =\ 0.88\ \times ml\ iodine\ solution \qquad (2)$$

2.3.6. Total soluble solids (TSS), pH, and titratable acidity (TA)

The pH and TSS of samples were measured at 20 °C using a refractometer (Mettler Toledo, Japan) and a pH meter (Mettler Toledo, Japan), respectively. The TA determination details are given in the **supplementary material**. All tests were performed in triplicate.

2.4. Sensory analysis of beverage samples

Sensory evaluation was performed by a panel of thirty semi-trained members using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely) (Arya & Shakya, 2021). These panelists (aged 23-40 years) were students of the Department of Food Science and Technology, University of Tabriz, Iran. Beverage samples (40 ml) were served at 4 °C in transparent polyethylene cups, coded with 3-digit random numbers. The samples were arranged in a randomized order and were asked to evaluate and score for taste, flavor, texture, color, and overall acceptance attributes. Potable water and salt-free crackers were provided as palate-cleansing agents. The reported values were the average of the three analyses.

2.5. Experimental design and statistical analysis

A D-optimal combined design having two mixture components (LEO (diluted 1:10) and ME concentrations), one numeric factor (lutein concentration), and one categoric factor (I/P mixture at two levels, absence (level 1) or presence (level 2) of fibers) was applied. 17 experimental points were obtained (**Table 1)**. The data were analyzed and the contour and 3D surface plots were created by Design-Expert package software (Version 10, Stat-Ease Inc., Minneapolis, USA). The following equation was fitted to the data:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (4)$$

where Y is a response variable, k is the number of variables, X_i and X_j are independent variables in coded units, β_{ij}, β_{ii}, and β_i are the measures of the X_i, X_j, X_i^2, and X_iX_j of linear, quadratic, and interaction effects, respectively, and β_0 is a constant coefficient.

The TPC, TFC, and antioxidant data were analyzed through analysis of variance (ANOVA) using SPSS software (Version 22, SPSS Inc., Chicago, IL, USA), and significant differences ($p \leq 0.05$) were identified by Duncan's multiple range tests.

2.6. Overall optimization of the variables

The graphical and numerical optimization methods were carried out using Design-Expert 10 software based on the desirability function approach for maximum sensory acceptance.

2.7. Verification experiments and validation of the model equations

Verification tests (Three replicates) using the optimal amounts of independent variables were used to confirm the adequacy of the equations obtained. The last optimal beverage formulation was developed using the same process mentioned above **(Section 2.2)**. The difference between the optimum point of the beverage sample and its repeat in terms of the studied sensory characteristics was evaluated by sensory analysis using the same group of panelists **(Section 2.4)**. To assess the validity of the regression models, the actual data were compared with the values predicted by the models.

3. Results and discussion

3.1. Optimization of the sensory properties of the beverages

3.1.1. Analysis of regression models

Table 1 presents the experimental design, independent variables levels, and the experimental values for the responses. Data indicated that all the sensory properties are generally affected by the formulation of the beverages. Various mixture-process combined models were fitted to the experimental data to obtain the regression equations. The sequential p-value, model summary statistic (MSS), and lack of fit tests (LOF) were used to evaluate the model adequacy **(Table 2)**. Based on the significant sequential p-value ($p<0.01$), insignificant LOF ($p>0.05$), and the highest determination coefficient (R^2), adjusted R^2 (adj-R^2) and predicted R^2 (pre-R^2), amongst the models tested, the quadratic × linear model was chosen as the most appropriate model for taste, color, and overall acceptance. In contrast, the quadratic × mean and linear × linear models were selected for flavor and texture, respectively **(Table 2)**.

The quality of fit and adequacy of developed models was then checked and verified by ANOVA and regression analysis **(Table 3)**. The highly significant model ($P \leq 0.001$) and non-significant lack of fit ($P>0.05$) showed the adequacy of models developed for all responses so used to constitute the correlation between variables and responses. High R^2 values (> 80%) suggest that all the models have a good fit and could describe the effect of variables on the responses. Reasonable agreement between the pre-R^2 and the adj-R^2 for all responses indicated the adequate accuracy and general availability of the models. The coefficient of variation (C.V.) was less than 10% for all responses which provided better reproducibility and indicated a high degree of precision and a good deal of reliability of the experimental values. The adequate precision values (measures the signal/noise ratio) greater than 4 for all responses indicate adequate signals (Fakhri, Ghanbarzadeh, Dehghannya, & Dadashi, 2021). The results showed that the models made in this study are reasonable for analyzing the responses.

The linear and interaction effects of X_1 and X_2 were the significant parameters associated with taste, flavor, and color models ($P<0.01$) **(Table 3)**. The interaction term of X_1 and X_4 also has a significant effect on taste. For texture, in addition to the X_1X_4, the X_2X_4 was also the significant model term ($P<0.01$). The interaction term of X_1 and X_3 also showed a significant influence on the color properties. The X_1X_2, X_2X_3, and X_2X_4 were significant parameters for the overall acceptance model. Finally, the results indicated that the interaction effect of mixture components (X_1X_2) was the most significant factor affecting taste, flavor, color, and overall acceptance, and the interaction effect of LEO (X_1) and prebiotic fibers (X_4) was the most significant parameter affecting the texture. When comparing mixture components coefficients, LEO was found to be a more influential factor in flavor and color **(Table 3 and Eqs. 7 and 9)**. Only the interaction effect of the LEO and prebiotics showed a negative effect on texture (P<0.01) **(Eq. 8)**. The final fitted equations in terms of L_Pseudo components, coded process factor, and coded categoric factor with the significance coefficients were:

$$Y_1 = 5.99\ X_1 + 5.42\ X_2 + 6.46\ X_1X_2 + 0.17\ X_1X_4 \qquad (5)$$

$$Y_2 = 6.80\ X_1 + 6.29\ X_2 + 5.28\ X_1X_2 \qquad (6)$$

$$Y_3 = -1.01\ X_1X_4 + 0.53\ X_2X_4 \qquad (7)$$

$$Y_4 = 6.35\ X_1 + 4.39\ X_2 + 3.59\ X_1X_2 + 0.65\ X_1X_3 \qquad (8)$$

$$Y_5 = 6.58\ X_1X_2 + 0.49\ X_2X_3 + 0.40\ X_2X_4 \qquad (9)$$

where Y_1, Y_2, Y_3, Y_4, and Y_5 are taste, flavor, texture, color, and overall acceptance, respectively.

3.1.2. Analysis of response surface

3.1.2.1. The interaction effects of functional ingredients on taste

A beverage containing intermediate levels of LEO and ME (approximately 55: 45 LEO: ME) showed, in general, the highest taste acceptance at all levels of lutein and in the presence or absence of fibers (p<0.05) **(Figs. 1a and 2a)**. The convex surface indicated a quadratic effect of LEO/ME combinations on the taste. At all amounts of lutein, taste improved with increasing LEO in the mixture up to a certain extent; but decreased with further increase. The samples containing 100% ME in the mixture had a minimum taste acceptability score. Except at very low concentrations of LEO, Lutein levels had a non-significant positive linear effect on taste acceptance of both fiber-free and fiber-enriched samples. The fibers improved taste acceptance at all lutein levels **(Figs. 1a, 2a, and 3),** and the LEO and fibers had a positive interaction effect on taste acceptance ($p<0.05$) **(Figs. 1a and 2a, and Eq. 5)**. When the effects of both lutein levels and fibers were considered, it was concluded that at all proportions of LEO, increasing lutein levels and adding fibers resulted in higher taste acceptance.

3.1.2.2. The interaction effects of functional ingredients on flavor

At all levels of lutein, the LEO/ME combination affected the flavor in a second-order manner. Up to a critical LEO proportion in the mixture, flavor acceptance increased, while at higher ratios, a negative trend occurred (**Figs. 1b and 2b)**. Flavor acceptance values behaved almost identically at all lutein levels. Neither the lutein level nor the addition of fibers caused a change in flavor acceptance (**Figs. 1b, 2b, and 3),** and only the composition of the mixture components affected this parameter. At all amounts of lutein and in the presence or absence of fibers, high flavor acceptance values were obtained using 30-80% LEO in the mixture.

3.1.2.3. The interaction effects of functional ingredients on texture

LEO/ME combination and lutein concentration demonstrated a linear effect on texture acceptance **(Figs. 1c and 2c)**. At all concentrations of lutein and in the absence of fibers, texture showed the highest acceptability at the highest proportion of LEO in the mixture ($p<0.05$). However, its values decreased linearly with increasing LEO in fiber-enriched beverages. The optimum ratio of LEO in the mixture was sharply reduced with adding fibers. The ME proportion and fibers had a positive interaction effect on this property ($p<0.05$) **(Figs. 1c, 2c, 3, and Eq. 7)** and at all lutein levels, fortifying with fibers increased texture acceptance at the ME concentrations higher than approximately 70% v/v (LEO $\leq$ 30% v/v) **(Fig. 3)**; however, at lower concentrations a rapid decrease was observed. Considering only the effect of lutein, at all combinations of LEO/ME and in both presence and absence of fibers, as the lutein increased, the response displayed a non-significant increase. Maximum texture acceptance was obtained in the samples containing no fibers and high amounts of LEO and lutein.

3.1.2.4. The interaction effects of functional ingredients on color

The LEO/ME combination indicated a second-order effect on this parameter **(Figs. 1d and 2d)**. Color acceptance of the fiber-free beverage increased up to a certain proportion of LEO followed by a decrease with its further increase. In the same mixture combination in fiber-free and fiber-enriched beverages, except at very low concentrations of LEO (approximately<10%), lutein significantly improved color acceptance. The LEO and lutein had positive interaction and synergistic effect on the color acceptance up to the optimal LEO/ME combination ($p<0.05$) **(Figs. 1d and 2d, and Eq. 8)**. Fortifying with fibers increased the color acceptance at high LEO proportions **(Figs. 1d, 2d, and 3)**. When the effects of both lutein and fibers were considered, it was concluded that an increased in lutein level and adding fibers yielded higher color acceptance only at low proportions of ME (synergistic and positive interaction effect) (**Figs. 1d, 2d, and 3**). The maximum color acceptance was yielded at 2.5-3 mg/100 ml lutein using LEO concentrations >50% in the absence of fibers and >60% in the presence of fibers. The minimum acceptability score was attained at high amounts of ME.

3.1.2.5. The interaction effects of functional ingredients on overall acceptance (OA)

A strong curvature of the surfaces pointed out the high significance of the quadratic effect of the LEO/ME combination on OA **(Figs. 1e and 2e)**. OA increased when LEO in the mixture was raised to a certain extent, which was the optimum mixture combination. Beyond this value, a decrease in OA with LEO proportion was observed. At the same mixture components level, the OA score of fiber-free and fiber-enriched beverages increased with increasing lutein content ($p<0.05$). This increase was more significant in the samples containing only

ME. The optimum mixture combination changed slightly depending on the lutein concentration, and with the rise of the lutein content, the optimum mixture had more ME. Unlike very low ME proportions, at the same mixture components level, fortifying with fibers at all lutein levels increased the OA (**Figs. 1e, 2e, and 3**). The ME and lutein, and ME and fibers had positive interaction and thus had a synergistic effect on OA ($p<0.05$) **(Eq. 9)**. Considering both the impact of lutein levels and fibers, it was concluded that increasing the level of lutein and adding fibers lead to higher OA values except at very high LEO ratios (**Figs. 1e and 2e**). The highest value of OA was obtained in the fiber-enriched beverage, using 27-65% LEO and lutein concentrations ≥1.75 mg/100 ml. The minimum acceptability score was attained for the fiber-free sample containing 1 mg/100 ml lutein and 100% ME in the mixture.

Other studies also reported the acceptable and improving effects of inulin and polydextrose (Cassani, Tomadoni, Moreira, Ponce, & Agüero, 2017; Nagarajappa & Battula, 2017), lutein (Domingos et al., 2014), and ME (Imran et al., 2021) on the sensory properties of various functional/fortified foods and beverages. Few studies have been conducted on the acceptability of the addition of prebiotics to juices (Rebouças, Rodrigues, & Afonso, 2014).

The physicochemical and sensory characteristics of a beverage result from individual components and physical and chemical interactions in the beverage matrix. Inulin is colorless and has a bland and neutral taste and aroma, without any off-flavor or aftertaste, which mixes easily with other ingredients without modifying their flavors. Its use offers the advantage of not compromising on taste while delivering nutritionally enhanced products (Kalyani Nair, Kharb, & Thompkinson, 2010). Polydextrose is tasteless and has a low impact on flavor. Sometimes it helps to mask off-flavors that may come from some ingredients (Beristain et al., 2006). In addition, these two ingredients are multifunctional as sweetness enhancers, carbohydrate-based sugar, and fat replacers. Removing sugar from beverages decreases viscosity and thus reduces mouthfeel and body. Inulin and polydextrose can interact with other dissolved or dispersed molecular species in the hydrated state and provide different technological advantages, such as texturizing, thickening, emulsifying, stabilizing, or suspending. Therefore, their use can improve the mouthfeel of low-calorie beverages, and cover off-flavors in them (Furlán, Baracco, Lecot, Zaritzky, & Campderrós, 2017; Kasapoğlu et al., 2019).

The orange-red color and sour taste of lutein (3,3′-dihydroxy-α-carotene) **(Fig. 4)** caused a favorable change in the taste and appearance of the beverages and the LEO and ME imparted a savory strong taste and flavor to the beverages. Researchers reported that aromatic compounds in geraniol and vanillin were responsible for improving the quality characteristics of fiber-enriched strawberry juice (Cassani et al., 2016). There are detailed discussions of bioactive components and the volatile and key aroma-contributing molecules in citrus EOs, including LEO (González-Mas, Rambla, López-Gresa, Blázquez, & Granell, 2019). The LEO has a refreshing and sweetness-enhancing aroma. Besides the aldehydes and esters that are considered potent aroma contributors, germacrene A, B, C, and D (sesquiterpenes) **(Fig. 4)**, which are described as potent, warm, sweet, woody-spicy, geranium-like odor, are very important to the LEO aroma. Citropten (5,7-Dimethoxycourmarin) and herniarin (7-methoxycourmarin) **(Fig. 4)** are other compounds in the LEO that have been described as sweet lactone-like and vanilla-like (S. Liu et al., 2022). ME have known for its peculiar aroma and is a refreshing flavoring agent for foods and beverages. A wide spectrum of bioactive phytochemicals such as flavonoids, phenolics, lignans, stilbenes, and EOs are expected to be responsible for their aroma effects (Mahendran & Rahman, 2020).

3.1.3. Overall optimization of the variables

In the optimization, the point with the maximum desirability is selected. The desirability range is between zero (completely undesirable response) and 1 (perfectly desirable response).

For this purpose, the desired target for each response and factor was set to "within the range", "minimum", or "maximum", and given each response's importance and the study aim, a value of importance was selected for each response (**Table S1**). Finally, by applying the desirability function method, a combination of independent variables levels was obtained that had the maximum desirability. Six solutions for two combinations of categoric factor levels with desirability values corresponding to "very good desirability" (>80%) were suggested by the software. Optimization criteria, optimum points calculated using CD, and desirability values are shown in **Tables S1 and 4**.

3.1.4 Verification experiments and validation of the model equations

The experimental and predicted acceptance scores of sensory properties obtained at the optimum points and the error percentage between them are tabulated in **Table 4**. Only a small percentage error was observed between the experimental and predicted values and these values were reasonably close to each other. Thus, an acceptable percentage error (< 30%) (Fakhri, Ghanbarzadeh, Dehghannya, Abbasi, & Ranjbar, 2018) indicated the validity and adequacy of the proposed response surface models and optimization method.

3.2. Determination of physicochemical properties

The data for the TPC and TFC of the six optimized formulations were in the range of 37.88-44.22 (mg GAE/L) and 20.04-25.49 (mg QE/L), respectively **(Fig. 5)**. All samples contained the highest amount of lutein used in beverage formulation. Increasing the ME proportion generally raised the TPC and TFC values of the beverages. This increment was expected given that ME is a rich source of these compounds. Sample Opt 6, which had the highest concentration of peppermint extract (ME) in the mixture, showed the maximum amounts of TPC and TFC ($p<0.05$). The prebiotic fibers had an increasing effect on antioxidant capacity of the beverages ($p<0.05$). On the other hand, the beverages containing intermediate levels of LEO and ME generally showed a non-significantly higher antioxidant capacity than the beverage containing a high proportion of ME. The sample Opt 3 containing prebiotic fibers, 3 mg/100 ml lutein and 48.24% LEO, and 51.76% ME, had the highest DPPH• scavenging capacity (40.03 ± 0.80%), but this value was not significantly different from value obtained for sample Opt 6 (38.30 ± 0.39%) ($p<0.05$). Thus, sample Opt 6 showed the highest TPC and TFC content and high antioxidant activity. According to the sensory evaluations, bioactive compounds content, and antioxidant capacity, sample Opt 6, which had prebiotics, 60.22% v/v ME, and 39.78% v/v LEO in the mixture and 3 mg/100 ml lutein, was chosen as the final optimal formulation. The TSS, pH, total acidity, and AA content of the optimized formulations are shown in **Table S2**. As it is expected, vitamin C degrades at 70°C and this temperature is less than the one needed for the steam distillation process which is used for the extraction of herbal extracts. Therefore, the proportin of ME did not affect vitamin C content of the beverages. Antioxidant properties of the developed functional beverage can be attributed to several used ingredients including lime juice, lime peel essential oil, peppermint extract, lutein and prebiotics.

Citrus (*Citrus L.* from Rutaceae) fruits, including *C. aurantiifolia*, are a rich source of nutrients and bioactive compounds, including AA and other vitamins, citric acid, essential minerals, and phenolic compounds (flavonoids and phenolic acids). Citrus flavonoids are particular nutrients in citrus because they are rare in other types of fruits. Flavanones are the major group of citrus phenolic compounds, among which hesperidin is the primary flavanone, followed by eriocitrin. Phenolic compounds are one of the significant contributors to antioxidant activity in citrus juice. Eriocitrin, which is stable even after the heat treatment process, has been found to have more potent antioxidant activity than other citrus flavonoids. AA is another crucial antioxidant and an efficient scavenger of reactive oxygen species (ROS) in citrus fruit juices. Unlike eriocitrin, it is thermolabile and is highly sensitive to light as well as to various processing conditions (Bhat, Kamaruddin, Min-Tze, & Karim, 2011;

Guimarães et al., 2010). There are several studies on the antioxidant potential of lutein (Domingos et al., 2014) and prebiotics (Shang et al., 2018). LEO is a complex mixture of organic compounds divided into three classes: terpenes (75%), oxygenated complexes (12%), and sesquiterpenes (3%). Limonene (monoterpene) has been reported to constitute the highest amount of volatile compounds. Additionally, there are approximately 20% of non-volatile chemicals in the cold pressed LEO. Besides colorants and wax, they are mainly coumarin and psoralene derivatives (Guimarães et al., 2010; S. Liu et al., 2022). The DPPH scavenging activity of the LEO has been found to range from 10.65–66.44% in 0.08-3.46 mg/mL, with an IC50 value of 2.36 mg/ml (Lin et al., 2019). The presence of terpenes, flavonoids, carotenes, and coumarins in citrus EOs is responsible for their strong antioxidative activities (Dosoky & Setzer, 2018).

ME is rich in phenolic and flavonoid antioxidants and contains lower amounts of vitamins and terpenes. ME contains 0.75 g/L of different classes of polyphenolic compounds, mostly flavonoids (530 g/kg), phenolic acids (420 g/kg), lignans, and stilbenes (25 g/kg). The most abundant phenolics are eriocitrin, rosmarinic acid, eriodictyol-glycopyranosyl-rhamnopyranoside, and luteolin 7-O-rutinoside (Cam et al., 2020). Antioxidant activity of *M. Piperita* extract and the direct positive correlation between its TPC and DPPH radical-scavenging have been reported (Kapp et al., 2013; Mahendran & Rahman, 2020; Oh, Jo, Cho, Kim, & Han, 2013). The considerable variation in the results of the antioxidant potential of ME is due to the different antioxidant assay methods and ways of its expression. Phenolic acids (for example, caffeic acids and rosmarinic), flavones (for example, luteolin glycosides), and flavanones (for example, eriocitrin glycosides) are probably the major antioxidants, and vitamins (for instance carotenoids and ascorbic acid) are minor contributors to the overall antioxidant potential (Riachi & De Maria, 2015).

Our results revealed that the developed functional beverage is an acceptable source of bioaccessible health-related compounds. The antioxidant phytochemicals of developed beverage (phenolic and terpenes, AA, prebiotics, lutein, etc.) have the potential for slowing or retarding and inhibiting the organic matter oxidation promoted by ROS and preventing the biological structures damage and development of oxidative stress- and inflammation-related diseases such as diabetic and cardiovascular disorders as well as some types of cancer (Middleton, Kandaswami, & Theoharides, 2000). Evidence suggests an inverse association between dietary fiber ingestion and inflammation and certain types of cancer, such as colon and breast (Dahl & Stewart, 2015). Inulin is capable of scavenging ROS, which can help to alleviate oxidative stress, reduce lipid peroxidation in the stomach (Shang et al., 2018) and protect against hepatotoxicity (Corrêa-Ferreira et al., 2017). Polydextrose is metabolized independently of inulin. It has health effects due to its laxative action and control of glucose and cholesterol levels in the blood. It helps to modulate appetite and satiety, causing a reduction in total caloric input and increasing antioxidant, antihypertensive, and antidiabetic activity (Ibarra et al., 2017; Wang et al., 2019).

4. Conclusions

New low-calorie fiber-enriched lime juice-based functional beverages containing prebiotics inulin/polydextrose, lutein, LEO, and ME were developed, and their sensory properties were optimized using the CD. The synergistic improving effects of these potential active components on the sensory properties resulted in beverages that were well preferred by consumers. ANOVA showed a good fit of the developed regression equations to the data. Among the six optimized beverage formulations with "very good desirability" (>80%), the beverage with the highest amount of bioactive compounds content and antioxidant capacity was selected as the last optimal beverage. This beverage was the one with inulin/polydextrose at 20% DRV, 3 mg/100 ml lutein, and 1.99:3.01 (ml: ml) LEO: ME mixture combination, which contained 44.22 mg GAE/L of TPC, 25.49 mg QE/L of TFC, and exhibited 38.30% $DPPH^{\bullet}$ scavenging activity. The validity and adequacy of the proposed models were verified experimentally. The newly designed beverage with good organoleptic properties has the potential to promote health for people with diabetes and hypertension and meets the consumer demand for nutritious and healthy beverages and therefore has good potential for commercialization.

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Declaration of competing interest

None.

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Figures captions

Fig. 1. The change in sensory acceptance based on different LEO and ME combinations under different lutein levels and in the absence of prebiotic fibers. *(in color)*

Fig. 2. The change in sensory acceptance based on different LEO and ME combinations under different lutein levels and in the presence of prebiotic fibers. *(in color)*

Fig. 3. The effect of the presence and absence of prebiotics on the sensory acceptance of beverages at specified lutein content acquired using a constant LEO/ME combination which is mentioned in the figures. *(in color)*

Fig. 4. Structures of germacrene A, B, C, and D, citropten, herniarin, and lutein. *(black and white)*

Fig. 5. Bioactive compounds content and in vitro antioxidant properties (phenolic and flavonoid content and the DPPH$^{\bullet}$ scavenging activity) of the optimized formulations (Different letters indicate statistically significant differences ($p \leq 0.05$)). *(in color)*

Table 1. Design of experiment for optimization of functional beverages formulation and their sensory attributes, levels of independent variables, and values of the responses

Design point	Components		Factors		Responses[e]				
	Mixture components		Numeric factor	Categoric factor	Taste	Flavor	Texture	Color	Overall acceptance
	X_1: LEO solution (0.1 v/v) %[a] (ml)[c]	X_2: ME %[b] (ml)[c]	X_3: Lu (mg/100 ml)	X_4: I/P mixture[d]					
1	50.00 (2.50)	50.00 (2.50)	3.00	Level 1	7.3 ± 1.5	7.9 ± 1.4	7.2 ± 1.4	7.1 ± 1.7	8.3 ± 1.2
2	50.00 (2.50)	50.00 (2.50)	3.00	Level 2	7.6 ± 1.2	7.7 ± 1.6	7.2 ± 0.7	6.6 ± 1.4	8.3 ± 1.2
3	0.00 (0.00)	100.00 (5.00)	3.00	Level 1	5.3 ± 1.6	6.4 ± 0.9	6.5 ± 2.1	4.3 ± 1.2	6.3 ± 1.7
4	100.00 (5.00)	0.00 (0.00)	3.00	Level 1	6.0 ± 1.5	6.9 ± 1.0	8.1 ± 1.7	7.1 ± 1.7	6.6 ± 1.1
5	75.00 (3.75)	25.00 (1.25)	2.00	Level 1	6.5 ± 0.9	7.6 ± 1.2	7.7 ± 1.2	6.8 ± 1.4	7.0 ± 0.9
6	100.00 (5.00)	0.00 (0.00)	1.00	Level 2	6.0 ± 0.7	6.8 ± 1.3	6.0 ± 1.6	5.9 ± 1.1	5.9 ± 0.9
7	0.00 (0.00)	100.00 (5.00)	1.00	Level 1	5.3 ± 0.8	6.5 ± 1.7	6.2 ± 1.3	4.5 ± 1.6	5.3 ± 1.2
8	50.00 (2.50)	50.00 (2.50)	3.00	Level 1	7.2 ± 1.1	7.8 ± 1.8	7.0 ± 1.0	6.9 ± 2.0	7.7 ± 1.3
9	50.00 (2.50)	50.00 (2.50)	1.00	Level 1	7.0 ± 1.3	7.9 ± 1.5	7.1 ± 1.3	5.8 ± 0.8	7.6 ± 1.2
10	50.00 (2.50)	50.00 (2.50)	1.00	Level 1	6.9 ± 1.9	7.8 ± 1.4	6.9 ± 2.3	5.6 ± 1.2	7.7 ± 1.7
11	100.00 (5.00)	0.00 (0.00)	3.00	Level 2	6.3 ± 1.1	6.6 ± 2.1	5.9 ± 1.8	6.8 ± 1.4	6.5 ± 1.6
12	0.00 (0.00)	100.00 (5.00)	1.00	Level 2	5.6 ± 1.5	6.1 ±1.8	7.5 ± 1.9	4.5 ± 1.7	6.3 ± 1.8
13	100.00 (5.00)	0.00 (0.00)	1.00	Level 1	5.8 ± 1.2	7.0 ± 1.5	8.0 ± 1.7	5.4 ± 1.2	6.2 ± 1.0
14	25.00 (1.25)	75.00 (3.75)	2.00	Level 2	7.2 ± 1.6	7.9 ± 1.4	6.4 ± 0.7	5.7 ± 1.5	7.7 ± 1.3
15	100.00 (5.00)	0.00 (0.00)	2.00	Level 2	6.3 ± 1.2	6.8 ± 1.9	5.9 ± 1.7	6.7 ± 1.3	6.3 ± 0.9
16	0.00 (0.00)	100.00 (5.00)	2.00	Level 1	5.3 ± 1.6	6.5 ± 0.8	6.3 ± 0.7	4.4 ± 1.3	5.8 ± 1.2
17	0.00 (0.00)	100.00 (5.00)	1.00	Level 2	5.4 ± 1.3	5.9 ± 0.5	7.3 ± 0.7	4.3 ± 1.3	6.0 ± 1.1

[a] This value represents the concentration distribution of the LEO concerning the total LEO+ME amount.
[b] This value represents the concentration distribution of the ME concerning the total LEO+ME amount.
[c] Amount added to 100 ml of beverage formulation.
[d] The levels of categoric factor indicate either absence (Level 1) or presence (Level 2) of I/P.
[e] Values are presented as mean ± SD, n = 3.

Table 2. Combined model mixture process fit summary and analysis of variance (partial sum of squares)

Source	Suggested models		Sequential p-value		Partial sum of squares		Lack of fit (LOF)	Model summary statistics (MSS)		
	Mix order	Process order	Mix	Process	Sum of squares	Mean square		R^2	Adj-R^2	Pred-R^2
Taste	Quadratic	Linear	< 0.001**	0.011*	10.04	1.26	0.27	0.98	0.96	0.92
Flavor	Quadratic	Mean	< 0.001**	-	7.03	3.52	0.11	0.91	0.90	0.87
Texture	Linear	Linear	< 0.001**	< 0.001**	7.07	1.41	0.13	0.90	0.86	0.80
Color	Quadratic	Linear	0.001**	0.003**	17.71	2.21	0.12	0.97	0.95	0.84
Overall acceptance	Quadratic	Linear	< 0.001**	0.033*	12.90	1.61	0.64	0.97	0.94	0.90

*, ** Significant at p-level<0.05 and p-level<0.01, respectively.

Table 3. Regression coefficients for the responses variables and analysis of variance of the regression models

Source	Taste			Flavor			Texture			Color			Overall acceptance		
	Reg. Co.	F-value	p-value	Reg. Co.	F-value	p-value	Reg. Co.	F-value	p-value	Reg. Co.	F-value	p-value	Reg. Co.	F-value	p-value
X_1	5.99	-	-	6.80	-	-	6.95	-	-	6.35	-	-	6.27	-	-
X_2	5.42	-	-	6.29	-	-	6.83	-	-	4.39	-	-	6.21	-	-
X_1X_2	6.46	235.68	<0.001**	5.28	125.28	<0.001**	-	-	-	3.59	31.63	0.001**	6.58	120.76	<0.001**
X_1X_3	0.14	2.92	0.126	-	-	-	0.05	0.19	0.672	0.65	27.87	0.001**	0.25	4.96	0.057
X_1X_4	0.17	5.44	0.048*	-	-	-	-1.01	93.16	<0.001**	0.09	0.66	0.440	-0.04	0.15	0.712
X_2X_3	0.05	0.21	0.660	-	-	-	0.10	0.53	0.482	-0.04	0.05	0.835	0.49	9.57	0.015*
X_2X_4	0.15	2.66	0.142	-	-	-	0.53	20.78	0.001**	0.01	0.01	0.941	0.40	9.56	0.015*
$X_1X_2X_3$	0.18	0.20	0.670	-	-	-	-	-	-	1.30	4.47	0.067	-0.79	1.87	0.209
$X_1X_2X_4$	0.57	1.82	0.214	-	-	-	-	-	-	-0.61	0.91	0.369	-0.10	0.50	0.500
Model	-	48.42	<0.001**	-	69.42	<0.001**	-	20.13	<0.001**	-	37.08	0.001**	-	30.77	<0.001**
Linear mixture	-	36.22	0.001**	-	13.55	0.003**	-	0.46	0.513	-	172.68	<0.001**	-	3.98	0.081
LOF	-	2.21	0.273	-	4.88	0.109	-	4.26	0.130	-	4.63	0.119	-	0.75	0.636
R^2	0.98	-	-	0.91	-	-	0.90	-	-	0.97	-	-	0.97	-	-
R^2_{adj}	0.96	-	-	0.90	-	-	0.86	-	-	0.95	-	-	0.94	-	-
R^2_{pred}	0.92	-	-	0.87	-	-	0.80	-	-	0.84	-	-	0.90	-	-
Adeq. Precision	21.66	-	-	16.67	-	-	13.52	-	-	15.44	-	-	17.36	-	-
C.V. %	2.57	-	-	3.19	-	-	3.85	-	-	4.23	-	-	3.38	-	-
Std. Dev.	0.16	-	-	0.23	-	-	0.26	-	-	0.24	-	-	0.23	-	-
PRESS	0.85	-	-	1.02	-	-	1.53	-	-	2.91	-	-	1.29	-	-

*, ** Significant at p-level<0.05 and p-level<0.01, respectively.

Table 4. Optimum points calculated using CD, desirability values, the actual and theoretical acceptance scores of responses obtained at the optimum points, and the percentage errors between these values

Optimized sample	**Optimum formula**				**Desirability**	**Responses at optimum point**					
	Mixture Components		**Numeric factor**	**Categoric Factor**							
	LEO solution (0.1 v/v) (%)	**ME (%)**	**Lutein (mg/100 mL)**	**I/P mixture**			**Taste**	**Odor**	**Texture**	**Color**	**Overall acceptance**
						TV^{a}	7.17	7.87	7.36	7.20	7.88
Opt 1	59.71	40.29	3.00	Level 1	0.87	***AV***^{b}	6.7 ± 1.4	6.9 ± 1.3	6.9 ± 1.1	7.4 ± 1.5	7.1 ± 1.6
						PE^{c} ***(%)***	-7.3	-14.6	-6.7	3.2	-10.4
						TV	7.18	7.87	7.34	7.17	7.89
Opt 2	58.01	41.99	3.00	Level 1	0.87	***AV***	6.5 ± 1.7	7.0 ± 1.4	7.6 ± 1.2	6.9 ± 1.1	6.9 ± 1.7
						PE (%)	-10.4	-12.8	2.8	-4.4	-13.8
						TV	7.75	7.86	6.75	6.75	8.22
Opt 3	48.24	51.76	3.00	Level 2	0.82	***AV***	6.5 ± 1.2	6.8 ± 1.6	7.1 ± 1.1	6.0 ± 1.5	6.6 ± 1.2
						PE (%)	-18.6	-15.3	4.3	-12.4	-24.0
						TV	7.76	7.87	6.72	6.79	8.21
Opt 4	50.00	50.00	3.00	Level 2	0.82	***AV***	6.9 ± 1.0	7.4 ± 1.4	6.3 ± 1.2	5.8 ± 1.6	7.2 ± 1.2
						PE (%)	-13.3	-6.8	-6.1	-17.8	-14.1
						TV	7.72	7.84	6.78	6.68	8.23
Opt 5	45.89	54.11	3.00	Level 2	0.82	***AV***	7.9 ± 0.7	7.5 ± 1.2	7.5 ± 1.3	7.0 ± 1.2	8.2 ± 0.6
						PE (%)	2.0	-4.7	9.6	4.9	-0.2
						TV	7.62	7.76	6.87	6.47	8.22
Opt 6	39.78	60.22	3.00	Level 2	0.81	***AV***	6.5 ± 1.4	7.1 ± 1.3	7.5 ± 1.1	6.7 ± 1.5	6.9 ± 1.5
						PE (%)	-16.9	-10.1	8.4	3.2	-19.1

Data are presented as mean ± SD, n = 3.
[a] Theoretical value
[b] Actual value (Mean ± S.D)
[c] Percentage error

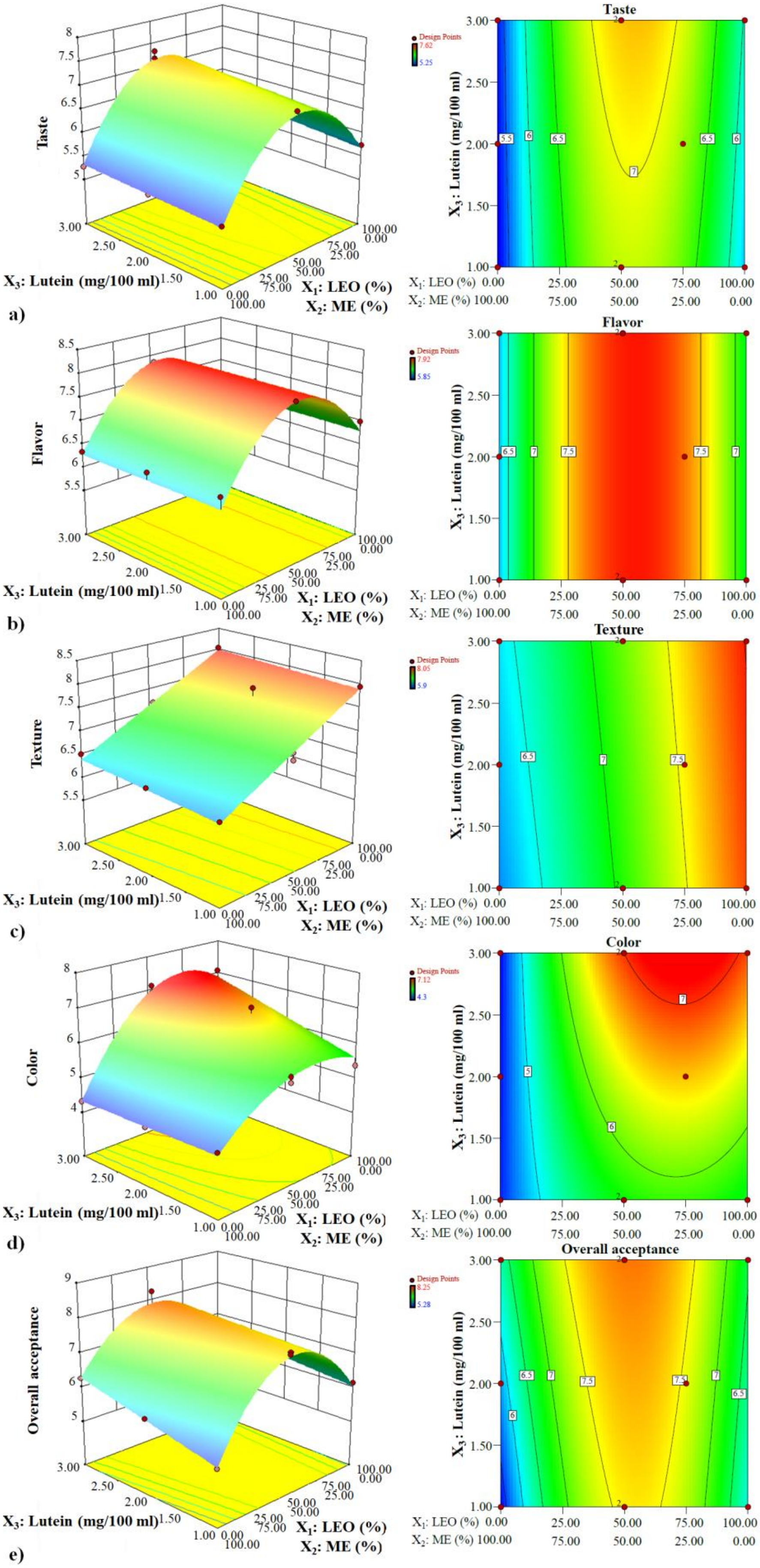

Taste
Flavor
Texture
Color
Overall acceptance
Design Points
X3: Lutein (mg/100 ml)
X1: LEO (%)
X2: ME (%)
a)
b)
c)
d)
e)

Fig. 1.

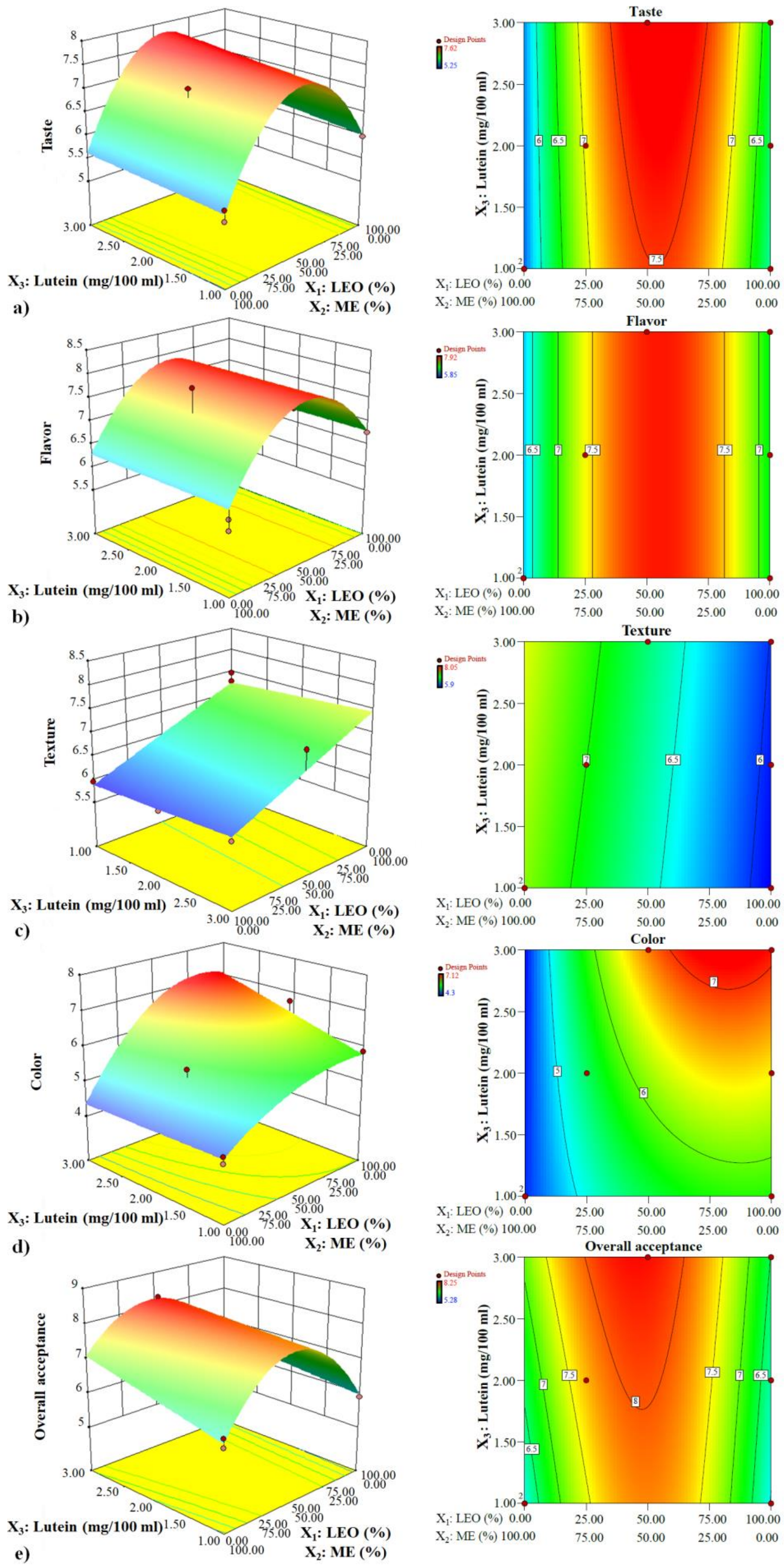
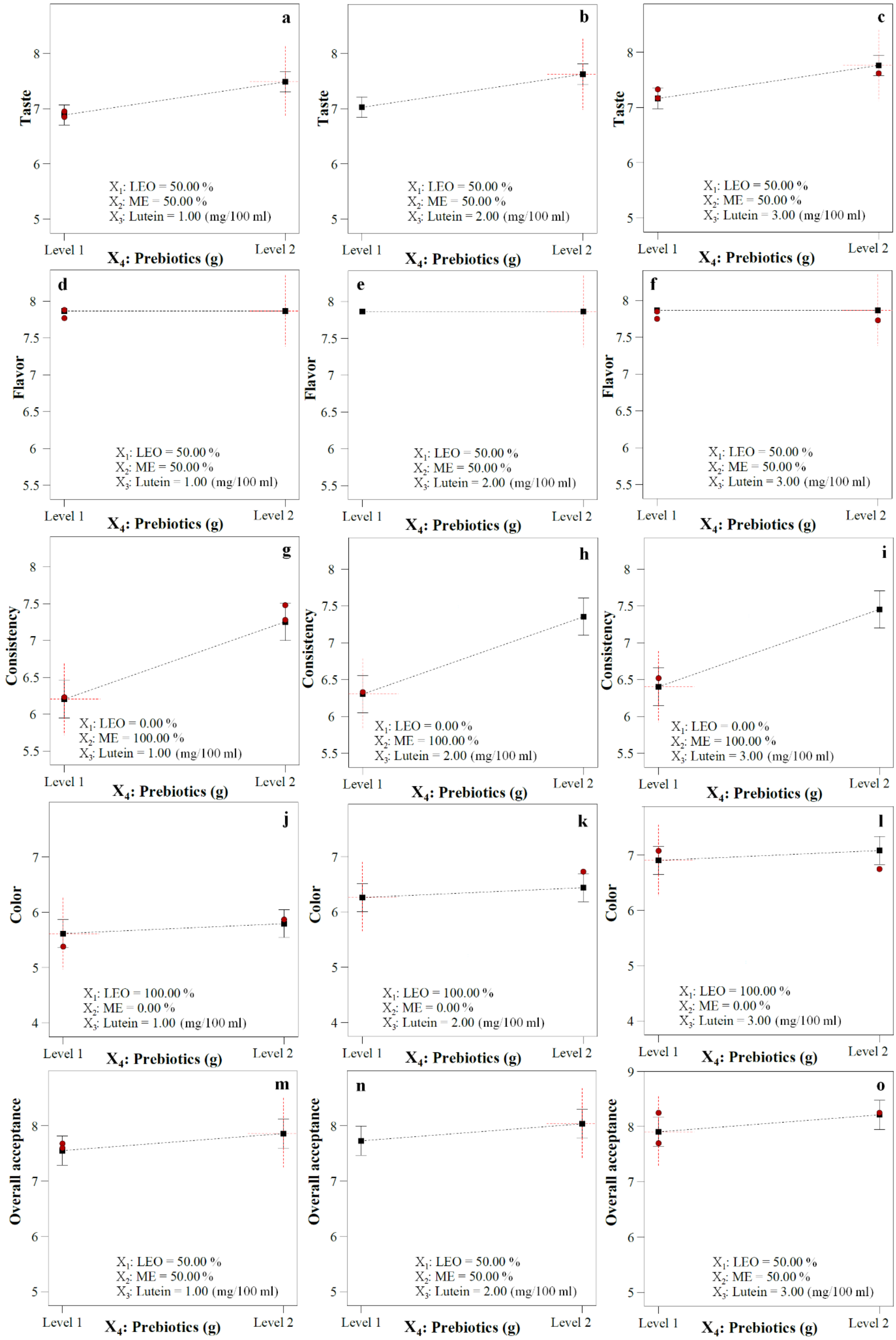

Taste
Flavor
Texture
Color
Overall acceptance
Design Points
X3: Lutein (mg/100 ml)
X1: LEO (%)
X2: ME (%)
a)
b)
c)
d)
e)

Fig. 2.



a
Taste
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 1.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
b
Taste
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 2.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
c
Taste
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 3.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
d
Flavor
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 1.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
e
Flavor
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 2.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
f
Flavor
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 3.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
g
Consistency
X_1: LEO = 0.00 %
X_2: ME = 100.00 %
X_3: Lutein = 1.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
h
Consistency
X_1: LEO = 0.00 %
X_2: ME = 100.00 %
X_3: Lutein = 2.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
i
Consistency
X_1: LEO = 0.00 %
X_2: ME = 100.00 %
X_3: Lutein = 3.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
j
Color
X_1: LEO = 100.00 %
X_2: ME = 0.00 %
X_3: Lutein = 1.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
k
Color
X_1: LEO = 100.00 %
X_2: ME = 0.00 %
X_3: Lutein = 2.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
l
Color
X_1: LEO = 100.00 %
X_2: ME = 0.00 %
X_3: Lutein = 3.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
m
Overall acceptance
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 1.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
n
Overall acceptance
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 2.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)
o
Overall acceptance
X_1: LEO = 50.00 %
X_2: ME = 50.00 %
X_3: Lutein = 3.00 (mg/100 ml)
Level 1
Level 2
X_4: Prebiotics (g)

Fig. 3.

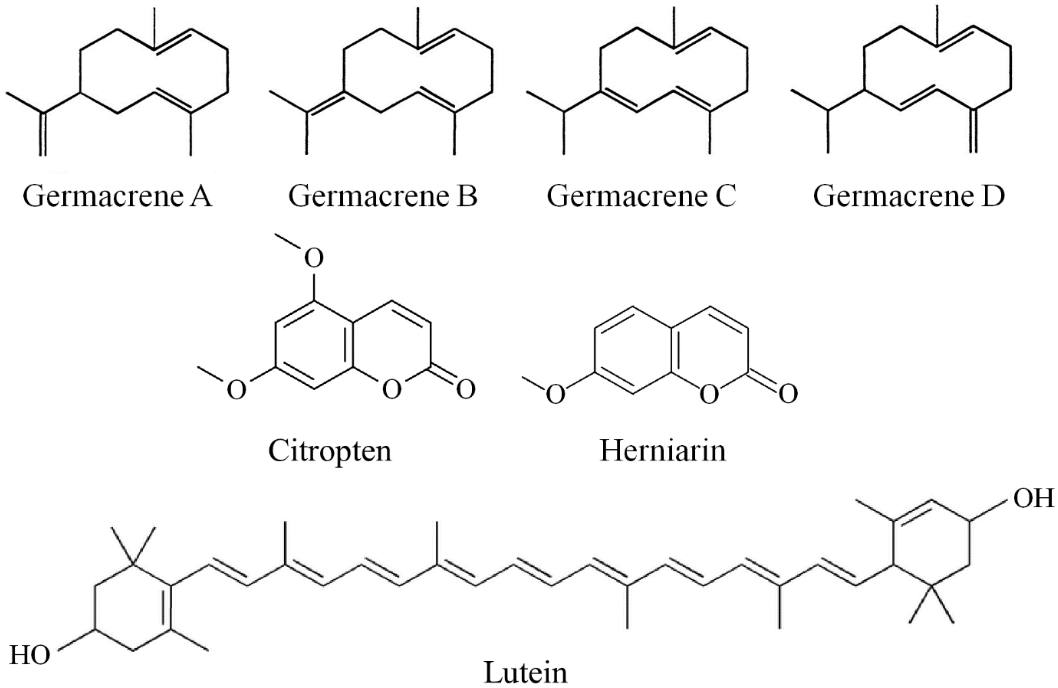
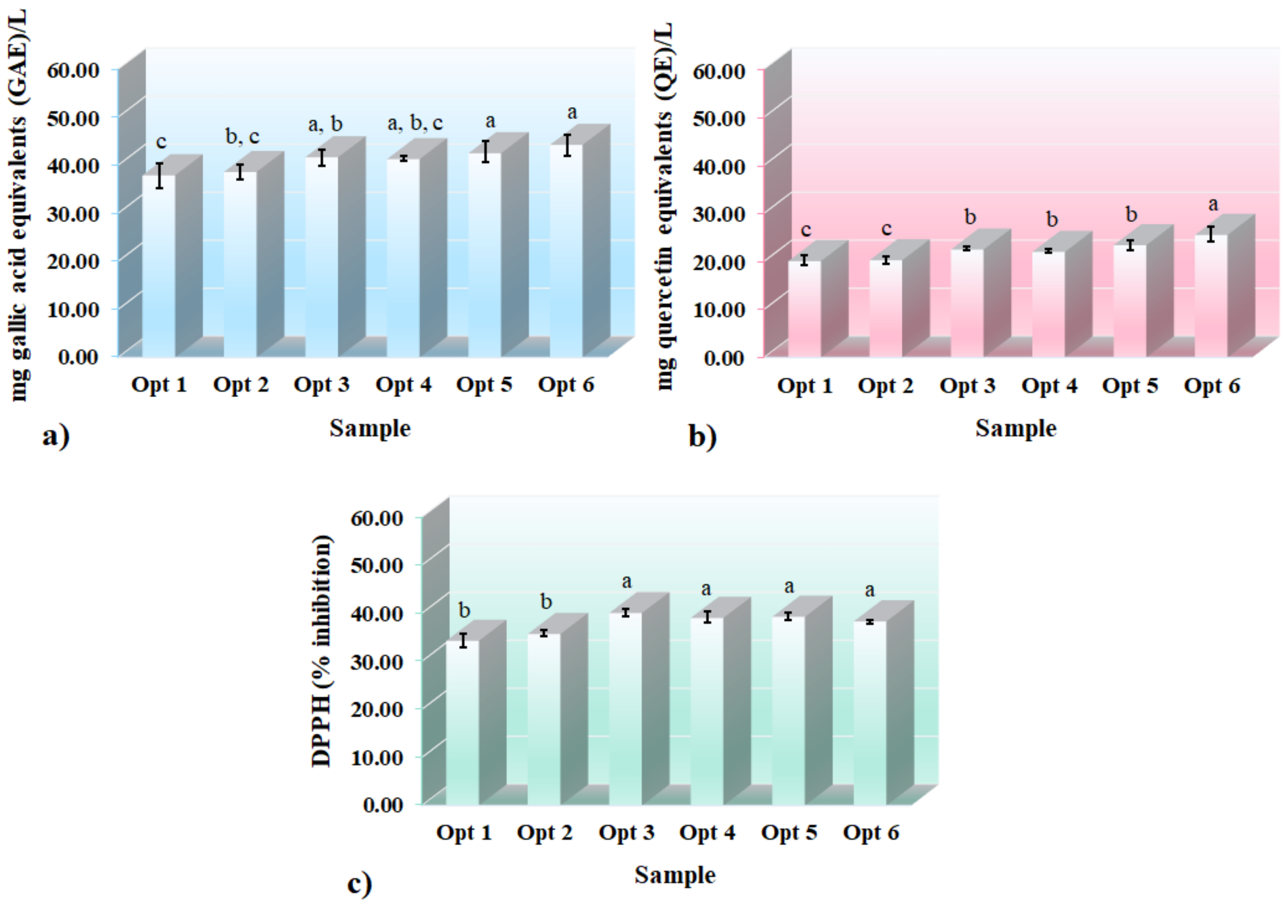

Germacrene A
Germacrene B
Germacrene C
Germacrene D
O
O
O
O
Citropten
O
O
O
Herniarin
OH
HO
Lutein

Fig. 4.



mg gallic acid equivalents (GAE)/L
60.00
50.00
40.00
30.00
20.00
10.00
0.00
c
b, c
a, b
a, b, c
a
a
Opt 1
Opt 2
Opt 3
Opt 4
Opt 5
Opt 6
Sample
a)
mg quercetin equivalents (QE)/L
60.00
50.00
40.00
30.00
20.00
10.00
0.00
c
c
b
b
b
a
Opt 1
Opt 2
Opt 3
Opt 4
Opt 5
Opt 6
Sample
b)
DPPH (% inhibition)
60.00
50.00
40.00
30.00
20.00
10.00
0.00
b
b
a
a
a
a
Opt 1
Opt 2
Opt 3
Opt 4
Opt 5
Opt 6
Sample
c)

688

689 **Fig. 5.**

Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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