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Production of Encapsulated Hydrogel Beads and Sugar-Free Beverage from Gilaburu Fruit Rich in Antioxidants, Antidiabetic Bioactives, and its Microwave-Assisted Extraction Optimization

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HIGHLIGHTS

- Gilaburu fruit functional enriched beverage was formulated for the first time.
- MAE conditions of fruit were optimized with RSM for the first time.
- The model was adequate for the accurate predictions of experiment results.
- GF, eGF, and sugar-free FB have promising antioxidant and antidiabetic activities due to their rich bioactive and fortified compounds.

Abstract: The study aimed to optimize microwave-assisted extraction (MAE) of phenolics from gilaburu fruit (GF) with the response surface method (RSM). GF was used to produce two functional products: The first one was encapsulated hydrogel beads GF (eGF) using ionic gelation and chitosan system, and the second one was a sugar-free functional beverage (FB) enriched with black carrot, riboflavin, and ascorbic acid. The optimal extraction conditions were determined to be 60 °C for temperature, 5 minutes for time, and 4.18 g/100 mL for solid/solvent ratio based on the highest total phenolic content (TPC) and extraction yield. The model was found to be sufficient for the successful prediction of experimental results. Chlorogenic acid was a major compound of GF, eGF, and sugar-free FB. The inhibition of α-glucosidase activity (%) for GF, eGF, and sugar-free FB was detected as 54, 92, and 77, respectively. These findings revealed that utilizing MAE may shorten the extraction of GF phenolics with low energy and maximum efficiency. Furthermore, GF, eGF, and

sugar-free FB have promising antioxidant and antidiabetic activities due to their rich bioactive and fortified compounds. They may be regarded excellent sources of compounds for the functional food industry. Besides, they can be used to provide health benefits to diabetic patients and future consumers.

Keywords: Gilaburu fruit; Response surface method; Microwave assisted extraction; Phenolic; Antidiabetic; Antioxidant

INTRODUCTION

Gilaburu (*Viburnum opulus* L.) is a red colored and chickpea-sized dietary fruit, has a wide distribution worldwide, and contains bioactive compounds that are pharmacologically important [1, 2]. The researchers reported its effect of antimicrobial, antimutagenic, antidiabetic, and antitumor due to its bioactive compounds [3, 4, 5, 6]. Therefore, a depth study of the extraction processes is required to extract its bioactive components. Especially in recent years, the demand for the use of green extraction techniques has been increasing due to some disadvantages of traditional extraction methods such as time consumption, environmental damage, and high cost [7]. MAE as a green technique is used extensively in the extraction of protein, carbohydrates, essential oils, and phenolics from foods due to its advantages such as better preservation of nutritional components, low cost, and shorter processing time compared to other extraction methods [8]. The response surface method (RSM), on the other hand, is a statistical approach widely used in the extraction process to reduce the number of trials, optimize the response affected by factors, and find the optimum extraction process parameters. To the best of our knowledge, this research is the first report regarding the optimization of MAE conditions with the RSM and Box-Behnken experimental design to extract bioactive components like phenolic substances from GF.

In this study, GF was used to produce both hydrogel beads and drink mix as functional products for extended shelf-life. Encapsulation is a method that allows different bioactive chemicals to be trapped in capsules to protect them from environmental impacts while also allowing them to release their contents under specified conditions [9, 10, 11]. This study is the initial research about the encapsulation of GF phenolics by ionic gelation method using sodium alginate, $CaCl_2$, and chitosan to increase the stability and bioavailability of eGF phenolics. Many studies have demonstrated that black carrot has health-promoting properties due to its phenolics and carotenoids content such as antidiabetic, anticarcinogenic, and antioxidant activity [12,13, 14, 15]. In addition, it is a great source of antioxidants including phenolics, anthocyanins, vitamins C and E, and phytoalexins [16]. The presence of rich bioactive compounds in GF and its enriched formulation with black carrot, ascorbic acid, and riboflavin content of the sugar-free FB may have a role as an antidiabetic activity due to its inhibition effect on the digestive enzymes (α-glucosidase). In addition, this sugar-free FB will decrease the carbohydrate intake due to no-added sugar formulation.

The first goal of this research was an optimization of gilaburu fruit water extraction process conditions by using MAE with RSM. The second was to develop a complete formulation for an antidiabetic functional drink that consisted of an optimized water extract of GF together with additional black carrot and hydrophilic vitamins (riboflavin and ascorbic acid) and without added sugar. The third was to encapsulate the GF for longer shelf life and controlled release. Also, the physicochemical properties and functional constituents of the extract, encapsulated extract and drink mix were analyzed for their antioxidant and antidiabetic effects.

MATERIALS AND METHODS

Gilaburu fruit

Gilaburu was obtained from Kayseri, Turkey at the maturity stage. Preparation process of the fruits included phases of cleaning, sifting, and drying in the oven (Eksis, TK-LAB, Turkey) at 70 ^{0}C until achieving the moisture contents of 4.5 %. Following that the dried fruits were ground to obtain fine powder (Waring Commercial Laboratory Blender, USA). After that samples were sifted and passed through a sieve (Kocintok, Turkey) with 500 µm mesh and was tightly packed in polyethylene bags to store at −45 °C for further experiments.

Experimental design: Optimization of microwave extraction (MAE) parameters

Response surface methodology (RSM) and Box-Behnken experimental design was used for determining optimal MAE (Milipore, Italy) conditions of dried GF phenolics. In this study water was used as solvent. Preliminary experiments were conducted to determine the independent variables based on the literature review. Therefore, appropriate ranges of independent process variables, namely temperature (°C), extraction

time (min), microwave power (W) and solid/solvent ratio (g/100 mL) were determined based on the response (TPC and extraction efficiency). Solid/solvent ratio ranged from 3-12 g/100 mL, microwave power varied between 200 to 500 W whereas time and temperature were fixed 7.5 min and 45°C, respectively. Based on the results of preliminary experiments microwave power fixed to 500 W and independent variables were determined as temperature (X1), extraction time (X2), and solid/solvent ratio (X3). A Box-Behnken design was applied with 3 center points and consists of 15 experiments, and the range of independent variable and their levels was presented in Table 1.

All runs were randomized in order and two experimental responses are presented (TPC and extraction efficiency [17, 18]. Minitab Statistical Software (Minitab 20.0) was used to perform RSM, and model adequacy and regression tests were evaluated by R^2 and adjusted R^2. To obtain the regression coefficients (β) experimental data were fitted to second-order polynomial model. To acquire the regression coefficients, the experimental data were fitted to a second-order polynomial model. In the RSM analysis, the extended second-order polynomial model $Z=\beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$ was used and Z is the dependent variable, X is the independent variable and constant coefficient is defined as β_0 for intercept, β_i for linear, β_{ii} for quadratic and β_{ij} for two factors interaction coefficient.

As a positive control maceration, a traditional extraction technique was used to GF phenolics at the optimized solid/solvent ratio (4.18 g gilaburu/100 ml water) for 12 h at room temperature and its TPC and extraction efficiency were measured as response. Three replicates were performed in each extraction.

Determination of extraction efficiency and TPC

The extracts obtained from Box-Behnken experimental design were centrifuged (2,6- Sigma, Germany). Then 10 mL of the supernatant were dried at 105 °C in incubators until the weight became constant. The efficiency of extraction was expressed as g extract per 100 g dried fruit.

The TPC was determined by the Folin & Ciocalteau colorimetric method (T70+UV/VIS spectrophotometer, PG Instruments, UK) [19]. TPC were expressed as mg of GAE per g of dry matter (DM). In addition, the TPC of eGF and sugar-free FB was detected using the above procedure. Three replicates were performed for each sample.

Development of the sugar free functional beverage (sugar-free FB) formulation

There were two types of functional drinks prepared: the control drink and the test drink. The test drink's basic formula was 4.18 g optimized GF water extract, 1.2 g black carrot, 0.01 g riboflavin, and 0.036 g ascorbic acid for 100 ml. The control sample contained simply infusing dried gilaburu fruit (4.18 g) without enrichment. All drink formulation ingredients were weighed and poured into a beaker with distilled water or eGF, then blended until completely dissolved at room temperature for 5-8 minutes. The prepared drink samples were refrigerated for one day.

Encapsulation of GF with chitosan coated calcium-alginat microcapsules (eGF)

External ionic gelation through calcium alginate matrix with a peristaltic pump was used for hydrogel beads (Ismatec, USA). The method of Najafi- Soulari [20] was modified slightly to prepare alginate hydrogel beads. Firstly, into the chitosan and calcium chloride mixture (a volume ratio of 1:2), alginate and GF mixture was dripped. The distance between alginate drops and chitosan with calcium chloride mixture was 10 cm. Secondly, the hydrogel beads were maintained in the mixture for 30 min under magnetic stirring. Following, hydrogel beads were filtered and washed with distilled water to stop the gelation process. Finally, the hydrogel beads (with/without extract) were weighted to 1 g and then mixed with 10 mL of sodium citrate solution (5% w/v) at 37 °C in a magnetic stirrer until the capsules dissolved (22 h) for further analysis.

Determination of encapsulation efficiency and moisture content of eGF

Encapsulation efficiency of hydrogel beads were determined by the amount of bioactive compound effectively retained in beads [20] and calculated by following equation: EE % = polyphenol content of the encapsulated samples/ polyphenol content of the extract. Through drying at 80 °C for 24 h, the moisture content was obtained gravimetrically [20]. The moisture content of hydrogel beads was expressed as percentage by following equation:

$$\text{Moisture Content}(\%) = ((\text{Wet weights} - \text{Dry weights}) / \text{Wet weights}) \times 100.$$

Chemical composition and functional properties of GF, sugar-free FB and eGF

DPPH radical scavenging

The DPPH activity was analyzed according to method developed by Dorman [21]. The mixture of 50 µL extract, 450 µL Tris-HCl buffer (50 mM, pH:7.4) and 1.00 mL of fresh methanolic solution DPPH (0.10 mM) was shaken and kept in dark condition at room temperature for 30 min. The % DPPH was calculated by the following equation after samples' absorbance was read at 517 nm: Inhibition % (DPPH)= [($Abs_{Control}$ – Abs_{Sample})/ $Abs_{Control}$] *100.

Abs_{Sample} indicates the absorbance of the DPPH in the presence of the sample and $Abs_{Control}$ shows the absorbance of DPPH solution in methanol without extracts. The results expressed as mg Trolox Equivalent per 100 g samples (GF, eGF and sugar-free FB).

Antidiabetic activity

Antidiabetic activities of the GF, eGF and sugar-free FB were analyzed according to method developed by Demircan [22]. As a control sample, 18 mM of sucrose (200 µl) and 15 mg/ml of acetone rat intestinal powder (200 µl) were mixed with 100 µl of sodium phosphate buffer (10 mM pH 7.0). To analyze the antidiabetic activity of extract and hydrogel beads, as a test sample 100 µl of sodium phosphate buffer were changed with 100 µl of extract/hydrogel beads solution. After that, the samples were incubated at 37 ºC for 15 min. To stop enzyme activity 750 µL of acetone were added to mixtures and immediately vortexed for 10 seconds and hold in ice to cool down at room temperature. The acetone was removed under nitrogen gas and centrifugation was repeated. Finally, the supernatant was filtered, and the amount of sucrose and its products (glucose and fructose) were determined using HPLC system with refractive index detector (RID) (Shimadzu, Japan).

Ascorbic acid content

Ascorbic acid contents of samples (GF, eGF and sugar-free FB) were analyzed after the preparation of the sample in a 1:10 (g/mL) ratio with 4.5 % meta-phosphoric acid. The injection volume was 20 µL which was loaded into the HPLC (Agilent 1260 infinity, USA) equipped with a diode array detector (Agilent 1260 MWD VL, USA) and a C18 column (250*4.6 mm, ID: 5 m ACE, UK). The mobile phase contained water and phosphoric acid (pH 2.2), and 30°C of the column temperature, 0.8 mL/min of the flow rate, and 30 minutes of the total run time were selected. For each sample, three replicates were done.

Carotenoid content

The carotenoid contents of samples (GF, eGF and sugar-free FB) were determined using a spectrophotometer at 470 nm [23]. Using Equations Carotenoid= (A470 x 106)/(2000 x 100 x L) total carotenoid (mg/100 g) contents were calculated. For each sample, three replicates were done.

Anthocyanidin content

The anthocyanidin contents of the samples (GF, eGF and sugar-free FB) were determined with a slight modification of Kelebek and Selli [24]'s method using HPLC. DAD detector, Beckman Ultrasphere ODS (Roissy CDG, France) analytical column (column length x inner diameter: 4.6mm x 250mm, particle size: 5 µm) and guard column (column length x inner diameter: 4.6 mm x 10 mm, particle size: 5 µm) were used. The oven temperature was 25 °C, and the injection volume was 20 µl. As a mobile phase ultrapure water:formic acid ((A) 95:5; v/v) and acetonitrile/A mixture (60:40; v/v) were used and the anthocyanidins were detected at a wavelength of 520 nm. Analyzes were made in three parallels.

Determination of phenolic composition by HPLC-DAD analysis

Analysis of phenolic compounds in samples (GF, eGF and sugar-free FB) were performed using by reversed-phase high performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Tokyo, Japan). A Diode Array Detector, SIL-10ADvp auto sampler, DGU-14A degasser, CTO-10Avp column heater, LC-10ADvp pump, and SCL-10Avp system controller are included in the system (Shimadzu Scientific Instruments, Columbia, MD).

The chromatographic separation was performed on a Agilent Eclipse XDB-C18 250 mm and 4.60 mm i.d., particle size 5 µm column and Agilent Eclipse XDB C18 guard column (4.6 x 12.5 mm i.d., particle size 5 µm) using gradient elution [25]. Solvent A was %2.0 acetic acid in water and solvent B was methanol.

The phenolic compounds were separated with modified gradient elution at 30 °C at a flow rate of 0.8 mL/min and 20 µL of injection volume [26]. A gradient elution was used. The phenolic components in the extract were measured in milligrams per gram of extract. The results of all extractions and chromatographic analysis were averaged after three replications.

Sensory evaluation

GF as a control drink and sugar-free FB as a test were sensory evaluated for overall acceptability, color, odor, flavor, and taste on a 5-point hedonic scale from 1=extremely bad to 5=most excellent by 16 trained panelists. Based on Lawless and Heymann [27] the trained panelists evaluated the test and control drink samples with a sample volume of 40 cc in codded containers in a closed room within 15 min [27]. Between samples, panelists' mouths were rinsed with distilled water.

Statistical analysis

All data were presented as means SD (standard deviation). Using the statistical program SPSS 20.0, the obtained data were statistically evaluated, and the analysis of variance was applied to determine the significance of the treatment differences using Duncan's multiple comparison test.

RESULTS AND DISCUSSION

Modeling and optimization of MAE parameters

In this study, three parameters were used in a three-level Box-Behnken design to optimize and examine the influence of process variables on the TPC and extraction efficiency of GF obtained using the MAE (Table 1).

Table 1 Experimental matrix and values of observed responses

Experiment No[a]	Solid/Solvent (g/100 mL)	Temperature (°C)	Time (min)	Efficiency (%)	Total Phenolic Content (mg GAE/g extract)
1	2	60	12.5	45.71	346.32
2	6	45	12.5	45.73	330.60
3	2	45	20.0	46.10	340.51
4	6	45	12.5	45.74	329.42
5	6	30	20.0	45.97	338.98
6	6	30	5.0	44.22	352.25
7	6	60	20.0	45.75	348.85
8	2	30	12.5	44.90	348.70
9	10	30	12.5	43.72	216.58
10	10	45	20.0	46.13	205.92
11	6	45	12.5	45.63	331.32
12	10	60	12.5	46.47	209.43
13	6	60	5.0	48.32	324.26
14	2	45	5.0	46.76	334.38
15	10	45	5.0	46.32	204.14

a, Randomly chosen

Extraction efficiency

The extraction yield ranged between 43.72 to 48.32 %. The minimum yield was recorded in the sample run 9 under the following experimental conditions: 12.5 min extraction time, 30°C temperature, and 10 g solid/100 mL solvent ratio, whereas maximum yield was detected in following conditions; 5 min extraction time, 60°C temperature and 6 g solid/100 mL solvent ratio. As a control sample a traditional method; maceration, was used for GF at 60°C with 60 min shaking, and the extraction yield was found as 45.07%. According to obtained results, it was found that MAE technique provides a higher extraction yield than maceration with 12-fold less time. In the literature there are different extraction yields that were reported depending on the extraction methods and solvent types such as 6.6% [28], between 36.75 to 70.34 [29] and 12.69 to 14.46 [5].

Table 2 Regression coefficients of polynomial function of response surface for TPC (mg GAE/g extract) and extraction efficiency (%)

Model Coefficients	Efficiency	Total Phenolic Content
β0	45.70	330.945
β1(Temperature)	0.9390***	-3.457***
β2(Time)	-0.2076***	-2.405**
β3(Solid/Solvent Ratio)	-0,1031**	-66.730***
β11	-0.3836**	9.766***
β22	0.7479***	ns
β33	-0.1184***	-60.081***
β12	-1.0795***	9.467***
β13	0.4835***	ns
β23	0,1189**	ns
Model	***	***
Regression coefficient (R^2)	99.87	99.95
Adjusted R^2 (Adj- R^2)	99.64	99.90
Predicted R^2 (Pred- R^2)	98.53	99.75
Lack of fit	0.454	0.188

*Statistically insignificant ($p \geq 0.05$); ** statistically significant at the 99% level ($p \leq 0.01$); *** statistically significant at the 99.9% level ($p \leq 0.001$), ns: statistically not significant.

Table 2 shows the predictive models derived by fitting the second-order polynomial model, and three responses were verified for adequacy and fitness using analyses of variance (ANOVA). The effect of independent (temperature and time), quadratic (time and solid/solvent ratio) variables and both interactions of extraction time-temperature (β12) and, temperature-solid/solvent ratio (β13) on extraction efficiency was statistically significant with predictive model ($p \leq 0.001$). Also, the extraction efficiency was effected from solid/solvent ratio and the temperature ($p \leq 0.01$). Also, the predicted value of the extraction efficiency (%) based on the temperature, time, and solid/solvent ratio parameters was found in good agreement with the data obtained in the experimental study (%99) and the model presented high predictive power in terms of extract yield.

The RSM interaction plots indicated that the influence of solid/solvent ratio, extraction temperature and time in the extraction yield (Figure 1). The shortest extraction time provided better extraction yield with the increase in the extraction temperature (Figure 1a). In addition, it was shown that the extraction efficiency increased with the raise in temperature and solid/solvent ratio (Figure 1b). Extraction efficiency increased correspondingly with the rise in variables temperature and solid/solvent ratio. Finally, based on the effect of solid/solvent ratio, the extraction efficiency was decreased between 5 to 15 min and the extraction efficiency was increased between 15 to 20 minutes (Figure 1c).

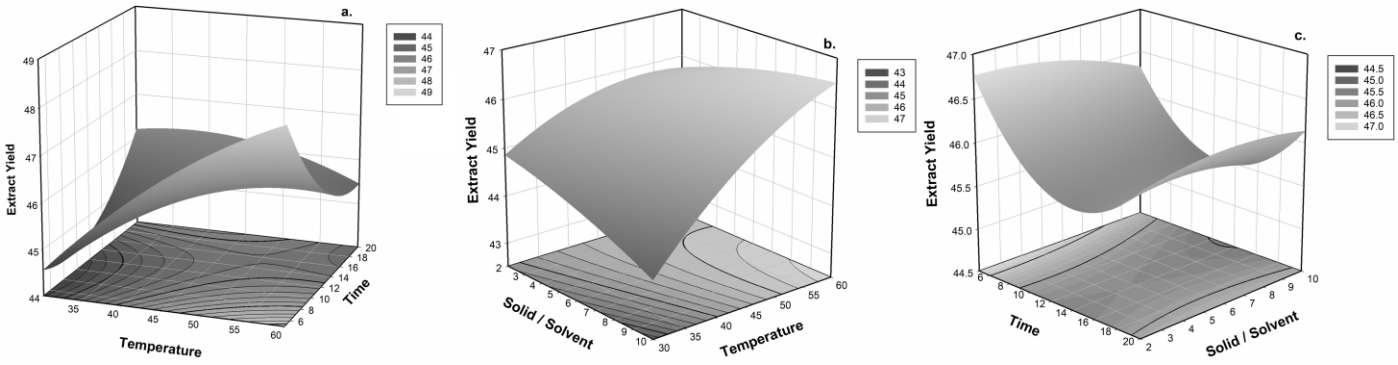

Figure 1 (a) Effects of extraction temperature and time, **(b)** effects of extraction temperature and solid/solvent ratio **(c)** effects of extraction time and solid/solvent ratio on extraction efficiency of the GF

To our knowledge, this is the first study about the detection of temperature, time, and solid/solvent ratio variables' effect on extraction efficiency optimization with RSM and using MAE. In accordance with the current study, increased extraction efficiency with the increasing solvent amount in the solid/solvent ratio was also reported in the literature and were shown that the increase of the extraction time effect the increase of the extraction efficiency up to a certain point and then slightly decreased insignificantly [30, 31].

Total phenolic content

Based on the model optimization results (Table 1), the TPC of GF was between 204.14 and 352.25 The lowest TPC was obtained at 45°C temperature, in 5 min, and 10.0 g solid/solvent ratio (run 10). On the other hand, the highest TPC was recorded in the sample run 6 with the extraction conditions of 30°C, 5 min, and 6.0 g solid/solvent ratio. As a traditional method, maceration, was used for GF phenolic water extract at 60°C and 60 min with shaking, and the TPC was found as 311.37 mg GAE/g extract. According to obtained results, it was found that MAE technique provides slightly higher TPC than a maceration. However, considering the extraction time for MAE as 5 minutes and maceration as 60 min, it may still conclude that MAE needs 12-fold less extraction time and low energy consumption. Therefore, MAE will be an efficient technique to extract GF phenolics. Also, different studies in the literature reported the TPC of GF with different solvents and extraction methods and the TPC were detected as 57.31 mg GAE/g extract [32], 40.9 mg GAE/g [28], 5.16 and 12.30 mg 100/g [33], 26.61 to 50.93 mg GAE/g km [29], 40.17 and 25.64 mg/g The GAE [5]. Those studies' TPC were lower than the current study results because of the MAE technique usage.

Table 2 shows the predictive models derived by fitting the second-order polynomial model, and three responses were verified for adequacy and fitness using analyses of variance (ANOVA). The effect of independent (temperature and solid/solvent ratio), quadratic (temperature and solid/solvent ratio) variables and interactions of extraction time-temperature (β12) on TPC was statistically significant with predictive model ($p \leq 0.001$). The TPC was effected from extraction time ($p \leq 0.01$), however β22, β13 and β23 was insignificant. In addition, the predicted value of the TPC based on the temperature, time, and solid/solvent ratio parameters was found in good agreement with the data obtained in the experimental study (%99) and the model presented high predictive power in terms of extract yield.

The effect of independent variables (temperature-time, temperature-solid/solvent ratio, and time-solid/solvent ratio) on TPC of the GF was investigated (Figure 2). Figure 2a showed that rising temperature caused a decrease in TPC followed by a slight increase at low extraction time. With the higher temperature, TPC was slightly increased after a decrease at longer extraction time. Also, TPC proportionally increased by temperature and time (Figure 2b and 2c). It was obtained that TPC decreased with the increase of solid/solvent ratio. Finally, it was found that the amount of TPC decreased based on the increase in solid/solvent ratio and extraction time did not affect the TPC (Figure 2c).

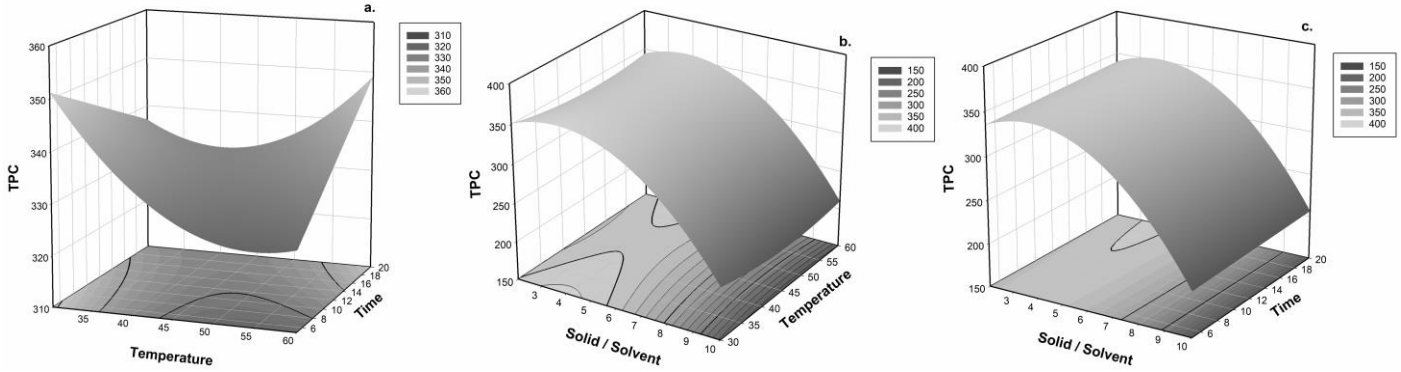

Figure 2 (a) Effects of extraction temperature and time, **(b)** effects of extraction temperature and solid/solvent ratio **(c)** effects of extraction time and solid/solvent ratio on TPC of the GF extract

The power of the MAE system and temperature has a directly proportional relationship. The increasing power causes a raise in the temperature which causes the amount of TPC to increase due to decrease of extract viscosity and increase of phenolic solubility, and then the amount of TPC decreases due to hydrolysis or oxidation of phenolics. Consistent with the result of current study, Durmaz and colleagues [31] indicated that increasing the extraction time cause a raise in the amount of TPC up to a point, and then it was observed that there was a slight decrease of TPC based on both power and solid/solvent ratio.

Determination and validation of optimum model conditions

To detect the optimal conditions of extraction parameters, Minitab Statistical Software was used. The RSM optimization was developed based on the initial experimental results, to maximize the extraction efficiency (%) and TPC (mg GAE/g extract) results of the extract with the highest level of importance. Under these conditions, the final optimization result's value for the three inputs; temperature, solid/solvent ratio, and time was determined as 60°C, 4.18 g dried fruit/100 mL water, and a 5 min extraction time. According to

optimized extraction conditions, the range of both extraction efficiency and TPC results were found as %47.98- 48.29 and 339.79- 346.81 mg GAE/g extract, respectively (Table 1).

The experiment was conducted according to the optimal conditions of temperature 60°C, 5 min, and solid/solvent ratio: 4.18 g dried fruit/100 mL water. The extraction efficiency was 48.03% and the total amount of phenolic content was detected as 344.31 mg GAE/g extract. These obtained results showed that the experimental values of the optimal conditions agreed with the predicted value ranges given above.

Encapsulation of GF (eGF)

Based on the preliminary results of the experimental design, the optimum encapsulation parameters of GF was detected as 3% sodium alginate, and 1:9 chitosan: $CaCl_2$. The hydrogel beads production rate was found 64 droplets/min and average diameter of 20 beads was measured as 4.5±0.00 mm. The encapsulation efficiency of these hydrogel beads was detected as 53.52 % and the moisture content was 91.26%. To the best of our knowledge, this is the first study about ionic gelation of GF phenolic extract with chitosan. According to literature, it was reported that encapsulation efficiency of hydrogel beads produced by ionic gelation and chitosan system from hydrophilic samples were low [34, 35]. In addition, Çoruhli [34] indicated that encapsulation of anthocyanin extraction from black mulberry using ionic gelation and chitosan system had a similar encapsulation efficiency (53.97%) to current study.

Chemical and physical analysis of GF, eGF and sugar-free FB

Phenolic composition

Phenolic composition of GF, eGF and sugar-free FB were analyzed by HPLC (Table 3). The major phenolics of all samples were detected as chlorogenic acid. Sugar-free FB has the highest concentration of chlorogenic acid whereas eGF contains lowest compared to GF. Enrichment of GF extract with black carrot cause a rise for the chlorogenic acid content in sugar-free FB compare to GF. In different studies major colorless phenolics of black carrot reported as chlorogenic acid followed with caffeic acid and quercetin derivatives [36, 37]. Therefore, phenolic content of sugar-free FB is more than GF.

Table 3 Phenolic composition of extract and hydrogel beads (mg/g extract)

Phenolic components	GF	eGF	Sugar-free FB
Chlorogenic acid	34.18±0.10	16.59±0.14**	57.01±1.21
Syringic acid	0.44±0.01	0.1±0.00	0.40±0.01
Catechin	4.50±0.02	1.40±0.00	4.91±0.21
Epicatechin	1.27±0.01	*	1.23±0.01
p-Hydroxybenzoic acid	0.51±0.01	*	1.36±0.07
Cinnamic acid	0.22±0.01	*	0.25±0.01
Gallic acid	0.05±0.00	*	0.05±0.00
Caffeic acid	*	*	39.97±1.14
Quercetin	*	*	21.56±1.09

*: Not detected.
** 56.45 g of hydrogel beads contain 1 g of phenolic extract.

Barak and colleagues [5] analyzed phenolic composition of fresh GF's methanol and water extracts using HPLC and chlorogenic acid amount of the extracts were reported as 34.433 and 26.76 mg/g, respectively. A recent study determined the phenolic composition of methanol extracted dried GF using ultra performance liquid chromatography (UPLC). The major phenolic was detected as hydroxycinnamic acid (763.32 mg/100g dw) and followed by chlorogenic acid with amount of 752.59 mg/100g dw [38].

According to the literature, the phenolic composition of GF extract reported in this study is mostly in agreement with the reports. It is thought that some of the slight differences may be due to solvent type and extraction method, growing and harvest conditions of the fruit. For the first time, gilaburu fruit extract was used to produce a fortified soft beverage, and also it was encapsulated using ionic gelation method.

Anthocyanidin content of sugar-free FB was detected for cyanidin-3-glucoside, cyanidin-3- rutinoside and pelargonidin 3-glucoside (mg/100g dw) 51, 34.4 and 4.7 respectively. Whereas cyanidin-3-glucoside, cyanidin-3-rutinoside and pelargonidin 3-glucoside (mg/100g dw) content of GF was detected in order of 13, 13.4 and 11.5. Similar to current study, there are several reports about the major anthocyanidin content of black carrot as cyanidin derivatives [36, 37]. Keskin and colleagues [39] investigated that fresh black carrot contains 180.5 mg/100g dw of cyanidin derivatives and 0.57 mg/100g dw pelargonidin derivative. Therefore

it may be concluded that due to the rich anthocyanidin content of black carrot sugar-free FB contains higher anthocyanidin than GF.

Functional properties

Functional properties such as total phenolic content, ascorbic acid and carotenoid content, radical scavenging and antidiabetic activity of GF, eGF and sugar-free FB was presented in Table 4. The TPC (mg GAE/ g) of sugar-free FB was significantly higher than GF. eGF indicated the lower TPC compared to GF. The TPC for eGF were lowest due to the amount of phenolics infused into the hydrogel beads. However ascorbic acid and total carotenoid content of the GF and eGF were not statically significant. Due to additional enrichment in sugar free FB, both ascorbic acid and total carotenoid content were higher than GF. A recent study analyzed the multilayered encapsulation of ascorbic acid and β-caroten using alginate and chitosan [40]. It was shown that multilayer gel microspheres for ascorbic acid and β-caroten improved their bioaccessibility and bioavailability. Therefore, encapsulation of GF may provide the infusion of ascorbic acid and total carotenoid content at the highest concentration to eGF. Nath and colleagues [16] investigated the TPC (mg GAE/100 g), anthocyanidin content (mg/L), ascorbic acid content (mg/100g) and DPPH (%) activity of fresh black carrot as 380.22, 1000.14, 108.51, 96.02, respectively. Due to the rich TPC of sugar-free FB with ascorbic acid fortification provides higher DPPH activity compared to GF and eGF. DPPH activity of hydrogel beads was lower than GF as it is directly proportional to TPC. In this regard the higher TPC displayed better free radical scavenging activity. In a study, water extraction of fresh GF was examined for its free radical scavenging activity and obtained between 7.21 to 13.89 mg DPPH/g dry matter [29]. Barak and colleagues [5] reported the free radical scavenging activity (mg BHTE/g) of both methanol and water extract of fresh gilaburu fruit 103.59 and 96.74, respectively. These results shown that extraction methods and solvents or water content of fruit (fresh or dried fruit) may affect the free radical scavenging activity. In addition, the current study results demonstrated higher activity for GF than literature. Also, this is the first study about the production of riboflavin and ascorbic acid fortified gilaburu beverage.

Table 4. Functional Properties of GF, eGF and sugar-free FB.

Functional Properties	GF	eGF	Sugar-free FB
TPC (mg GAE/g extract)	344.31±3.29	174.3±1.05	1452,3±4.71
DPPH (mg TE/100 g)	317.19±2.12	116.26±2.80	673.52±2.91
Total carotenoid (mg/100 g dw)	1.85±0.01	1.07±0.01	66.09±0.03
Ascorbic acid (mg A.A/100 mL)	677.85±3.90	648±8.91	3834.3±15.30
Antidiabetic activity (%)	54±1.55	92±0.51	77±1.37

For the first time, current study reported the antidiabetic activity of microwave-assisted water extract of GF, eGF and sugar-free FB. According to Table 4, GF was inhibited the α-glucosidase activity (%) 54 whereas eGF and chemical drug acarbose (1 g) inhibited almost 90 and 92, respectively. The phenolic content of GF indicated promising amount of antidiabetic activity. Kajszczak and colleagues [6] reported that chlorogenic acids, proanthocyanidin polymers and dicafeoylquinic acids increase inhibitory activity against α-glucosidase. Thereby the phenolic composition of gilaburu may have antidiabetic activity. However eGF, demonstrated the highest antidiabetic activity. The reason of this result was due to the effect of coating materials to α-glucosidase activity and hydrogel beads without extract inhibited α-glucosidase activity 72.9%. Also, in a study it was discovered that low molecular weight chitosan inhibited both α-glucosidase activity and glucose transporters (SGLT1 and GLUT2) [41]. Therefore, it was thought that even with a low amount of phenolics in hydrogel beads indicated higher antidiabetic activity than extract. In addition, it was reported in different studies that oligosaccharide and chitosan complex (COS) indicated an antidiabetic activity [42, 43]. Therefore, it was thought that the interaction between oligosaccharide content of GF and coating material chitosan may increase the inhibition of α-glucosidase activity. These effects may provide the highest α-glucosidase activity for eGF compared to GF and sugar-free FB. Also the quercetin and caffeic acid content of the sugar-free FB may support its inhibitory activity for the digestive enzymes. Caffeic acid is reported as another important bioactive component that possesses an important role in the management of diabetes [44]. Quercetin was reported as a strong antidiabetic agent as it caused a reduction in hyperglycemic and blood glucose levels in Streptozotocin-induced diabetic Wistar rats [45]. In different studies black carrot indicated antidiabetic activity [46, 47]. Esatbeyoglu and colleagues [46] reported that acarbose and black carrot has IC_{50} (g/L) value for α-glucosidase activity in order of 5.96 and 7.32. In the same study intestinal glucose uptake of Caco-2 cells was decreased to approximately 90% at concentration of 0.1 mg/mL. Antidiabetic

activity of GF, eGF and sugar-free FB is similar to literature. Especially eGF inhibited α-glucosidase activity as good as acarbose. These results shows that gilaburu and its products may use in the management of diabetes.

Sensory evaluation of the sugar-free FB

Organoleptic evaluation of the microwave-assisted water extract obtained at optimum conditions of GF extract supplemented with ascorbic acid, riboflavin, and black carrot was carried out for color, odor, flavor, taste, and overall acceptability shown in Figure 3A and B.

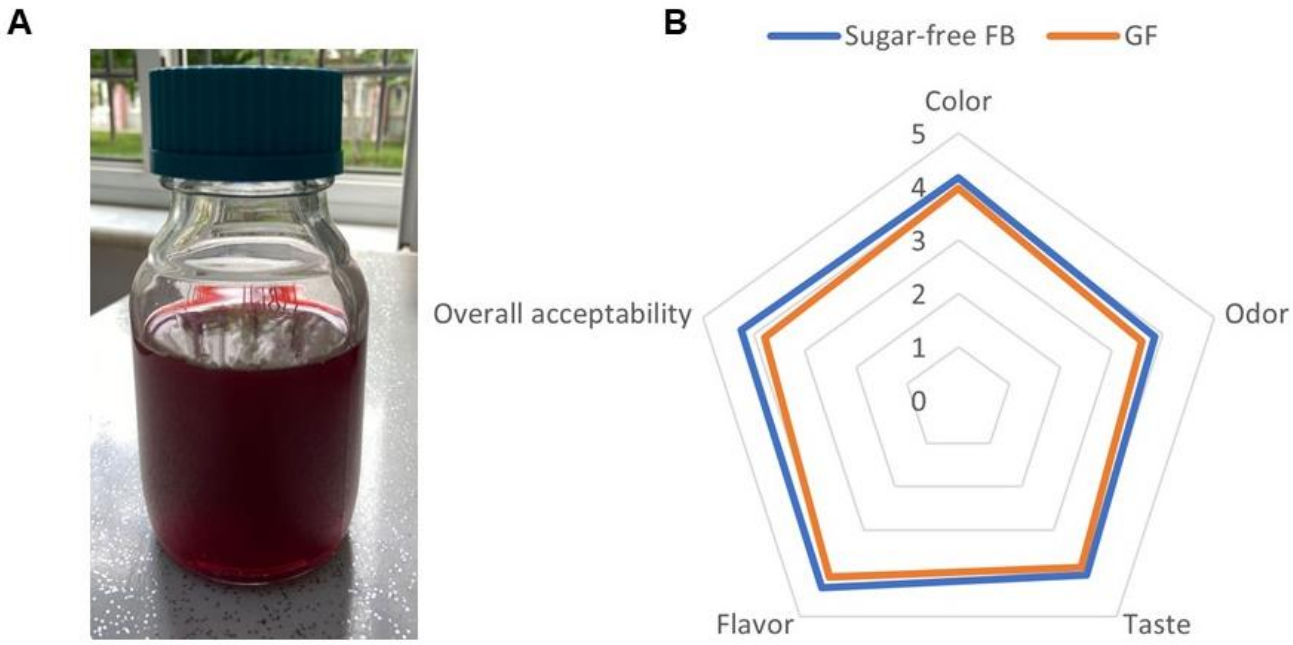

Figure 3. A: Picture of sugar-free FB, B: Radar charts for sensory analysis of sugar-free FB and GF groups.

As a control sample, microwave -assisted water extract obtained at optimum conditions of GF extract was provided to the panelists. The most representative and intense odor impression perceived in sugar-free FB compared to control. The intense purple color of the black carrot enriched beverage was more attractive than the control as it was evaluated as a brighter and more homogeneous color. In addition, the extract was found blurrier than sugar-free FB. The taste and overall acceptability were significantly better for sugar-free FB than extract without enrichment for the panelists.

CONCLUSION

In the current research, primarily the MAE conditions of phenolic compounds from gilaburu were optimized using the RSM. This is the first report about MAE phenolic extraction process optimization from gilaburu fruit with RSM. The objective of this investigation was to develop a complete antidiabetic and antioxidant sugar-free functional drink formulation and producing encapsulated forms of GF for longer shelf life. The results of the current study indicated that sugar-free FB and eGF has high antidiabetic activity compared to GF. According to literature, the highest inhibitory α-glucosidase activity of eGF may be due to its alginate and chitosan content. In addition, fortification of GF with black carrot and hydrophilic vitamins was significantly increased its antioxidant activity. These effects of sugar-free FB may be related to extracts rich in phenolic composition together with ascorbic acid, riboflavin, and black carrot fortification. Therefore, sugar-free FB may be used to provide health benefits. The results of the study show that further *in vivo* and clinical trials need to be considered to confirm these products' effect on the metabolism of diabetic patients. Finally, it may be considered that the consumption of developed sugar-free FB with the meal may decrease postprandial blood glucose level with an acute effect.

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