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Antidiabetic Property Optimization from Green Leafy Vegetables Using Ultrasound-Assisted Extraction to Improve Cracker Production

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ABSTRACT: Here we test a method of incorporating of plant extracts into popular snack foods to help control diabetes. Since some fresh vegetables contain antidiabetic compounds, ultrasound-assisted extraction was used to optimize their extraction of from spring onions, bunching onions, and celery for later incorporation into crackers. We compared various concentrations of ethanol used during extraction, after which they were exposed to an ultrasound processor whose amplitude and sonication time were also varied. The optimal extraction conditions were found to be an ethanol concentration of 44.08%, an amplitude of 80%, and a sonication time of 30 min. This resulted in the highest level of α-glucosidase inhibitory activity (i.e., 1,449.73 mmol ACE/g) and the highest extraction yield (i.e., 24.16%). The extract produced from these optimum conditions was then used as a constituent component of crackers at 0.625%, 1.25%, or 2.5% w/w. These biscuits were then produced at baking temperatures of 140°C, 150°C, or 160°C. We then measured the physical characteristics and bioactivities of sample biscuits from each treatment. We found that biscuits containing 2.5% vegetable combination extract and baked at 140°C had the highest total phenolic content, the strongest antioxidant performance, and showed the most substantial antidiabetic and antiobesity effects. Here we establish conditions for the effective extraction of antidiabetic functional ingredients via ultrasound from green leafy vegetables. We also provide a method of using these ingredients to prepare crackers with the aim of developing a functional antidiabetic snack food.

Keywords: baking temperature, Box-Behnken design, functional food, glycoside hydrolase inhibitors

INTRODUCTION

Diabetes mellitus is a complex metabolic disease characterized by increased blood glucose levels due to impaired insulin resistance or production (Bashkin et al., 2021). One aspect of diabetes management involves α-glucosidase regulation during carbohydrate digestion and absorption (Javadi et al., 2014). α-Glucosidase acts on carbohydrates such as starches and disaccharides and breaks them down into simpler sugars such as glucose, which are then absorbed into the bloodstream (Javadi et al., 2014). Medications to inhibit α-glucosidase are therefore frequently prescribed to manage diabetes (Li et al., 2022), but unfortunately α-glucosidase inhibitors are not suitable for everyone (Hedrington and Davis, 2019) since they pose side effects that limit their usage, including gastrointestinal discomfort, diarrhea, and flatulence (Liu et al., 2022). Recent studies have shown that natural inhibitors derived from plant-based sources offer promising blood sugar level regulation, improve insulin sensitivity, have fewer side effects, and reduce the risk of other complications associated with diabetes medications (Li et al., 2020; Li et al., 2022; Liu et al., 2022).

Many vegetables, especially green leafy vegetables (GLVs), contain bioactive compounds that offer diverse health-promoting properties (Sarkar et al., 2023). Several compounds found in GLV extracts, including essential oils, quercetin, and berberine, have been shown to modu-

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late glucose metabolism, improve insulin signaling, and reduce insulin resistance (Randhawa et al., 2015). Plant extract-based formulations have also been studied (Parveen et al., 2021), and plant-derived compounds have been found to have synergistic effects by targeting multiple metabolic pathways (Martel et al., 2017). Recent research indicates that a combination of treatments may improve the effectiveness of specific medical products in controlling diseases and disorders, including atherosclerosis, cancer, and diabetes, each of which have complex pathophysiologies and etiologies and are therefore challenging to manage using single-agent strategies (Zhou et al., 2016). For example, a combination of the ethanolic extract *Eugenia uniflora* and *Clonorchis sinensis* was found to synergistically affect the inhibitory and lipid peroxidation activity of α-glucosidase (Vinholes and Vizzotto, 2017). By harnessing the potential synergistic effects of these bioactive compounds, the combination of GLVs can offer a holistic and natural approach for promoting diabetes management.

Ultrasound-assisted extraction (UAE) is emerging a promising technique for extracting bioactive compounds from various plant sources (Chotphruethipong et al., 2019). Esclapez et al. (2011) reported that UAE can save a considerable amount of energy compared to alternatives. Because it uses only moderate temperatures, its use can be advantageous when dealing with heat-sensitive compounds. Albero et al. (2019) also reported that UAE may be a more environmentally friendly method because it uses less solvent required and has a shorter extraction time than others. To maximize the extraction of desired compounds, UAE must be optimized with respect to extraction parameters, including the solvent-to-sample ratio, extraction time, and ultrasonic power (Li et al., 2020; Yancheshmeh et al., 2022).

Crackers are a common snack consumed throughout the world (Olagunju et al., 2018; Hu et al., 2022). However, there is increasing awareness that snack foods should not only satisfy consumer tastes but also contribute to overall health (Paciulli et al., 2023). One innovative approach is to incorporate plant extracts containing bioactive compounds with potent antidiabetic properties into snack foods (Olagunju et al., 2018; Indiarto et al., 2023). Other studies have tried to successfully incorporate plant extracts into crackers to influence α-glucosidase and α-amylase activities, including extracts from species such as *Armoracia rusticana*, *Digitaria exilis*, *Cajanus cajan*, and *Prunus cerasus* (Tumbas Šaponjac et al., 2016; Olagunju et al., 2018; Tomsone et al., 2020). The objective of this study was to develop crackers with antidiabetic properties by using UAE to produce an extract from a combination of GLVs, then adding this extract to the cracker formulation. UAE was optimized to obtain potential antidiabetic metabolites from GLVs using a Box-Behnken design (BBD) [using a response surface methodology (RSM)] and was then compared with conventional extraction techniques. In addition, we also investigated the effect of baking temperature on the physical properties and bioactivities of crackers. These activities were quantified by measuring antioxidant activity [2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating, and ferric reducing antioxidant power (FRAP)] and enzyme inhibitory activity (α-amylase, α-glucosidase, and lipase), and by conducting a proximate analysis of crackers.

MATERIALS AND METHODS

Materials

Enzymes (i.e., α-glucosidase, α-amylase Type IV-B, and lipase Type II sourced from porcine pancreas), p-nitrophenyl-α-D-glucopyranoside (p-NPG), acarbose, and 4-methylumbelliferyl oleate (4MUO) were purchased from Sigma-Aldrich. DPPH was obtained from Thermo Fisher Scientific, and 2,4,6-tri(2-pyridyl)-s-triazine, gallic acid monohydrate, ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p-disulfonic acid monosodium salt hydrate (Ferrozine), L-ascorbic acid, and Folin-Ciocalteu reagent were purchased from Acros Organics. All chemicals employed were of analytical quality. Ten kilogram each of fresh GLVs, including spring onions (*Allium cepa*), bunching onions (*Allium fistulosum*), and celery (*Apium graveolens*), along with hen eggs, sucrose, soybean oil, wheat flour, sodium chloride, and baking powder (i.e., sodium bicarbonate), were obtained from the Hua Takhe market (Bangkok) then taken directly to King Mongkut's Institute of Technology Ladkrabang. Before use, the aerial part of all GLVs was carefully rinsed with tap water to remove dirt and then dried on screens.

Preparation of GLV powder

GLV powder was prepared using a method described by Maser et al. (2023b). Briefly, GLVs were first dehydrated in a WRH-100 drier (IKE Industrial Co., Ltd.) at 45°C until they reached a constant weight. The dried GLVs were then blended in a Philips HR2222 blender.

Preparation of extracts by conventional and UAE processes

Three types of GLV powder (100 g each) were mixed in equal proportion (1:1:1; w:w:w) then mixed with 0, 40, or 80% ethanol (1:10 w/v ratio). Samples were then homogenized at 4,000 *g* at room temperature (about 25°C) for 2 min using an IKA T25 ULTRA-TURRAX homogenizer then macerated at 4°C for 24 h. Ground samples were then centrifuged at 2,500 *g* using an Eppendorf 5910 R centrifuge at 25°C for 15 min. Next, samples

were filtered using Whatman No. 1 filter paper and concentrated under vacuum at 40°C using a Buchi Rotavapor R-300. The resulting extracts were then freeze-dried at −50°C using a Kinetic LD0.5 freeze dryer (Kinetic Engineering Co., Ltd.) for 48 h.

The UAE process was optimized using a BBD with three factors (Table 1). Briefly, equal proportions (i.e., 100 g each) of powdered samples from spring onions, bunching onions, and celery were first combined (1:1:1; w:w:w) and mixed with 200 mL of solvent containing 0%, 40%, or 80% ethanol at a sonication amplitude of 40%, 60%, or 80%. Samples were then sonicated for 10, 20, or 30 min in an inert atmosphere using an ultrasound processor (Sonics & Material Vibra-Cell model), equipped with a 13-mm tip probe. Ultrasonic sonication proceeded at a power of 750 W and a frequency of 20 kHz, at 35°C± 5°C; the pulsing mode used was 10 s on and 5 s off. After extraction, sample mixtures were centrifuged, filtered, concentrated, and dried as described above. The dependent variables measured to evaluate the UAE and conventional extraction processes include yield (%) and α-glucosidase inhibitory (AGI) activity [mmol acarbose equivalent (ACE)/g extract].

RSM

Next, RSM was used to analyze and optimize extraction. RSM was performed using Design-Expert version 7.0.0 (Stat-Ease). Specifically, we used an ANOVA to determine the coefficients of regression, *F*-test, and probability (*P*) of statistically significant differences between means. A polynomial model of precision was determined by calculating the coefficient of determination (R^2), the coefficient of adjusted determination (adj-R^2), and the coefficient of variance (CV%). The model, expressed as a quadratic Equation (1), is as follows:

$$Y^e = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

Here Y^e denotes the response to either extraction yield or AGI activity. The coefficients b_0, b_1, b_2, and b_3 represent the constants of the intercept, linear, and quadratic coefficients of the independent variables X_1, X_2, and X_3 (i.e., ethanol concentration, amplitude percentage, and sonication time, respectively). This polynomial equation was then used to generate a contour plot to explain the relationships between the experimental and dependent variables.

All data were then subjected to multiple regression analysis using Design-Expert version 7.0.0 to develop another polynomial model. This model described the relationship between the independent experimental conditions and response variables (e.g., extraction yield and AGI activity) and was represented using the Equations as follows:

$$Y_{Yield} = 25.73 - 3.42X_1 + 0.22X_2 - 0.49X_3 - 0.05X_1X_2 + 0.74X_1X_3 - 1.19X_2X_3 - 3.80X_1^2 + 0.25X_2^2 + 0.004X_3^2$$

$$Y_{AGI\ activity} = 951.20 + 126.21X_1 + 80.15X_2 + 120.31X_3 + 76.27X_1X_2 + 90.92X_1X_3 + 66.00X_2X_3 + 92.18X_1^2 + 143.50X_2^2 + 68.92X_3^2$$

Table 1. Box-Behnken design parameters for ultrasound-assisted extraction of green leafy vegetable using response surface methodology

Run	Standard	Independent variable			Dependent variable	
		Factor X_1 Ethanol concentration (%)	Factor X_2 Amplitude (%)	Factor X_3 Sonication time (min)	Yield (%)	α-Glucosidase inhibitory activity (mmol ACE/g extract)
1	2	80	40	20	20.01±0.18^{d}	1,161.74±27.70^{b}
2	1	0	40	20	25.20±2.44^{abc}	1,070.39±60.08^{bcd}
3	10	40	80	10	27.99±2.42^{a}	1,045.24±39.03^{d}
4	12	40	80	30	25.70±2.15^{abc}	1,460.96±16.17^{a}
5	8	80	60	30	17.45±1.28^{d}	1,432.46±76.72^{a}
6	14	40	60	20	25.50±1.70^{abc}	964.46±59.73^{de}
7	15	40	60	20	26.49±1.34^{abc}	919.42±63.20^{e}
8	4	80	80	20	19.07±2.43^{d}	1,455.93±80.55^{a}
9	6	80	60	10	18.03±0.72^{d}	1,053.10±53.71^{cd}
10	5	0	60	10	27.90±2.20^{ab}	974.00±26.41^{de}
11	3	0	80	20	24.46±2.40^{abc}	1,059.49±53.77^{cd}
12	11	40	40	30	26.37±1.41^{abc}	1,150.01±66.64^{bc}
13	9	40	40	10	23.89±1.16^{c}	998.31±57.90^{de}
14	7	0	60	30	24.36±2.35^{bc}	989.66±49.17^{de}
15	13	40	60	20	25.22±1.38^{abc}	969.72±56.06^{de}

Values are presented as mean±SD of three technical replicates (i.e., n=3).
Different superscripts in the same column (a-e) indicate statistically significant differences (P<0.05).
ACE, acarbose equivalent.

To optimize the process, a desirability function (D) was employed for multiple response analysis, which was calculated using Equation (2):

$$D=d_n(Y_i) \quad (2)$$

Here, $d_n(Y_i)$ denotes the normalized values of each response.

Cracker preparation

Next, we prepared crackers using the obtained extracts. For all tests, acarbose (0.1% w/w) was used as a control. We tested different percentages of combination extracts (i.e., 0.625%, 1.25%, and 2.5% w/w; Table 2) during optimization testing. A kneading machine was then used to combine the ingredients, and the resulting dough was allowed to rest for 30 min at 4°C. After resting, the dough was then rolled into a 2 mm layer, cut into 35-mm disks, then baked for 15 min at one of the following temperatures: 140°C, 150°C, or 160°C. The crackers were then removed from the oven, allowed to cool to room temperature, and vacuum sealed in flexible polylaminate pouches for storage. Crackers were stored in the dark until further analysis.

Analysis of the combination extract

***Extraction yield*:** The yield (%) of the GLV extract was determined using the following Formula (3):

$$\text{Extraction yield (\%)}=(w_1/w_2)\times 100 \quad (3)$$

Here, w_1 is the dry weight of the obtained extract and w_2 is the dry weight of the initial GLV sample.

***AGI assay*:** An AGI assay was performed using the method described by Maser et al. (2023b). First, extracts (10 μL in 10 mg/mL dimethylsulfoxide concentration) were mixed with 50 μL of 0.1 M phosphate buffer at pH 6.9, 25 μL of 0.1 U/mL α-glucosidase, and 25 μL of 10 mM p-NPG. The resulting mixture was left at 37°C±2°C for 30 min. At this time, 100 μL aliquot of 0.2 M sodium carbonate was added to stop the reaction. We then examined absorbance at 410 nm using a spectrophotometer. A calibration curve was established using acarbose. Inhibition activity was determined as an ACE (mmol ACE/100 g crackers).

Cracker analysis

***Color*:** The color of each cracker was determined using a Konica Minolta CR-400 colorimeter. Color measurements were reported using the Commission Internationale de l'Eclairage system, and included L^* for lightness, a^* for redness, and b^* for yellowness, as described by Ali et al. (2020).

***Texture analysis*:** Next, the texture of each biscuit was measured using the procedure described by Devi et al. (2023). Briefly, crackers were tested for hardness (g) and crispiness (mm) using a TA.HDplusC texture analyzer (Stable Micro Systems) in compression mode. A blade-cutting probe was employed at speeds of 1 mm/s (for pre-testing), 3 mm/s (during testing), and 10 mm/s (posttest) at a trigger force of 50 g.

***Moisture content*:** We subsequently, measured moisture content following the AOAC International (2000) procedure. Each biscuit was initially weighed (w_1) and then dried at 105°C±2°C in an oven until a constant weight was reached (w^2). The moisture content was then calculated using Formula (4).

$$\text{Moisture content (\%)}=[(w_1-w_2)/w_1]\times 100 \quad (4)$$

***Water activity*:** Next, the water activity (a_w) of each cracker was measured using an Aqualab 4TEV Water Activity Meter (Addium, Inc.). All measurements were taken at 25°C.

***Total phenolic content (TPC)*:** TPC was determined using the procedure described by Bavisetty and Venkatachalam (2021) with minor adjustments. Briefly, crackers were first dissolved in 1 mg/mL of 50% methanol. Next, 100 μL of the dissolved sample was combined with 200 μL of 10% Folin-Ciocalteu reagent. This was followed by the addition of 800 μL of a 700 mM sodium carbonate solution and the resulting mixture was incubated at 25°C for 2 h, after which the absorbance was measured at 765 nm. A calibration curve was established using gallic acid, and TPC was then quantified in terms of gallic acid equivalents (i.e., mg GAE/100 g cracker).

***DPPH assay*:** We then measured DPPH activity by using a DPPH assay protocol described by Ali et al. (2021) using 0.2 mM DPPH solution. The absorbance was then measured at 517 nm, and a calibration curve was established using L-ascorbic acid. DPPH activity was quantified in terms of L-ascorbic acid equivalent antioxidant capacity

Table 2. Ingredients of crackers enriched with green leafy vegetable extracts

Ingredient (% w/w)	Control	0.1% acarbose	0.625% extract	1.25% extract	2.5% extract
Egg	38.36	38.36	38.36	38.36	38.36
Soybean oil	8.70	8.70	8.70	8.70	8.70
Wheat flour	52.23	52.17	51.90	51.57	50.92
Combination extract	0.00	0.05	0.33	0.65	1.31
NaCl	0.30	0.30	0.30	0.30	0.30
Baking powder	0.40	0.40	0.40	0.40	0.40

(i.e., mg AEAC/100 g cracker).

***Metal chelating assay*:** Metal chelating analysis was conducted using the method described by Maser et al. (2023b) using 0.6 mM $FeCl_2$ and 5 mM Ferrozine. For all experiments, the absorbance was measured at 562 nm and a calibration curve was established using EDTA. Metal chelating activity was then expressed in terms of EDTA equivalent chelating capacity (i.e., mg EECC/100 g cracker).

***FRAP assay*:** A FRAP assay was performed using the FRAP reagent as described by Ali et al. (2021). For all experiments, absorbance was measured at 593 nm. A calibration curve was established using L-ascorbic acid. FRAP activity was then expressed in terms of L-ascorbic acid equivalent antioxidant capacity (i.e., mg AEAC/100 g cracker).

***α-Amylase inhibitory (AAI) assay*:** An AAI assay was conducted using the protocol described by Maser et al. (2023a). Each extract (i.e., 20 μL in 1 mg/mL 0.2 M phosphate buffer at pH 6.9) was mixed with 20 μL 20 U/mL α-amylase solution then incubated at 37°C±2°C for 10 min. The resulting mixture was then combined with 30 μL 0.5% starch solution and left for 8 min at 37°C±2°C. To stop this reaction, 20 μL of 1 M HCl and 100 μL of 0.25 mM iodine solution were added. The absorbance was subsequently measured at 565 nm, and a calibration curve was established using acarbose. The inhibition activity was then expressed in terms of ACEs (i.e., mmol ACE/100 g cracker).

***AGI assay*:** An AGI assay was conducted as described in subsection AGI assay of combination extract analysis.

***Lipase inhibitory (LPI) assay*:** LPI analysis was performed as per the method described by Maser et al. (2023b). Briefly, extracts (25 μL of a concentration of 1 mg/mL) were mixed with 25 μL of 50 U/mL lipase solution and 50 μL of 0.5 mM 4MUO. This mixture was then incubated for 30 min at room temperature after which 100 μL of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The fluorescence intensity was then measured at 460 nm (emission) and 355 nm (excitation). The inhibition activity was subsequently determined using the following Formula (5):

$$\text{Percentage inhibition (\%)} = [(A_1 - A_2)/A_1] \times 100 \quad (5)$$

Here, A_1 represents the fluorescence intensity in the absence of the extract and A_2 represents the fluorescence intensity in the presence of the extract.

***Proximate composition*:** Next, crude protein content was evaluated using a protein analyzer (KB8S/VAP30S, Gerhardt). Ash content was determined using a muffle furnace (LT40/11/B170, Nabertherm GmbH), total fat content was determined using an automated Soxhlet method (SOX406, Gerhardt), and carbohydrate content was then determined by subtracting the percentages of protein, moisture, fat, and ash from 100% (AOAC International, 2000).

Statistical analyses

All measurement results were presented as a mean plus or minus standard deviation. ANOVAs were then performed on the data, and significance levels were determined using Duncan Multiple Comparison tests. Here $P<0.05$ was used as the threshold of statistical significance. All analyses were performed using IBM SPSS version 28.0 (IBM Corp.).

RESULTS AND DISCUSSION

GLV extraction using UAE

Regarding the BBD experiment, 15 treatments were performed using three independent variables: ethanol concentration (X_1), amplitude (X_2), and sonication time (X_3) (Table 1). Next, the *F*-value and associated *P*-value ($P<0.05$) of the variance analysis indicated that variation in response variables may be associated with a minimum of one model parameter (Table 3). However, the lack of fit, as evidenced by the nonsignificant value for the overall model ($P>0.05$), validated the accuracy of the model obtained.

***Optimization of extraction yield*:** We found that the UAE process generated a higher yield than conventional extraction methods (17.21%~23.89%; Table 4). This can be attributed to the cavitation effect, which enhanced mass transfer and accelerated the release of target compounds (Ali et al., 2019). The impact of the independent variables on the response (i.e., extraction yield) was assessed by examining both the linear and interactive effects, as visualized in three-dimensional (3D) plots. These plots were used to determine the optimal conditions for achieving maximum extraction yield (Fig. 1).

The polynomial model was determined to not be significant ($P>0.05$; Table 3), thereby confirming the findings (Yancheshmeh et al., 2022). Furthermore, the R^2 value for extraction yield was 0.9334, indicating a strong correlation between actual and predicted data, which was also consistent with the findings of Yancheshmeh et al. (2022). Moreover, the adj-R^2 parameter confirmed the adequacy of the model. Yancheshmeh et al. (2022) suggested that a CV value below 10% indicates suitable reproducibility. Therefore, the CV observed here (i.e., 6.31%) supports the reproducibility and precision of our model.

Overall, our results demonstrated that lower ethanol concentrations resulted in AGI activity (Fig. 1). This finding confirmed the finding that ethanol concentration can have different effects on yield and inhibitory activity. This effect was observed in *Phaleria macrocarpa* (Easmin et al., 2017) and *Cosmos caudatus* (Javadi et al., 2014). Moreover,

Table 3. ANOVA to evaluate fitted linear and quadratic polynomial models with respect to parameter optimization and desirability value

Source	Degrees of freedom	Sum of squares	Mean square	*F*-value	*P*-value
Yield (%)					
Model	9	158.46	17.61	7.78	0.0180[s]
Lack of fit	3	10.42	3.47	7.79	0.1159[ns]
Pure error	2	0.89	0.45		
Total error	14	169.77			
R^2	0.9334				
adj-R^2	0.8134				
CV (%)	6.31				
AGI activity (mmol ACE/g extract)					
Model	9	478,996.67	53,221.85	43.74	0.0003[s]
Lack of fit	3	4,556.11	1,518.70	1.99	0.3520[ns]
Pure error	2	1,528.45	764.22		
Total error	14	485,081.23			
R^2	0.9875				
adj-R^2	0.9649				
CV (%)	3.13				
Desirability value[1)]					
Ethanol concentration	Amplitude	Time	Yield	AGI activity	Desirability
44.08	80.00	30.00	24.21	1,460.96	0.801
43.65	80.00	30.00	24.24	1,457.60	0.800
43.31	80.00	30.00	24.27	1,454.95	0.800
44.42	80.00	29.92	24.19	1,460.96	0.800
44.38	79.89	30.00	24.18	1,460.96	0.799
41.72	80.00	30.00	24.40	1,442.85	0.798
44.95	80.00	29.80	24.16	1,460.96	0.798
44.76	79.76	30.00	24.15	1,460.96	0.797
45.40	80.00	29.69	24.14	1,460.96	0.796
45.38	79.53	30.00	24.10	1,460.95	0.794

[s]Significant. [ns]Not significant.
[1)]Only the 10 highest of 43 values are shown.
adj-R^2, the coefficient of adjusted determination; CV, coefficient of variance; AGI, α-glucosidase inhibitory; ACE, acarbose equivalent.

Table 4. Yield and α-glucosidase inhibitory activity of green leafy vegetable extracts produced using conventional processes at different ethanol concentrations

Ethanol concentration (%)	Extraction yield (%)	α-Glucosidase inhibitory activity (mmol ACE/g extract)
0	23.89±1.16[a]	794.27±36.84[b]
40	22.83±1.56[a]	869.19±72.51[b]
80	17.21±0.08[b]	974.46±81.57[a]

Values are presented as mean±SD of three technical replicates. Different superscripts in the same column (a,b) indicate statistically significant differences (P<0.05).
ACE, acarbose equivalent.

the combined influence of ethanol concentration, amplitude, and sonication time led to an increase in extraction yield via ultrasonic-induced cavitation, and thereby resulted in a higher yield at 40% than at 0% ethanol. Cavitation is due to the energetic implosion of bubbles within a system and promotes solvent diffusion into plant material and causes enhanced mass transfer rates. This effect ultimately enhanced extraction efficiency, as evidenced by higher yield (Chotphruethipong et al., 2019).

We observed the maximum yield (27.99%) at an amplitude of 80%, an ethanol concentration of 40%, and a sonication time of 10 min. Moreover, our findings suggested that an increase in amplitude resulted in higher extraction yield. This finding is consistent with Calliari et al. (2020), who optimized *Hibiscus sabdariffa* extract by extracting at 80% amplitude. In general, amplitude tends to enhance yield due to intensified heat generation and acoustic cavitation, which resulted in the disruption of plant cells, particle reduction, and improved mass transfer (Chotphruethipong et al., 2019). Applying ultrasonic processing has also been found to facilitate the transfer or extraction of soluble constituents into solvents (Chotphruethipong et al., 2019). This is why the UAE process is influenced by the amplitude used during extraction.

We further found that the extraction yield increased with sonication time at low amplitudes. However, increasing sonication time decreased the yield at high amplitudes. In general, cavitation is pivotal in disrupting cell walls and accelerating the extraction process (Ali et al., 2019). For example, Petcharat et al. (2021) reported that

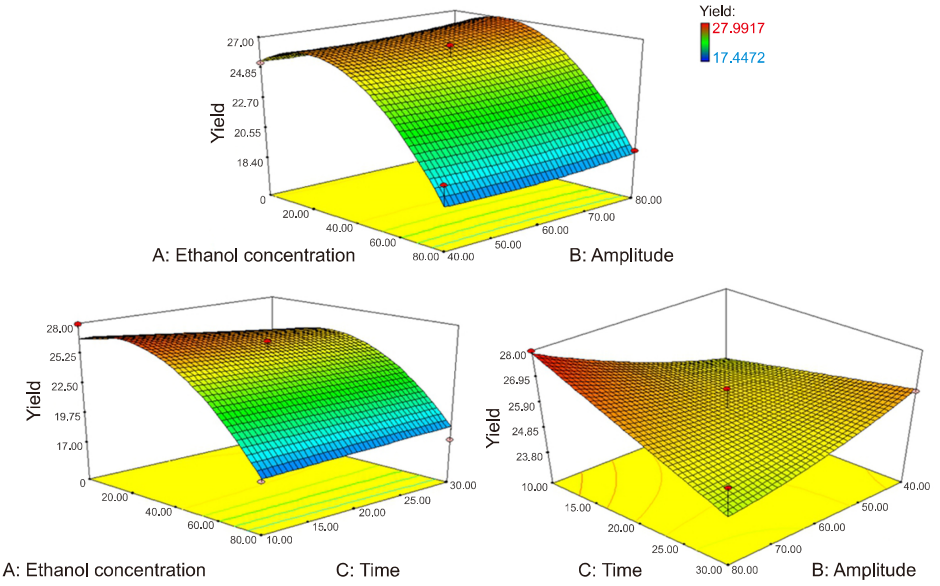

Fig. 1. Response surface plots indicate the effect of ethanol concentration, amplitude, and sonication time on green leafy vegetable extraction yield using ultrasound-assisted extraction.

UAE under suitable conditions could enhance the yield when extraction was performed at 80% amplitude for 10 min. Moreover, extended exposure may result in the deterioration and impairment of the structural characteristics of the extracted compounds (Ali et al., 2019). Moreover, the strong molecular association between metabolites and the cell wall can hinder solvent penetration, resulting in incomplete extraction and reduced efficiency (Chotphruethipong et al., 2019).

Our results observed that the ultrasound-induced cavitation effect was the primarily regulator of the extraction of compounds from GLV cells. Intense cavitation waves have been shown to promote solvent infiltration into the tissue matrix, aiding in mass transfer (Ali et al., 2019). In addition, we also found that the yield was higher under the UAE treatment than the conventional method. High-intensity ultrasound appeared to effectively disrupt cell membranes and cause the release of metabolites from complex tissues, which also led to relatively high efficiency (Table 1 and 4).

***Optimization of AGI activity*:** The use of UAE demonstrated greater AGI activity (Table 1) relatively to conventional extraction methods (i.e., 794.27 to 974.46 mmol ACE/g extract; Table 4). This result is consistent with Li et al. (2020), who reported that UAE facilitates solvent-biomass contact, thereby liberating specific compounds and augmenting AGI activity. Similarly, Duangjai et al. (2021) reported that UAE is an effective tool for enhancing the bioactive compounds associated with α-glucosidase inhibition. Here, the influences of independent variables on AGI activity were assessed by analyzing both linear and interactive effects, as visualized through 3D plots. These plots were then used to determine the optimal conditions for achieving maximum AGI activity, as illustrated in Fig. 2.

Next, the reliability of the model in predicting responses was evaluated by examining the lack of fit value, which was determined to be insignificant ($P>0.05$) with a P-value of 0.3520 (Table 3). This finding suggests that the model developed here can be trusted in predicting the response of AGI activity (Yancheshmeh et al., 2022). Moreover, the extraction yield showed a robust correlation between actual and predicted data, as evidenced by an R^2 value of 0.9875 (Yancheshmeh et al., 2022) and an adj-R^2 value of 0.9649. In addition, the CV value of the AGI activity response (i.e., 3.13%) was found to be less than 10%, indicating a high level of model precision and reproducibility (Yancheshmeh et al., 2022).

Our findings also indicated that higher ethanol concentrations can result in increased AGI activity, as illustrated in Fig. 2. In addition, the combined effect of ethanol concentration, sonication time, and amplitude was also found to contribute to AGI activity. Interestingly, cavitation led to higher AGI activity at an ethanol concentration of 40%, which was higher than the activity observed at other concentrations (Table 1). Due to the principles of phase solubility and similarity, the release of compounds from the cell wall is enhanced when the solute and solvent have similar polarities (Li et al., 2022). Moreover, UAE influences the level of surface contact between the solvent and solute, thereby facilitating the general diffusion of target compounds (Li et al., 2022). UAE also promotes improved extraction, resulting in the optimal production of desired AGI-active compounds at a concentration of 40% ethanol. Similarly, Liu et al. (2022) reported that in a pomegranate peel system combined with UAE, the use of 40%～50% ethanol enhanced the yield of compounds responsible for AGI activity.

We found that the highest level of AGI activity (i.e., 1,460.96 mmol ACE/g extract) was achieved at an am-

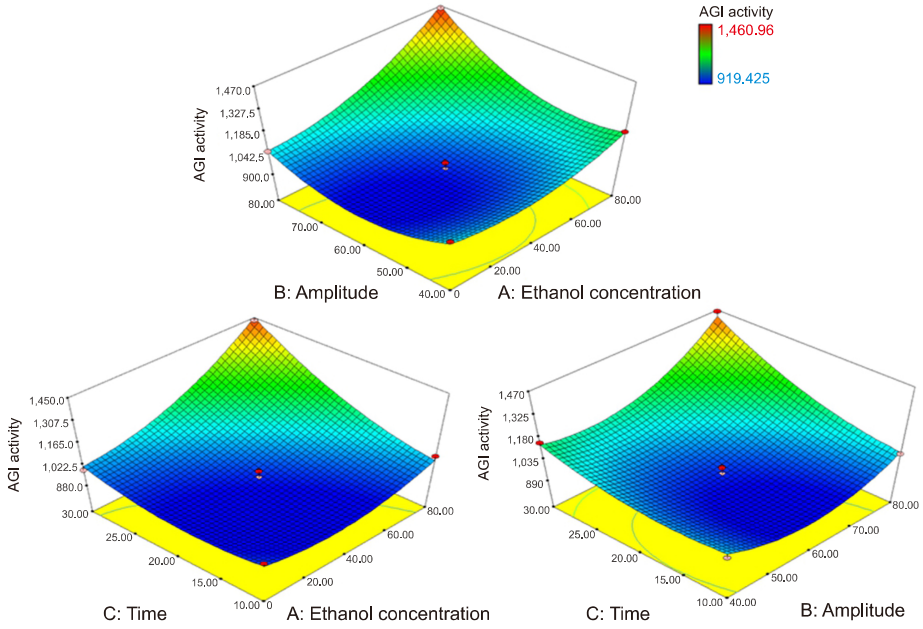

Fig. 2. Response surface plots indicating the effect of ethanol concentration, amplitude, and sonication time on α-glucosidase inhibitory (AGI) activity of green leafy vegetable extracts obtained using ultrasound-assisted extraction.

plitude of 80% using an ethanol concentration of 40% and a sonication time of 30 min. These findings indicated that increasing the amplitude resulted in an increase in AGI activity. Likewise, Gulzar and Benjakul (2019) reported the use of UAE at an ultrasonic amplitude of 80% to obtain extracts from *Litopenaeus vannamei*, thereby offering a promising approach for increasing yield and preserving enzyme inhibitors. Finally, we also note that the intensified collision of cavitation bubbles can facilitate solvent infiltration, thereby enhancing mass transfer rate (Chotphruethipong et al., 2019).

In general, we found that AGI activity increased as the sonication time increased. These findings are consistent with those of a study conducted by Wang et al. (2018), who reported that the extraction of metabolites from apple pomace peaked following being subjected to a sonication treatment for 30 min. Furthermore, Ma et al. (2022) reported that the AGI activity of glycosylated α-lactalbumin and α-lactalbumin digestive products in the intestine and stomach was significantly enhanced ($P<0.05$) at longer ultrasound times. Li et al. (2020) also reported that AGI activity augmented with increasing power and sonication time. Considering the significance of the regression coefficients corresponding to these variables, time may be the most important factor influencing inhibitory activity.

***Validation of optimal conditions*:** Next, a desirability function was used to validate our experimental results and assess the accuracy of the regression equation reported here. In general, desirability functions combine all responses into a single index ranging from 0 to 1 (Ali et al., 2019). Here, a desirability value (D) of 0.801 [calculated using Equation (2)] was achieved when the optimal conditions were applied—this consisted of an ethanol concentration of 44.08%, an amplitude percentage of 80%, and a sonication time of 30 min (Table 3). Under these ideal circumstances, the observed extraction yield was 24.16% and the AGI activity was 1,449.73 mmol ACE/g extract. These values closely matched the predicted extraction yield of 24.21% and the predicted AGI activity of 1,460.96 mmol ACE/g extract. These findings confirm the validity of the RSM model reported here. Hence, we conclude that the ultrasound-facilitated process reported here holds promise as a tool for manufacturing antidiabetic agents, particularly α-glucosidase inhibitors, from GLVs.

Conventional extraction yields of green vegetable combinations

The extraction yields of combined GLV samples at different concentrations of ethanol (Table 4) suggest that the polarity of the solvent plays a pivotal role in determining extraction yield, with more polar solvents resulting in higher yields. This finding indicates that water was the most effective solvent for extracting the desired yield. Similarly, previous studies have also reported that higher ethanol concentrations decrease yield (Javadi et al., 2014; Easmin et al., 2017). In addition, the AGI activity was found to be higher at higher ethanol concentrations (Table 4), which was consistent with the findings of Javadi et al. (2014) and Easmin et al. (2017). Since ethanol has a lower polarity than water, lipophilic compounds can be more easily extracted in ethanol than in water (Easmin et al., 2017). The highest activity was observed in the 80% ethanol extract, thereby suggesting that the presence of more lipophilic compounds in GLV samples may have contributed to the presence of inhibition.

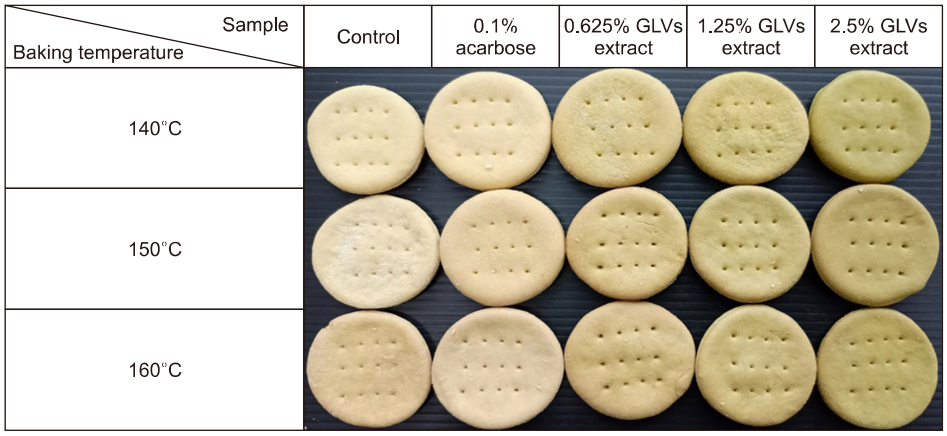

Fig. 3. Green leafy vegetable (GLV) extract-enriched crackers produced at different baking temperatures.

Properties of cracker enriched in GLV extract

The results of our baking experiments revealed that crackers with higher amounts of GLV extract showed a significantly greener color relative to those with lower extract content (Fig. 3). Moreover, as the baking temperature increased, cracker color was darker (Fig. 3). Furthermore, our color analysis of crackers produced at different baking temperatures was performed using the following parameters: L^* (lightness), a^* (redness), and b^* (yellowness), as presented in Table 5. Quantified color analysis of different crackers was found to be consistent with the visual observations reported in Fig. 3. Analysis of L^* values also revealed that the addition of GLV extract led to darker crackers ($P<0.05$) relative to either control and 0.1% acarbose crackers, which did not differ significantly from each other ($P\geq0.05$). With respect to a^* and b^*, we observed a low intensity of red color (ranging from −3.06 to 6.63), and the addition of GLV extract caused the product to become measurably greener (i.e., darker, ranging from 33.45 to 38.26). This effect may be attributed to the higher chlorophyll content of crackers with a higher GLV content. Similarly, Nabil et al. (2020) reported that the addition of *Opuntia ficus-indica* flour to biscuits also resulted in a darker green color (L^* value of 53.22 to 66.74).

Higher baking temperatures generally resulted in darker crackers (Table 5). A darker hue is common in baked biscuits (Al-Ansi et al., 2019). This is because the non-enzymatic Maillard reaction occurs between amino acids and reducing sugars during baking, leading to a brownish effect (Nabil et al., 2020). Moreover, at higher temperatures the green color of high-GLV crackers (Table 5) further because of the degradation of chlorophyll pigments. This agrees with the results of Galla et al. (2017) who reported that chlorophyll levels decline during cooking. In addition, Zheng et al. (2023) reported that elevated temperatures resulted in the increased chlorophyll release, which then undergoes conversion into a brown substance known as pheophytin.

Table 5 illustrates the hardness and crispiness values

Table 5. Properties of green leafy vegetable extract-enriched crackers

No.	Formulation	Baking temperature (°C)	Color			Hardness (g)	Crispiness (mm)
			L^*	a^*	b^*		
1	Control	140	70.39±0.44^{a}	3.65±0.24^{d}	34.43±0.93^{hi}	4,542.75±118.90^{c}	0.48±0.10^{de}
		150	68.43±0.75^{b}	4.56±0.18^{bc}	34.57±1.46^{ghi}	2,664.70±265.08^{f}	1.35±0.30^{abc}
		160	65.47±0.36^{e}	6.53±0.65^{a}	35.73±0.59^{defg}	1,409.91±364.03^{h}	1.64±0.09^{a}
2	0.1% acarbose	140	70.95±0.51^{a}	3.34±0.14^{d}	33.45±0.28^{i}	4,561.14±178.49^{c}	0.30±0.25^{def}
		150	67.04±0.63^{cd}	4.93±0.36^{b}	35.32±0.89^{fgh}	2,765.63±182.85^{f}	1.30±0.06^{abc}
		160	66.06±0.97^{de}	6.63±0.65^{a}	36.27±0.18^{cdef}	1,467.14±264.07^{gh}	1.59±0.34^{a}
3	0.625% extract	140	67.48±0.96^{bc}	1.32±0.30^{e}	38.26±1.00^{a}	4,808.20±239.32^{bc}	0.22±0.06^{efg}
		150	61.69±0.85^{f}	3.38±0.14^{d}	37.25±0.39^{abc}	3,229.86±185.40^{e}	1.10±0.30^{bc}
		160	59.90±0.60^{g}	4.01±0.26^{cd}	36.87±0.18^{bcde}	1,725.92±42.05^{gh}	1.48±0.10^{a}
4	1.25% extract	140	61.57±1.18^{f}	−1.41±0.62^{g}	38.02±0.16^{ab}	5,119.05±204.98^{b}	0.09±0.04^{fg}
		150	59.19±0.89^{g}	−0.01±0.52^{f}	36.05±0.59^{cdef}	3,295.08±206.15^{e}	1.03±0.25^{c}
		160	56.90±0.33^{h}	0.60±0.38^{f}	36.02±0.37^{cdef}	1,830.40±97.04^{g}	1.40±0.09^{ab}
5	2.5% extract	140	59.02±0.57^{g}	−3.06±0.42^{h}	36.95±0.45^{bcd}	5,606.44±359.02^{a}	0.02±0.09^{g}
		150	57.47±0.11^{h}	−1.95±0.35^{g}	35.65±0.70^{efg}	4,005.99±282.98^{d}	0.62±0.07^{d}
		160	54.80±0.44^{i}	0.18±0.08^{f}	33.71±0.39^{i}	2,764.07±58.80^{f}	1.35±0.12^{abc}

Mean±SD of three technical replicates.
Different superscript letters in the same column (a–i) indicate statistically significant differences ($P<0.05$).

of crackers produced at different baking temperatures. The findings of this study differ slightly from those of Dhal et al. (2023), who showed a hardness level of approximately 5,872 g and crispiness of about 0.6 mm for their control crackers. These differences may be attributed to differences in constitutive ingredients. Furthermore, cracker hardness also increased with increased extract levels ($P<0.05$), whereas the crispiness levels were lower relative to either the control and 0.1% acarbose crackers, which did not significantly differ from each other ($P\geq0.05$). Previous studies have also noted a reduction in hardness that is related to increased crispiness (Dias-Faceto et al., 2020). In another study, Galla et al. (2017) reported that the inclusion of spinach (*Spinacia oleracea* L.) in crackers resulted in increased hardness, while Indiarto et al. (2023) reported that TPC can influence cracker texture. Polyphenols can serve as reducing agents for disulfide bonds, and lead to the augmentation of thiol groups and other covalent bonds (Indiarto et al., 2023). These changes can affect dough morphology and thereby influence factors such as viscosity, elasticity, and strength (Indiarto et al., 2023). In addition, the presence of phenolic compounds during gelatinization can impede the absorption of water by starch granules since starch competes with hydrophilic polyphenols for water molecules (Indiarto et al., 2023). As a result, increases in TPC can lead to higher product hardness (Indiarto et al., 2023). Fig. 4 demonstrates that the higher extract numbers of crackers correspond to higher TPC levels, while increasing baking temperatures reduced TPC.

Higher baking temperature resulted in reduced hardness and an enhancement in the crispiness of crackers (Table 5). These results are consistent with those of Sazesh and Goli (2020), who reported that cracker hardness decreased when the baking temperature was lowered from 160°C to 185°C. This was thought to occur due to the protein network coagulating more rapidly as well as an increase in carbon dioxide gas entrapment within the cracker at higher baking temperatures, two processes that resulted in increased porosity and decreased cracker density (Sazesh and Goli, 2020). Here, these factors likely also affected the crispiness of the crackers. Furthermore, the low moisture content of the high-baking-temperature crackers also contributed to their increased crunchiness, as depicted in Fig. 5. Water plays a crucial role in many manufacturer foods, since it significantly influences the overall texture, appearance, and flavor (Setyaningsih et al., 2019).

The moisture content of crackers at various baking temperatures is illustrated in Fig. 5. Generally, an increase in the amount of extract used led to a rise in moisture content. In addition, higher moisture content is known to be related to hydrophilic content (Bolarinwa et al., 2019). Here, the use of the GLV extract may cause crackers to contain a higher level of hydrophilic compounds, including polyphenols and polyol (Maser et al., 2023a), thereby resulting in a greater ability to retain water (Al-Ansi et al., 2019). These moisture content results align with those reported by Jiang et al. (2022), who found that crackers supplemented with papaya seed and peel had moisture contents ranging from 7.83% to 15.42%. However, when the baking temperature increased, the moisture content of the crackers decreased (Fig. 5). During the baking process, the moisture within the dough vaporizes from the surface of the cracker, causing moisture deep within the cracker to migrate to the surface, after which it to vaporizes (Setyaningsih et al., 2019). This leads to a steep reduction in moisture content that is proportional to the temperature increase (Ranjbar et al., 2014).

Strongly correlated with moisture content was the water content of crackers, which also increased as the amount of the GLV extract used rose (Fig. 6). Similarly, as the baking temperature increased, we observed a correspond-

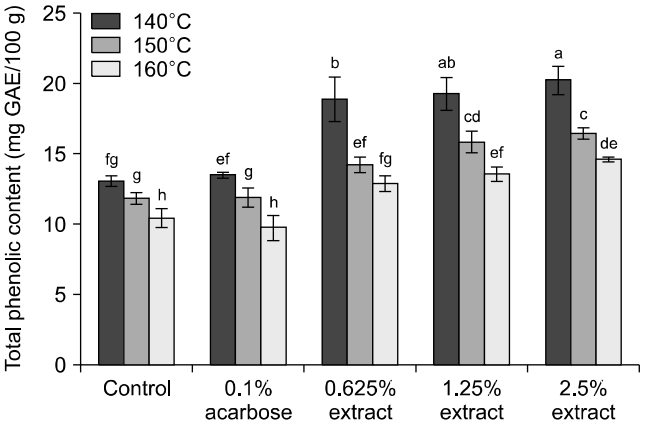

Fig. 4. Total phenolic content of green leafy vegetable extract-enriched crackers produced at different baking temperatures. Bars represent standard deviation (n=3). Different lowercase letters (a-h) indicate statistically significant differences ($P<0.05$).

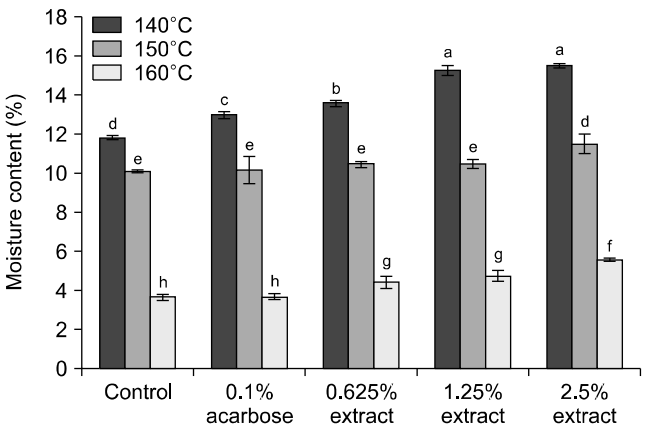

Fig. 5. Moisture contents of green leafy vegetable extract-enriched crackers produced at different baking temperatures. Bars represent SD (n=3). Different lowercase letters (a-h) indicate statistically significant differences ($P<0.05$).

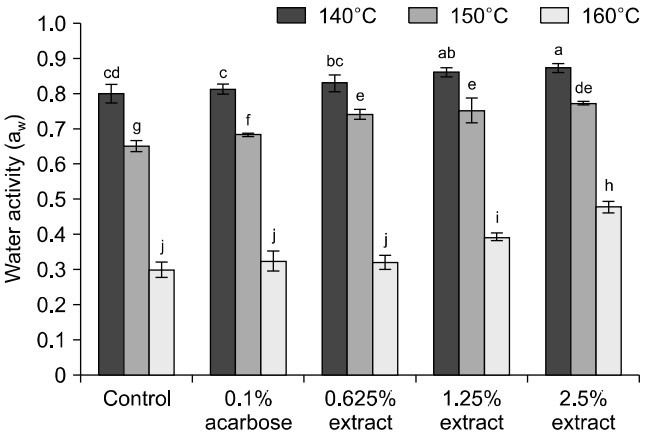

Fig. 6. Water activity (a_w) of green leafy vegetable extract-enriched crackers produced at different baking temperatures. Bars represent standard deviation (n=3). Different lowercase letters (a-j) indicate statistically significant differences (P<0.05).

ing decline in water activity. It is likely that reduced water activity is connected to the reduction in moisture content (Renshaw et al., 2019). Taken together, these findings align with previously reported results for olive leaf extract crackers, in which crackers with a moisture content of approximately 3.73 had a water activity of approximately 0.3 (Paciulli et al., 2023).

Antioxidant activities of crackers enriched with GLV extract

Fig. 4 illustrates the TPC of crackers enriched in GLV extract at various baking temperatures. In general, a higher amount of GLV extracts corresponded to higher TPC values of the crackers. Moreover, the TPC results recorded here were slightly lower than those found in a prior study where wheat cookies exhibited a TPC of 19.23 mg GAE/100 g (Sharma et al., 2016). The observed differences in phenolic acid content can be accounted for by diverse factors, including variation in geographical conditions, species, and other environmental influences (Nabil et al., 2020). Furthermore, higher baking temperatures led reduced TPC content across all cracker batches. Similarly, Nabil et al. (2020) reported that baking Moroccan cladode flour biscuits at 160°C for a few minutes resulted in minimal phenolic compound (e.g., ferulic acid) content. This may be due to structural alterations in phenolics caused by heating, and the degree of polyphenol degradation is contingent on the material composition and processing conditions (Nabil et al., 2020).

Next, the DPPH activity of GLV extract-enriched crackers is presented in Table 6. Overall, we found that the DPPH activity of the crackers increased as the amount of extract increased. Similarly, Paciulli et al. (2023) noted that adding olive leaf extract significantly boosted DPPH activity relative to an unenriched control group. Next, a direct link between TPC (Fig. 4) and DPPH (Table 6) was established, which confirmed the antioxidative potential of the polyphenols present in the GLV extract. However, higher baking temperatures also decreased the DPPH activity of crackers. In one paper, Zheng et al. (2023) reported that the loss of antioxidant activity occurs due to the poor thermal stability of certain antioxidant compounds under prolonged exposure to heat. Changes in heat-sensitive antioxidants therefore led to an overall decline in antioxidant activity.

Table 6. Antioxidant and antidiabetic activities of green leafy vegetable extract-enriched crackers

No.	Formulation	Baking temperature (°C)	DPPH (mg AEAC/100 g)	Metal chelating (mg EECC/100 g)	FRAP (mg AEAC/100 g)	AAI (mmol ACE/100 g)	AGI (mmol ACE/100 g)	LPI (% inhibition)
1	Control	140	11.76±0.06^{c}	0.79±0.08^{f}	10.77±0.51^{d}	40.07±3.38^{c}	134.68±11.70^{de}	22.26±0.97^{bcd}
		150	10.16±0.04^{e}	0.40±0.03^{h}	8.73±0.11^{f}	35.47±2.38^{de}	127.00±12.69^{e}	19.61±1.27^{fgh}
		160	9.92±0.22^{e}	0.24±0.02^{i}	8.54±0.21^{f}	29.79±2.20^{f}	77.67±7.11^{f}	17.73±1.24^{h}
2	0.1% acarbose	140	12.28±0.16^{b}	0.80±0.05^{f}	11.06±0.49^{d}	40.69±2.26^{bc}	209.94±11.28^{a}	22.32±0.56^{bcd}
		150	10.24±0.36^{e}	0.42±0.04^{h}	10.04±0.29^{e}	37.43±2.64^{cd}	152.25±9.58^{cd}	19.69±1.62^{fgh}
		160	9.87±0.07^{e}	0.21±0.03^{i}	8.61±0.11^{f}	30.67±0.71^{f}	83.13±6.95^{f}	19.13±1.08^{gh}
3	0.625% extract	140	12.42±0.84^{b}	1.45±0.06^{c}	13.04±0.35^{b}	41.25±1.64^{bc}	225.30±15.21^{a}	22.84±1.97^{bc}
		150	11.03±0.26^{d}	0.97±0.10^{e}	12.09±0.41^{c}	39.81±1.98^{c}	170.69±14.14^{bc}	20.20±1.04^{def}
		160	9.98±0.21^{e}	0.67±0.01^{g}	11.23±0.42^{d}	31.70±1.71^{ef}	91.15±6.60^{f}	19.19±1.37^{gh}
4	1.25% extract	140	13.04±0.13^{a}	1.59±0.01^{b}	14.62±0.32^{a}	44.45±4.19^{ab}	226.84±19.76^{a}	23.31±1.26^{b}
		150	11.11±0.22^{d}	1.08±0.03^{d}	12.14±0.43^{c}	40.07±1.73^{c}	183.83±17.66^{b}	20.58±0.95^{cdef}
		160	10.16±0.04^{e}	0.74±0.05^{fg}	11.23±0.52^{d}	32.11±1.17^{ef}	98.83±4.11^{f}	19.28±1.30^{gh}
5	2.5% extract	140	13.38±0.05^{a}	1.86±0.08^{a}	14.95±0.17^{a}	47.76±0.63^{a}	227.35±12.29^{a}	26.70±0.75^{a}
		150	11.29±0.20^{cd}	1.10±0.02^{d}	14.72±0.46^{a}	45.59±2.06^{ab}	189.46±12.33^{b}	21.94±0.70^{bcde}
		160	10.94±0.40^{d}	0.83±0.07^{f}	13.69±0.86^{b}	34.85±1.48^{de}	142.70±9.97^{de}	19.45±1.91^{gh}

Values are presented as mean±SD of three technical replicates.
Different superscript letters in the same column (a-i) indicate statistically significant differences (P<0.05).
DPPH, 2,2-diphenyl-1-picrylhydrazyl; AEAC, L-ascorbic acid equivalent antioxidant capacity; EECC, ethylenediaminetetraacetic acid equivalent chelating capacity; FRAP, ferric reducing antioxidant power; AAI, α-amylase inhibitory; ACE, acarbose equivalent; AGI, α-glucosidase inhibitory; LPI, lipase inhibitory.

Like DPPH activity, the metal chelating activity of the crackers was found to increase with increasing GLV extract but decreased with higher baking temperature (Table 6). This occurrence may be associated to the presence of phytochemical antioxidants within the GLV extract. We also observed that the metal chelating activity correlated with cracker TPC content (Fig. 4). In one recent study, Maser et al. (2023b) also reported a positive correlation in GLV extracts between metal chelating activity and TPC ($P<0.01$), which suggests that phenolic compounds such as flavonoids, condensed tannins, and phenolic acids may significantly contribute to metal chelation. Similarly, Arogundade et al. (2023) found that metal chelating ability increased with the addition of plantain flour and bambara groundnut blends to biscuits. In general, metal chelation activity stems from various antioxidant mechanisms, in which the ability of antioxidants to chelate metals prevents transition metals from instigating lipid peroxidation and oxidative stress during metal catalysis (Arogundade et al., 2023).

Next, Table 6 shows the FRAP activity of crackers enriched GLV extract baked at different temperatures. In general, higher GLV content correlated with higher FRAP activity, but higher baking temperatures were associated with lower FRAP activity. In this study, we identified a connection between TPC and FRAP activity; this is similar to the results of a prior investigation, which reported a linear correlation ($P<0.01$) between FRAP activity and TPC (Maser et al., 2023b). This finding suggests that polyphenols found in natural products, which are capable of neutralizing radicals by providing hydrogen, serve as major antioxidants (Maser et al., 2023b). These outcomes further align with the findings of Olagunju et al. (2018), who reported that the addition of pigeon pea flour heightened the FRAP activity of another type of biscuit. Moreover, an increase in the ability of crackers to transform Fe^{3+} into Fe^{2+} was found to correspond to the augmented inclusion of bioactive compounds. This ability may be due to the potential of these compounds to generate reducing substances that react with free radicals (Olagunju et al., 2018).

Antidiabetic activities of GLV-enriched crackers enriched with the combination extract

Table 6 presents the AAI activity of crackers enriched with GLV combination extracts. The control cracker demonstrated a relatively favorable AAI activity of 40.07 mmol ACE/100 g cracker. Similarly, a study by Olagunju et al. (2018) reported that their control (100% wheat) cracker exhibited an AAI activity of approximately 54.22% inhibition. Furthermore, we also found that AAI activity was contingent on the concentration of the combination extract. For example, incorporation of 0.1% acarbose displayed an inhibitory efficacy equivalent to the use of 0.625% GLV extract ($P\geq0.05$). Acarbose, a drug that has been clinically validated for hyperglycemia control, functions as a mixed-type inhibitor against α-amylase (Yang et al., 2021). Increasing the amount of GLV extract led to further escalation in AAI activity. These findings align with those of Olagunju et al. (2018), who reported that use of pigeon pea flour led to enhanced cracker AAI activity. However, we also found that higher baking temperatures decreased AAI activity and observed that there was a notable correlation between TPC and AAI activity. Maser et al. (2023b) reported a positive association between AAI activity and TPC in response to the incorporation of various GLVs ($P<0.01$). Taken together, our results emphasize the importance of phenolic compounds for the inhibition of carbohydrate metabolism. This importance arises from the ability of phenolic compounds to chemically bind to various enzyme, thereby inducing altered enzyme activity (Maser et al., 2023b).

Next, we measured the inhibition of α-glucosidase in crackers enriched with GLV extracts at various baking temperatures; these results are presented in Table 6. The control crackers displayed a moderate AGI activity of 134.68 mmol ACE/100 g. This result slightly exceeds that reported by Olagunju et al. (2018), who documented 15.56% AGI activity in 100% wheat crackers. This difference may be attributed to variation in ingredients used, including the presence of soybean oil, which is known for its high oleic and linoleic acid contents and is widely recognized as increasing AGI activity (Su et al., 2013). Moreover, we also observed that the addition of 0.1% acarbose resulted in a significant improvement relative to control crackers ($P<0.05$). This compound competitively binds to the enzyme's active site, resulting in diminished glucose absorption and thereby preventing increases in glycemic index (Yang et al., 2021).

The addition of various concentrations of GLV extract was associated with different increases in AGI activity, although this effect was not statistically significantly different from the addition of 0.1% acarbose ($P\geq0.05$). This phenomenon may be attributed to bioactive compound degradation at high baking temperatures. Moreover, AGI activity did not correlate with TPC, which suggests that other bioactive substances may be responsible for this inhibitory activity. For instance, Allium species (e.g., spring and bunching onions) contain primary organosulfur compounds that have also shown potential for diabetes treatment (Maser et al., 2023b). However, these compounds are susceptible to degradation during heating, as demonstrated by Wongsa et al. (2023). In that study, the authors observed a 66.11% loss of organosulfur compounds in *Allium sativum* L. after heating to 80°C. Moreover, higher baking temperatures were associated with decreased AGI activity. Similarly, Wongsa et al. (2023) also reported that increased hot-air drying temperatures reduced

the inhibitory activity against α-glucosidase.

Table 6 presents the LPI activity of crackers enriched with GLV extract at various baking temperatures. Here, adding 0.1% acarbose did not yield a significant difference in LPI activity compared to control crackers ($P \geq 0.05$). However, the enhancement conferred by the combination extract led to increased activity, whereas the increase in baking temperature resulted in a notable decrease in activity ($P<0.05$). Similarly, Irondi et al. (2021) reported a significant decrease in IC_{50} LPI activity following the supplementation of biscuit flour with *Vigna unguiculata* L. Walp and *Zea mays* L. instead of using 100% refined wheat flour. In this study, LPI activity measurements were found to correlate with cracker TPC measurements. In another study, Maser et al. (2023b) reported a correlation between LPI activity and TPC ($r=0.804$, $P<0.01$) in GLV, which suggests that phenolic compounds within these extracts are likely responsible for observed differences in LPI activity.

Proximate composition of crackers

Next, we selected the cracker formulation featuring the most potent antidiabetic, antiobesity, and antioxidant properties and determined their proximate composition. First, all crackers baked at 140°C were selected for proximate composition analysis (Table 7). The moisture content of these crackers was found to increase following supplementation with 0.1% acarbose and GLV extract ($P<0.05$). This may occur because acarbose, a highly hydrophilic polyol (Zhang et al., 2012), elevates the water content. In addition, the GLV extract may contain a significant proportion of hydrophilic compounds such as polyphenols (Fig. 4), which may also enhance water retention within the structure (Al-Ansi et al., 2019). Similarly, Jiang et al. (2022) noted that crackers enriched with papaya peel exhibited a moisture content of 15.42%. Like moisture content, the ash content was found to increase following the addition of the GLV extract ($P<0.05$). In contrast, adding 0.1% acarbose showed no significant difference in ash content relative to the control crackers ($P \geq 0.05$). It has been widely reported that GLVs contain elevated mineral levels (Sarkar et al., 2023). Similarly, Galla et al. (2017) reported that increased *S. oleracea* L. leaves had a relatively high ash content. Furthermore, our ash content results were consistent with the findings of Jiang et al. (2022), who reported an ash content of 1.20% in control biscuits supplemented with papaya peel and seed. Furthermore, Gupta et al. (2021) reported that *A. cepa* was found to contain aluminum (Al), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), calcium (Ca), magnesium (Mg), sodium (Na), phosphorus (P), and potassium (K), and the presence of Ca, K, Mg, P, Fe, Mn, nickel (Ni), and Al was reported in *A. fistulosum* (Wang et al., 2020). In another study, Singh et al. (2023) reported the presence of P, K, Ca, Fe, Zn, Mn, Cu, and Na in *A. graveolens* L. Given these results, we assume that the incorporation of GLV extracts into crackers may enhance their mineral content.

Unlike moisture content, we found no significant differences in crude protein and fat content among cracker types ($P \geq 0.05$). This makes sense, since GLVs are not significant sources of protein or fat (Sarkar et al., 2023). In contrast, we observed a notable decline in carbohydrate content following incorporation of the GLV extract ($P<0.05$). This reduction can be attributed to the fact that the inclusion of the GLV extract led to a decrease in the quantity of wheat flour per cracker and resulting in lower carbohydrate content. Similarly, Al-Ansi et al. (2019) and Jiang et al. (2022) also reported carbohydrate contents of 49%~51% in biscuits containing *Foeniculum vulgare* and *Nigella sativa*, as well as in biscuits supplemented with papaya seed and peel.

In summary, in this study we report an optimized ultrasound-assisted process for extracting antidiabetic metabolites from GLVs. Overall, this method was more effective than conventional extraction methods in extracting these metabolites. The optimal extraction conditions for samples tested here were 44.08% ethanol concentration, 80% amplitude, and 30 min sonication time. These conditions resulted in a 24.16% increase in extraction yield compared to a conventional extraction method and an AGI activity of 1,449.73 mmol ACE/g extract. In addition, sonication improved yield while reducing solvent consumption and showed no detrimental effects on AGI activity. Next, when the GLV extract was used for the production of crackers at different baking temperatures, we observed a general increase in the amount of total phenolic compounds and their associated bioactivi-

Table 7. Proximate composition of green leafy vegetable extract-enriched crackers produced at a baking temperature of 140°C

No.	Formulation	Moisture (%)	Ash (%)	Crude protein (%)	Fat (%)	Carbohydrate (%)
1	Control	11.80±0.12^{d}	1.41±0.03^{c}	18.12±0.62^{a}	17.75±0.62^{a}	50.92±0.47^{a}
2	0.1% acarbose	12.97±0.19^{c}	1.43±0.02^{c}	17.80±0.79^{a}	17.74±0.25^{a}	50.06±0.42^{a}
3	0.625% extract	13.57±0.16^{b}	1.53±0.01^{b}	17.52±0.14^{a}	17.64±0.70^{a}	49.74±0.34^{ab}
4	1.25% extract	15.28±0.37^{a}	1.54±0.01^{b}	17.32±0.59^{a}	17.46±0.85^{a}	48.40±0.61^{b}
5	2.5% extract	15.51±0.12^{a}	1.69±0.00^{a}	17.22±0.06^{a}	17.28±0.62^{a}	48.30±0.27^{b}

Values are presented as mean±SD of three technical replicates.
Different superscript letters in the same column (a-d) indicate statistically significant differences ($P<0.05$).

ties, including antioxidant capacity and inhibitory effects on enzymes such as α-glucosidase, α-amylase, and lipase. However, higher baking temperatures (e.g., 160°C) reduced the amount of total phenolic compounds present as well as their bioactivity, and therefore lower baking temperatures such as 140°C or 150°C are recommended. Further research is recommended to examine the textural and sensory evaluations of GLV-supplemented crackers as well as to evaluate food safety and consumer acceptance.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: AMMA, SCBB. Analysis and interpretation: WHM, NM, NH. Data collection: WHM, NM, SK. Writing the article: WHM, SK, PN. Critical revision of the article: PN, AKT, NH, AMMA, SCBB. Final approval of the article: all authors. Statistical analysis: WHM, NM. Obtained funding: WHM, AMMA, SCBB. Overall responsibility: PN, AMMA, SCBB.

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