Heliyon 10 (2024) e24443

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Development of functional cookies form wheat-pumpkin seed based composite flour

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A R T I C L E I N F O

Keywords:
Cookies
Pumpkin seed
Wheat
Food process
Nutritional quality
And optimization

A B S T R A C T

To develop high quality cookies, even seemingly smallest changes depended on factors that can affect taste, texture, and nutritional value. In this light, this study aimed to investigate the upshot of refined wheat flour and pumpkin seed flour on properties of cookies such as antioxidant activity, thermal and oxidative stability. In view of the foregoing, the roasted pumpkin seeds of particle size below 500 μm were blended with wheat flour at different ratios (BR) to bake at selected pre-determined temperatures (T) and time durations (TD). The synergetic effect of aforesaid parameters on cookie development, BR, T, and TD was studied by varying the parameters between the range 6–15 %, 180–200 °C and from 8 to 12 min, respectively, for the baking process of cookies. Further, the process was modelled and scrutinized using numerical optimization to achieve a highly acceptable product. On that account, it was deduced that the optimal condition for BR, T, and TD were 12.87 %, 186 °C and 9.5 min, respectively, that could pave to beget the excellent quality cookies with overall acceptance score of 8, protein content 14.28 %, fat 17.85 %, ash 2.23 %, moisture 2.46 %, fiber 2.38 % and total color difference 12.01. The optimized cookies (OCs) were found to have higher protein (11.49–14.28 %), fiber (0.93–2.41 %), ash (2.19–1.77 %), total antioxidant activity (38.7158–43.1860 %), oxidative stability (28.61–51.24 h), Zn (1.42–2.63 mg/100g), and Fe (2.12–3.20 mg/100g) content as compared to the control. Laconically, the study results provided the optimized processing condition for developing high quality cookies with respect to improved nutritional value and comparable overall acceptability.

1. Introduction

Undeniably, imparting the enrichment of food quality to accomplish the prime quality product is serious concern for managing the

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https://doi.org/10.1016/j.heliyon.2024.e24443
Received 7 August 2023; Received in revised form 22 December 2023; Accepted 9 January 2024
Available online 10 January 2024
2405-8440/

food security and preventing specific chronic diseases and dietary shortcomings as well as promoting communal well-being of various populations. In light of this, food technologists are still looking for developing a better quality food products using native bioavailable fortifying agents [1,2]. Among the different food products, confectionery and pastries products offer various nutritional contentious ingredients that may serve as appropriate paradigms for food quality enrichment. In consequence, the use of natural ingredients, such as polyphenols (cumin, sorghum, pomegranate peel, and ginger), high fiber cereal (oat), carotenoids (pumpkin seed powder), and monounsaturated/polyunsaturated fat (canola, olive, soy, and sunflower), exogenous amino acids (lysine, tyrosine, tryptophan, and methionine), minerals (pumpkin seeds) are recognized for upgrading the sensory, rheological, and nutritional attributes of confectionery food products [3,4].

However, among aforementioned food ingredients for value added purpose, the pumpkin (*Cucurbita pepo*) seeds are seem to be one of the underutilized food values [4]. Pumpkin seeds are the edible kernels of fruit that are obtained as plant by-products. The seeds are semi-flat features, typically ovoid shape with a conical tip. Inside its kernels feature olive-green color, sweet, buttery in texture and nutty in flavor and have a white outer hull. In general, the seeds are benefited as plant by-product to utilize as food and to extract edible oil since centuries.

In recent years, pumpkin seeds have received a considerable attention due to its health protective and nutritional benefits. Pumpkin seeds are characterized by different nutrients and medicinal properties, hence, these seeds are used for remedial purposes across many countries [5]. Customarily, pumpkin seeds are earthy in flavor and utilized for preparing variety of dishes like snacks, soups, and salads. pumpkin seeds are ascertained to be a good source of calories, proteins, carotenoids, minerals (iron, zinc, magnesium and calcium), fiber and phytosterols. Furthermore, pumpkin seeds are proven to be one of the important sources of omega 3 and omega 6 fatty acids that are crucially needed for brain function and healthy skin [6].

In the world of bakery products, cookies emerge as a prime candidate for incorporating new and innovative ingredients. They adhere to a trifecta of desirable qualities: affordability, extended shelf life, and widespread appeal across all age groups. With a global consumption of a staggering 10 million tons annually, cookies present a significant opportunity for the food industry to introduce novel and nutritious ingredients [7]. The growing demand for healthier products with functional properties has prompted the food industry to explore the use of unconventional flours in cookies. Whole wheat flour has become a popular choice, offering enhanced fiber content and other nutrients. However, researchers have ventured beyond traditional grains, investigating the potential of pulse flours derived from legumes [8].

Keeping in view of the significance on pumpkin seeds, the aim of this study was focused to optimize cookies processing by supplementing pumpkin seed flour in wheat flour as an alternative value-added product to provide nutritionally enhanced cookies with improved dietary fiber, protein, zinc, iron, calcium and magnesium and reduced carbohydrate content.

2. Materials and methods

2.1. Sample collection and preparation

The pumpkin seeds (*Cucurbita pepo*) and wheat flour and additional ingredients like vegetable oil, sugar, baking soda, baking powder and salt were procured from the local market in Addis Ababa, Ethiopia. The all-purpose wheat flour was used in this research (colloquially, Kojj wheat flour). The sample was kept at room temperature in sterilized polyethylene bags. Clean pumpkin seeds were singled out from immature seeds and washed to eliminate sands, and soil. Followed by washing with tap water, seeds were oven dried at 50 °C then roasted at 100 °C for 15 min. Further they were milled and sieved to separate the particles having sized below 500 μm [9]. The flour was stored at 4 °C using in a polyethylene bags further use and analysis.

2.2. Preparation and optimization of cookies processing using pumpkin seed flour

The cookies were prepared by adopting standard method AACC 116 as reported by Sibian, with slight modifications [10]. In line with this, a 100 g flour of flour mixture which was blended with pumpkin seed and wheat flours, 37.5 g palm oil, 43.75 g sugar, 1 g $NaHCO_3$, 1.25 g salt, 1 g backing powder, and 22 g water were mixed with pre-calculated blended flour of pumpkinseed and wheat. Different blending ratios were used to make cookies dough that was kneaded with fixed thickness of 3 mm and 4.5 cm diameter. The cookies were baked in free stand baking oven at different operating conditions. Then, cooled off to room temperature. Baked cookies were packed in sealed plastic bags and stored at −20 °C until further analysis [11]. Using response surface method via Design Expert software (Stat-Ease Inc., Minneapolis, MN55413, USA), the combinations of selected parameters were set and the baking process was optimized using statistical modelling and optimization for better quality cookies [12]. To study the effect of each operating condition, a preliminary experiment had been conducted. In addition, the optimized product was characterized with referring to different standardized techniques.

2.3. Experimental design

A central-composite experimental design, with three variables (blending ratio, baking temperature, and baking time, here after, BR, T, TD, respectively) were chosen to examine the response pattern for moisture, ash, protein, fat, fiber, total color difference and overall acceptance of cookies. Experiments studies were performed in order to follow the pre-determined experimental boundaries. Based on the preliminary study conducted, BR ranged from 6 to 15 %; T, 180–200 °C; and TD of 8–12 min were considered. Table 1 shows levels for each selected parameter focused in this study.

Each set of experimental runs was conducted in triplicate, average result values were deduced and considered. Using RSM, A statistical equation of model was generated that correlates the dough formulations and baking process in terms of selected parameters. Response surface regression for the expected response was analyzed using model generated by Design Expert as presented in Equation (1).

$$Yi = B_O + B_1X_1 + B_2X_2 + B_3X_3 + B_{11}{X_1}^2 + B_{22}{X_2}^2 + B_{33}{X_3}^2 + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{23}X_2X_3 \quad (1)$$

where Y_i refers predicted value of response, B_o known as offset term, B_1, B_2 and B_3 are nothing but the terms correlated to linear effect, B_{11}, B_{22} and B_{33} are referred to squared effects and B_{12}, B_{13} and B_{23} are the interaction effects.

2.4. Model validation and optimization of process variables

The significance of the developed model of equation was evaluated for its statistical significance using analysis of variance (ANOVA). Table 2 presents the setting criteria of selected parameters that were applied for numerical optimization.

2.5. Characterization of cookies acquired from optimized conditions

2.5.1. Proximate and mineral analyses of cookies

By adopting the optimized condition, the cookies were prepared and analyzed for their characteristics using Association of Official Analytical Chemists standard procedure [13].

2.5.2. Moisture content determination

Presence of moisture content in the cookies prepared at optimal condition was ascertained using the 2 g of cookies sample which was oven dried (Binder, Germany) at 105 ± 3 °C for 24 h. The mass of the sample before and after drying was weighed [14]. The moisture content was determined using Equation (2).

$$\mathbf{Moisture}\ (\%) = \frac{\mathbf{W_1 - W_2}}{\mathbf{W_s}} * \mathbf{100} \quad (2)$$

Where; W_1 is a weight of cap and a fresh sample, W_2 is a weight of dry sample and cap and W_S is sample weigh.

2.5.3. Ash determination

The ash content of the sample was evaluated by igniting 4 g of sample at 550 °C for the samples are igniting until light gray results or to constant weight obtained in a muffle furnace (Nabertherm, Germany). The sample was cooled in a desiccator at room temperature and weighed. It was determined by dividing the residue mass by the initial mass as is given in Equation (3) [15].

$$\boldsymbol{Ash}\ (\%) = \frac{\boldsymbol{W_1 - W_2}}{\boldsymbol{W_s}} * \mathbf{100} \quad (3)$$

Where; W_1 is a weight of ash + crucible after formation of ash, W_2 is a weight of empty crucible and W_S is a weight of sample taken.

2.5.4. Crude protein determination

The crude protein of cookie sample was determined by digesting 1 g of dried sample in 8 mL concentrated sulfuric acid (98 %) and mixture of potassium and copper sulphate (1:9) using Kjeldahl flask. The flask was swirled in order to mix the contents thoroughly then placed on heater to start digestion till the mixture become clear. After 2 h, the digest was cooled off. Then, 30 mL of distilled water, 10 mL sodium hydroxide solution (35 %) were added and the mixture was under taken to distillation using Kjeldahl unit (Tecator Kjeltec BSXT-06, Zhuanznan, China). Distillation was continued for at least 5 min and the produced NH_3 was collected as ammonium hydroxide in a conical flask containing 20 mL of 2 % boric acid solution with few drops of modified methyl red indicator and yellowish color appear due to ammonium hydroxide. The distillate was titrated against standard 0.1 N HCl solution until it turned to pink color. Percent of nitrogen content was calculated using Equation (4) and the crude protein was deduced using Equation (5) [16].

$$\%\ \mathbf{N} = \frac{\mathbf{T - B * N * 14}}{\mathbf{W_s}} * \mathbf{100} \quad (4)$$

Table 1
Chosen variables and their ranges with actual and coded levels.

Independent variables	Variable symbol	Unit	Level of coded variables		
			Low	Center	High
			−1	0	+1
Blending ratio	A	%	6	10.5	15
Baking temperature	B	°C	180	190	200
Baking time	C	min	8	10	12

Table 2
Summary of criteria for selected parameters to be optimized.

Parameters	Goals	Lower limits	Upper limits	Importance
Blending ratio	in range	6	15	3
Baking temperature	in range	180	200	3
Baking time	in range	8	12	3
Overall acceptability	maximize	6.66	8.15	3
Protein content	maximize	10.7	16.8	3
Fat content	in range	14.6	19.23	3
Ash content	maximize	1.91	2.5	3
Moisture content	minimize	2.25	3.01	3
Fiber content	maximize	2.02	2.6	3
ΔE	minimize	5.55	16.33	3

$$\textbf{Crude protein}\ (\%) = \%\ \mathbf{N} * \mathbf{6.25} \tag{5}$$

Here, T refers the volume of titration for sample (mL), B is known as volume of titration for blank (mL). N refers the normality of HCl.

2.5.5. Crude fat determination

In the present study, the total fat content present in the optimized cookies was ascertained using solvent auto extractor (SER 158, VELP Scientifica, Italy). In this regard, an 8 g of dried powder sample within cellulose thimble and 100 mL diethyl ether within extraction cap were added to extraction chamber and the extraction process proceeded for 3 h. The extract was put in an oven (Binder, Germany) at 105 °C for 1 h to evaporate the solvent. The amount of fat was determined gravimetrically using Equation (6) [16].

$$\textbf{Fat}\ (\%) = \frac{\mathbf{W_2 - W_1}}{\mathbf{S_w}} * \mathbf{100} \tag{6}$$

Where; W_2 is the weight of extraction cup after extraction process with fat, W_1 is the weight of empty extraction cup and S_W is sample weight.

2.5.6. Crude fiber determination

The total dietary fiber (TDF) present in the sample of cookies was determined by adopting the standard procedure as per AOAC 962.09 [13]. In compliance with this, 2 g of grounded cookies sample has been boiled in 200 mL of H_2SO_4 (0.128 %) for 30 min. The solution was filtered and the obtained residue has been rinsed using distilled water followed by it was boiled in NaOH (0.313 %) for 30 min. Again, obtained residue was rinsed with water and made dried using a hot air oven (Binder, Germany) at 230 °C for 2 h. Further, it was heated at 550 °C for 2 h using the muffle furnace (Nabertherm, Germany). As signified in Equation (7) [16], presence of crude fiber (%) was determined.

$$\text{Crude fiber}\ (\%) = \frac{(W_1 - W_2)}{W_s} * 100 \tag{7}$$

Here; W_1 refers weight of dry sample, W_2 denotes the weight of ash sample and W_S is known as weight of sample.

2.5.7. Determination of total carbohydrate

The estimation of total carbohydrate present in the cookies was ascertained according to standard method [13]. In this line, the fat content, crude protein, ash and moisture were deducted from the organic matter, besides, the remained at hand can be considered as carbohydrates.

2.5.8. Determination of gross food energy

The energy value of cookies was considered for the three nutrients groups, such as crude protein, crude fat and carbohydrates. The energy values of gross nutrients was deduced using appropriate conversion factors [13]. In this case, the energy value of the cookies was calculated according to Equation (8) [17].

$$\text{Food energy}\ (Cal) = [\text{TC}(\%) - \text{CF}] * 4 + [\text{TF}(\%) * 9) + [\text{CP}(\%) * 4)] \tag{8}$$

Where, TC is total carbohydrate, TF denotes the total fat, CP is known as crude protein, CF is referred to crude fiber.

2.5.9. Determination of mineral content

The analyses for minerals content was examined using Atomic Absorption Spectrophotometric (Analytika jena AAS 400P, Germany) technique. In this respect, the digested sample was subjected to analyze selected mineral elements (Fe, Zn, Ca, and Mg). In compliance with AOAC (Method 985.35), the sample (1 g) was of [13] was digested with 5 mL of concentrated HNO_3 and 1 mL of $HClO_4$. The mixture was kept in oven at 100 °C for 6 h. After ensuring the complete digestion, the solution was filtered using a filter paper. Further, the resulted clear solution was subjected to examine for mineral content detection [18]. The results for chosen minerals were expressed in mg per 100 g of cookies sample on a dry–weight basis.

2.5.10. Determination of water activity

The water activity of cookies was perceived using an instrument for water activity analyzer (AQUALAB 4 TE, USA). Prior to analysis, the instrument was calibrated against appropriate standards. In this sense, holder was ensured with full placement of sample to keep the dew point water activity meter at 25 °C [19].

2.5.11. Fourier-Transform Infrared Spectroscopy (FTIR) analysis

Functional group analysis was carried out using FTIR instrument (Thermo Scientific iS50 ABX, USA) equipped with attenuated total reflection (ATR) accessory, detector. The sample was placed under Infrared (IR) light source and the collected data at 32 resolutions and 16 number of scans. IR spectrum was analyzed in the range of 4000 and 400 cm^{-1} wavenumber.

2.5.12. Determination of texture profile

In the present study, texture analysis machine (TA1SH-203V, LLOYD Materials Testing, UK) was used to assess the texture profile for cookie samples [13]. The hardness of cookies was determined by adopting cutting-shear test. During experimentation, stainless-steel probe was operated in perpendicular direction to the primary dimension of cookie sample. In this regard, a 4 mm wide slot surface was employed at a fixed speed of 2 mm/s. Further, the highest demanded force (N) to shear and the maximum distance were recorded as a measure of hardness [20].

2.5.13. Cookies color

A spectrophotometer (Model CM-600d, Konica Minolta, INC, Japan, 2012) was used to measure the color of the cookie samples that measure values L*, a*, and b*. The L*-value indicates the lightness index, the a* and b* values indicate the degree of redness (−a*) or greenness (+a*), and the degree of yellowness (−b*) or blueness (+b*), respectively. Initially, a white reference standard with respect to white duplicating paper sheet (80 g/m^2) was used to standardize the instrument (L* = 99.27, a* = −1.5, and b* = 0.41) [21]. Total color difference, $\triangle E^*$, was estimated using Equation. 9 [22]. Browning Index (BI) due to the substitution of pumpkin seed flour in the cookies, as per the methodology of Sung &Chen [23] Equation (10).

$$\sqrt{(L^* - L^*o)^2 + (a^* - a^*o)^2 + (b^* - b^*o)^2} \tag{9}$$

$$BI = \frac{\left[100 * \left(\frac{a^* + 1.79L^*}{5.645L^* + a^* - 3.012b^*} - 0.31\right)\right.}{0.17} \tag{10}$$

Where; L^* is lightness of enriched cookies sample; L^*o is lightness of control sample; a^* is redness/greenness of enriched cookies sample; a^*o is redness/greenness of control sample; b^* is yellowness/blueness of enriched cookies sample; and b^*o is yellowness/blueness of control sample.

2.5.14. Oxidative stability analysis for cookies using rancimat

For the determination of oxidation stability, the Rancimat method using an 892 professional (Metrohm, 892 Herisau, Switzerland) instrument was performed for the cookies sample [24]. In this context, the analysis was carried out at 100 °C with an airflow rate of 20 L/h. A sample of 0.5 g of cookies powder was taken to examine and the data were recorded. Oxidative stability of sample was expressed as induction time (h).

2.5.15. Thermal properties of cookies

The thermal properties of cookies were analyzed using Differential Scanning Calorimetry (DSC) (SKZ 1052B, China). Distilled water was added to ground cookies in a ratio 3:1 and left to equilibrate for 24 h at room temperature. The sample was heated at a rate of 5 °C/min in a range of temperature from 25 to 125 °C [21] with a void pan as reference. The enthalpy (ΔH, J/g) and the onset (T_{on}, °C), peak (T_p, °C), and offset (T_{off}, °C) temperatures of the observed transitions were computed from the thermal curves using the Universal Analysis Program 2000 (TA Instruments).

2.5.16. Free radical scavenging using DPPH assay

The cookies sample was extracted using soaking extraction according to the methods documented by Msaddak et al. [25], and Bazezew et al. [26], with some modification. According to this, the cookies samples were made into fine powder using pestle and mortar. Further, 5 g of sample powder was homogenized with 50 mL methanol for 2 h at room temperature with the use of orbital shaker which was rotated at 120 rpm. Then, the extracted sample was filtered with Whatman No.1 filter paper and dried using rotary evaporator. The extract sample was kept in a refrigerator at 4 °C for analysis.

The DPPH free radical scavenging was investigated using the methods demonstrated by Braca et al. [27]. About 4 mL of 0.004 % DPPH radical solution in methanol was mixed with 1 mL of various concentrations (100, 200, 300, 400, and 500 μL/mL) of sample extract in methanol and was mixed using vortex mixer and then the mixed solution was incubated in a dark place for 30 min at room temperature. The scavenging capacity was read using jasco V-770 UV–Visible/NIR spectrophotometer (V-770, Japan) at 517 nm using

UV and ascorbic acid standard solution (100, 200, 300, 400, and 500 μg/mL) was prepared and the scavenging activity was calculated using Equation (11) [28].

$$AA\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control} * 100 \tag{11}$$

2.6. Phytochemical determination

2.6.1. Phytates

The phytate content (mg/100 g) was determined according to the previous report by Lapteva [29], method. Dried cookie sample (0.1 g) was extracted using 10 mL of HCl (0.2 N) for 1 h at room temperature. Further, it was subjected to centrifuge (Centrifuge CE 800) at 3000 rpm for 30 min. 3 mL of resulted supernatant was mixed with 2 mL of Wade reagent in distilled water. Further, the solution was well-mixed using vortex mixer (Taiwan- Gemmy industrial corp, VM-300P). Using a UV–Vis spectrophotometer (DU64, Beckman, USA), absorbance was recorded at 500 nm.

The concentration of Phytate was determined using the absorbance difference between blank and cookie sample. In this line, pre-determined amount of Phytic acid (PA) was utilized for preparing the standard curve [30]. Using the standard curve, Phytate mineral in terms of molar ratio was ascertained using molecular weight of PA (660). The phytate content was calculated using Equation (12) [31].

$$Phytic\ acid\ in\ \frac{\mu g}{g}\ of\ sample = \frac{[(A_b - A_s) - intercept] * 10}{slope * 3 * W_s} \tag{12}$$

Where; A_S is absorbance of sample, A_b is absorbance of blank and W_S denotes the sample weight.

2.6.2. Tannins

The tannins content was investigated by the procedure presented by Hawa et al. [30]. In such a way, the flour and cookies sample (1 g) was subjected to extract using 10 mL of HCl (1 %) and methanol for 24 h through mechanical shaker (Edmund Buhler, USA) at room temperature, then, the solution was clarified by using centrifuge (3000 rpm, 5 min). Then, vanillin-HCl reagent (1 % HCl and 2 % vanillin) was used for color development. As per the procedure reported by Anhwange et al., the tannin content determined using Equation (13) [32].

$$Tannin\ content, \frac{\mu g}{100}\ of\ sample = \frac{[(A_s - A_b) - intercept] * 100}{slope * \rho * W_s} \tag{13}$$

Where; A_S is absorbance of sample, A_b absorbance of blank, ρ is known as solution density (in this case, 0.791 g/mL) and W_S refers to sample weight.

2.6.3. Oxalates

The oxalates content was determined using the dye technique according to the procedure AOAC, 2005. In this context, 1 g cookie sample taken into a 100 mL conical flask. After that, 75 mL H_2SO_4 (3 mol/L) was added. The solution was well-mixed by hand shaking. The resulted precipitate was collected and washed with 75 mL of H_2SO_4 (25 %). Then, the solution was agitated for 1 h using 25 % before being filtered through Whitman No.1 filter paper. Further, the filtrate (25 mL) was titrated against a hot (80–90 °C) $KMnO_4$ (0.1 N) solution to get light pink color [32]. The presence of oxalates in mg/100g was deduced with the use of titration volume. The oxalate content was determined using Equation (14).

$$\text{Oxalate}\ (\%) = \frac{\text{Volume of KMnO}_4 * 0.006303 * 100}{\text{weight of fresh sample}} \tag{14}$$

2.6.4. Statistical data analysis

The relationship between independent and dependent variables and corresponding Analysis of Variance (ANOVA) was analyzed to test its appropriate significance by using Design-Expert® software, version 12 (Stat-Ease Inc., Minneapolis, MN55413, USA). In this line, numerical optimization was implemented by desirable minimization or maximization of the necessary variable to find a good set of conditions.

3. Results and discussion

3.1. Interaction effect among the parameters and optimization

The selected variables were studied for their interaction effect using RSM-CCD combination. The ranges of BR (6–15 %), T (180–200 °C), and TD (8–12 min) were set as cookies baking process parameters. Within the given levels, 20 runs of experimental combinations were carried out and their corresponding effects were analyzed by developing the statistical model. Further, the parameters were optimized and validated through ANOVA.

3.2. Fitting of process model analyses of variance

The developed model was further used to execute for generate 3D response surface graphs and optimize the chosen variables. Among the developed models, quadratic model was ensured to be the best fit while examine with the p-values and R^2 values. The model significance was ensured with acceptable P-values and R^2 values. The developed quadratic equations that predict the respective responses as blending ratio, baking temperature and baking time are given in Table 3.

Where; A is blending ratio (%), B is baking temperature (°C) and C is baking time (min).

Furthermore, the statistical significance via P- and F-value for the main variables and their corresponding interactive terms were also ensured to be sound for fit. The values of sum of squares, df, mean square, F-values, P-values and R^2 of the models for all responses are shown in Table 4.

Overall acceptance of cookies as the sum of various quality characteristics (appearance, color, aroma, taste, crispiness and texture) can be used to assess the quality of the cookies prepared. The overall acceptance score of cookies varied from 6.66 to 8.15 (Table 5). When the cookies prepared at optimized condition were compared with the control sample (100 % wheat flour), run 8 had showed a ameliorated quality with respect to sensory evaluation. This may be due to the increased level of pumpkin seed flour that enhances the taste, flavor, aroma, texture and color of the cookies. In this line, Kanwal et al. [33], showed that pumpkin seed flour is incorporated successfully to partial replacement with wheat flour to produce biscuits with improved nutritional value and wholesome biscuit without disturbing their overall acceptability. Likewise, the total color difference (ΔE) of cookies supplemented with pumpkin seed flour (5.55–16.33) is presented in Table 5. However, blending ratio, baking temperature and time affected total color difference of cookies. The results showed that low blending ratio, baking temperature and baking time of cookies resulted in low color difference values when compared with the control sample. Similarly, the responses for protein, fat, ash, moisture, and fiber are shown in Table 6.

3.3. Interaction effect among the selected variables

The interaction effect for the overall acceptability of the cookies prepared at optimal condition as functions of blending ratio and baking temperature at fixed baking time of 10 min is illustrated in Fig. 1 (a). It can be observed from the figure that the overall acceptability seems to be increased from 7.2 to 8.04 while blending ratio increases from 6 to 12 % and T increases from 180 °C to 185 °C. However, the overall acceptability showed a decreasing trend to 7.3 when the blending ratio further increases to 15 % and baking temperature increases to 200 °C. The cookies seem to be more acceptable by the panelists as the blending ratio increases (percent increase of pumpkin seed flour in the cookies). The positive effect can be due to the reason that Pumpkin seeds acts as flavor and taste enhancer and it gives better mouth feel. Similarly, baking temperature can have a positive effect due to its potential for improvement of texture, color and crispiness of cookies. Further, increase of blending ratio and baking temperature decrease the overall acceptability of cookies owing to reduction of the color (*L*-*value) and increase of hardness of cookies.

In a similar fashion, Fig. 1 (b) demonstrates the overall acceptability with respect of blending ratio and baking time at a given baking temperature of 190 °C. The Figure reveals that the overall acceptability of the prepared cookies increases from 7.4 to 8 as the blending ratio increases from 6 to 12 % and baking time increases from 8 to 10 min. Nevertheless, the overall acceptability of the cookies declines back to 7.6 when both parameters increase to 15 % and 12 min, respectively. The increase in overall acceptability of the cookies may attribute to sufficient baking time of the mixed dough to assume standard properties that are acceptable by the panelists. At low baking time, the cookies may not be properly baked at the given temperature of 190 °C and at high baking time, the cookies would be over baked to a point where the panelists dislike some of the properties. Be it low or be it high, the baking time would have an influence on color, texture, mouth feel, aroma and the like.

Fig. 1 (c) depicts overall acceptability of cookies by 20 panelists as baking temperature and time varied to 180–200 °C and 8–12 min at constant blending ratio of 10.5 %. The graph unfolds that the overall acceptability increases from 7.4 to 8.0 when the baking temperature decreases from 200 to 185 °C and baking time decreases from 12 to 10 min based on the panelists' evaluation and the model prediction. When these two variables further decrease to 180 °C and 8 min respectively, the overall acceptability falls all the way down to 7.6. It is evident from the graph that the acceptance of the cookies lies in vicinity of 185 °C and 10 min operating condition. Besides, it seems that at high or low operating condition of both parameters the quality of the cookies declines sharply to a level the panelists evaluated it relatively lower.

A more or less similar result can be seen from the work of Desalegn & Olika [34]. The authors produced biscuits from common bean, pumpkin and wheat flour and claimed 75 % wheat, 15 % common bean and 10 % pumpkin would produce an overall acceptability of

Table 3
Responses and the corresponding model equations.

Response	Model equation
OAA	$OAA = -63.92 + 1.17A + 0.69B + 0.22C - 0.01AB + 0.02AC + 0.01BC - 0.02A^2 - 0.002B^2 - 0.08C^2$
Protein	$Protien = +52.21 + 2.68A - 0.97B + 8.14C - 0.013AB - 0.01AC - 0.022BC + 0.024A^2 + 0.003B^2 - 0.202C^2$
Fat	$Fat = +6.41 + 0.60A + 0.096B - 0.091C - 0.0025AB + 0.0024AC + 0.0013BC + 0.0122A^2 - 0.0003B^2 - 0.011C^2$
Ash	$Ash = -3.07 - 0.042A + 0.0548B - 0.0024C - 0.0004AB + 0.0001AC - 0.00006BC + 0.0085A^2 - 0.0001B^2 + 0.0004C^2$
Moisture	$Moisture = +15.50 - 0.276A - 0.098B - 0.192C - 0.0012AB + 0.0007AC + 0.00069BC - 0.00034A^2 + 0.00018B^2 + 0.004C^2$
Fiber	$Fiber = -5.25 + 0.162A + 0.08B - 0.089C - 0.0009AB - 0.00014AC + 0.000062BC + 0.0029A^2 - 0.0002B^2 + 0.0036C^2$
ΔE	$\Delta E = -2.80 + 2.20A - 0.148B - 0.346C - 0.0061AB + 0.0356AC - 0.0074BC - 0.033A^2 + 0.0011B^2 + 0.079C^2$

Table 4
Analysis of variance (ANOVA) for the regression models of overall acceptance, protein, fat, ash, moisture, fiber content and total color difference.

Response	Sum of squares	df	Mean square	F-value	P-value	R^2
OAA	2.95	9	0.3272	52.98	<0.0001	0.983
Protein	54.45	9	6.05	89.88	<0.0001	0.990
Fat	36.22	9	4.02	1279.88	<0.0001	0.995
Ash	0.8021	9	0.0891	450.79	<0.0001	0.998
Moisture	0.6159	9	0.0684	173.92	<0.0001	0.994
Fiber	0.4585	9	0.0509	606.81	<0.0001	0.998
ΔE	123.94	9	13.77	439.71	<0.0001	0.998

Table 5
Effect of baking process variables on overall acceptability and total color difference of enriched cookies.

Run	Pumpkin seed flour (%)	Baking temperature	Baking time	Response			
				Overall acceptance		*ΔE**	
				Actual	Predicted	Actual	Predicted
Control	0	190	10	8.00 ± 0.65	–	–	–
1	6	200	12	6.85 ± 0.73	6.90	9.24	9.25
2	15	200	8	6.66 ± 0.72	6.68	14.42	14.42
3	15	180	12	7.50 ± 0.54	7.52	13.49	13.61
4	10.5	190	10	7.94 ± 1.09	7.85	11.02	10.97
5	10.5	190	10	7.84 ± 1.17	7.85	11.11	10.97
6	6	180	8	7.12 ± 0.82	7.11	5.55	5.62
7	15	200	12	7.31 ± 0.59	7.32	16.33	16.26
8	10.5	190	10	8.15 ± 0.18	8.11	11.72	11.61
9	6	180	12	6.73 ± 1.02	6.72	6.77	6.78
10	10.5	190	10	8.00 ± 0.23	8.11	11.32	11.61
11	6	200	8	7.40 ± 0.81	7.38	10.08	9.96
12	15	180	8	7.92 ± 0.35	7.87	12.46	12.47
13	6	190	10	7.50 ± 0.76	7.49	7.46	7.49
14	10.5	190	10	7.90 ± 1.01	7.95	11.26	11.32
15	15	190	10	7.80 ± 0.51	7.80	13.85	13.79
16	10.5	200	10	7.71 ± 1.32	7.65	12.67	12.86
17	10.5	190	12	7.65 ± 0.88	7.58	12.13	12.06
18	10.5	190	8	7.66 ± 1.03	7.72	11.16	11.21
19	10.5	190	10	7.98 ± 0.72	7.95	11.31	11.32
20	10.5	180	10	7.83 ± 0.91	7.88	10.21	10.00

Table 6
Effect of baking process on protein, fat, ash, moisture and fiber of enriched cookies.

Run	Pumpkin seed flour (%)	Baking temperature	Baking time	Response				
				Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Fiber (%)
1	6	200	12	10.95 ± 0.18	14.60 ± 0.04	1.91 ± 0.02	2.64 ± 0.01	2.02 ± 0.03
2	15	200	8	15.20 ± 0.10	18.15 ± 0.02	2.42 ± 0.01	2.33 ± 0.02	2.35 ± 0.00
3	15	180	12	16.80 ± 0.01	19.11 ± 0.01	2.47 ± 0.03	2.39 ± 0.02	2.56 ± 0.03
4	10.5	190	10	13.24 ± 0.18	16.60 ± 0.04	2.03 ± 0.03	2.54 ± 0.05	2.20 ± 0.05
5	10.5	190	10	13.10 ± 0.02	16.59 ± 0.03	2.04 ± 0.01	2.55 ± 0.05	2.21 ± 0.04
6	6	180	8	10.70 ± 0.01	15.34 ± 0.05	1.94 ± 0.01	3.01 ± 0.03	2.12 ± 0.05
7	15	200	12	13.39 ± 0.00	18.13 ± 0.03	2.40 ± 0.03	2.25 ± 0.04	2.33 ± 0.05
8	10.5	190	10	13.13 ± 0.12	16.71 ± 0.00	2.02 ± 0.03	2.54 ± 0.04	2.23 ± 0.03
9	6	180	12	10.98 ± 0.03	15.13 ± 0.01	1.92 ± 0.02	2.85 ± 0.02	2.10 ± 0.05
10	10.5	190	10	13.20 ± 0.01	16.59 ± 0.01	2.03 ± 0.03	2.52 ± 0.04	2.23 ± 0.02
11	6	200	8	11.50 ± 0.12	14.70 ± 0.00	1.95 ± 0.04	2.60 ± 0.02	2.05 ± 0.04
12	15	180	8	15.98 ± 0.09	19.23 ± 0.01	2.50 ± 0.03	2.38 ± 0.04	2.60 ± 0.03
13	6	190	10	11.00 ± 0.10	14.99 ± 0.00	1.93 ± 0.04	2.75 ± 0.05	2.08 ± 0.04
14	10.5	190	10	12.70 ± 0.05	16.58 ± 0.01	2.04 ± 0.03	2.53 ± 0.02	2.22 ± 0.02
15	15	190	10	15.20 ± 0.05	18.69 ± 0.01	2.47 ± 0.02	2.30 ± 0.03	2.46 ± 0.02
16	10.5	200	10	12.85 ± 0.09	16.25 ± 0.04	2.01 ± 0.01	2.46 ± 0.04	2.13 ± 0.03
17	10.5	190	12	11.30 ± 0.05	16.50 ± 0.04	2.03 ± 0.05	2.50 ± 0.02	2.21 ± 0.01
18	10.5	190	8	12.30 ± 0.00	16.60 ± 0.01	2.03 ± 0.01	2.58 ± 0.03	2.25 ± 0.00
19	10.5	190	10	12.46 ± 0.01	16.63 ± 0.01	2.01 ± 0.05	2.53 ± 0.02	2.22 ± 0.03
20	10.5	180	10	13.05 ± 0.03	16.87 ± 0.02	2.02 ± 0.02	2.64 ± 0.03	2.26 ± 0.03

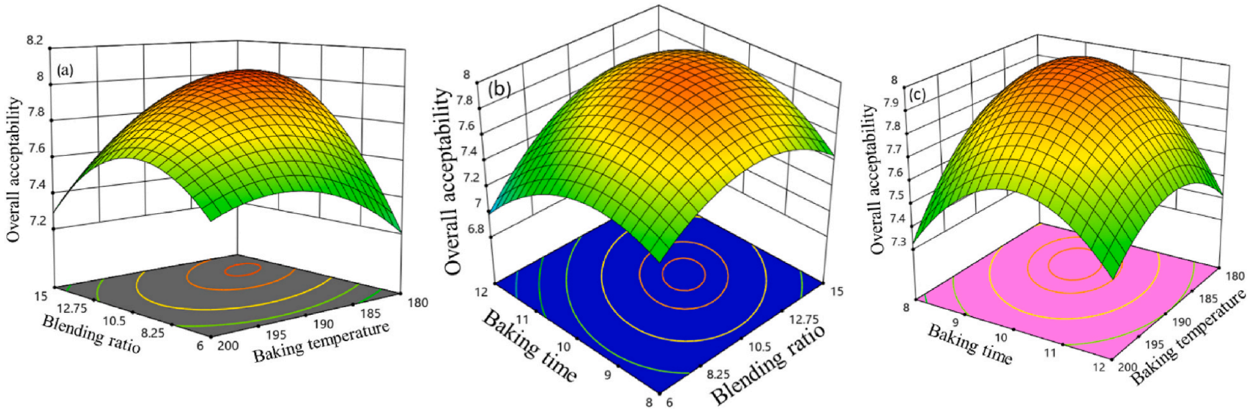

Fig. 1. Overall acceptability of cookies in terms of surface plot as functions of (a) blending ratio and baking temperature, (b) baking time and blending ratio and (c) baking time and temperature.

5.01–6.56.

The interaction effect of total color difference (ΔE) with respect of blending ratio, baking temperature and time is shown in Fig. 2. Fig. 2 (a) is the total color difference as function of blending ratio and baking temperature at fixed baking time of 10 min. It can be seen from the Figure that the color values increased from 6 to 15 as blending ratio increases from 6 % to 15 % and baking temperature increased from 180 °C to 200 °C. As is shown in Fig. 2 (b) and (c), the response total color difference was found to increase from 7.7 to 14.8 and 9.7–13.4 when the blending ratio increases from 6 to 15, baking time from 8 to 12 and baking temperature from 180 to 200 °C, respectively. It can be inferred from these results that the increase of color difference with respect of the control cookies is due to addition of pumpkin seed flour. Besides, the baking temperature and time might take effect on the color difference of the enriched cookies as compared to the control one.

The effect of blending ratio, baking time and temperature on the proximate composition of enriched cookies had been studied. The protein content of the cookies enriched with pumpkin seed flour was studied by varying blending ratio, baking temperature and time as shown in Fig. 3 (a), (b) and (c). It was found that the protein content was highly influenced by addition of pumpkin seed flour and T, and to some extent by baking time. The change in protein content of the cookies varied from 10.4 to 16.8 as the aforementioned variables changed. It can be said that the pumpkin seed has more protein that causes the cookies to have higher protein content relative to the control which is 11.49. Moreover, at higher baking temperature, the protein content could be denatured and the cookies would have lower protein content.

The results showed that the protein content and fat content were considerably affected by change with the pumpkin seed flour addition. When the blending ratio increases, it was found that the fat content of the cookies enriched with pumpkin seed increases proportionally, it has gone as high as 19 % from 14.7 %. The baking temperature and baking time had less effect on the fat content of the cookies prepared. As would be expected, the blending ratio is the main reason to the increment of fat content. Fig. 4 (a), (b) and (c) illustrated the fat content of the cookies enriched with pumpkin seed as the aforementioned variables change with their respective

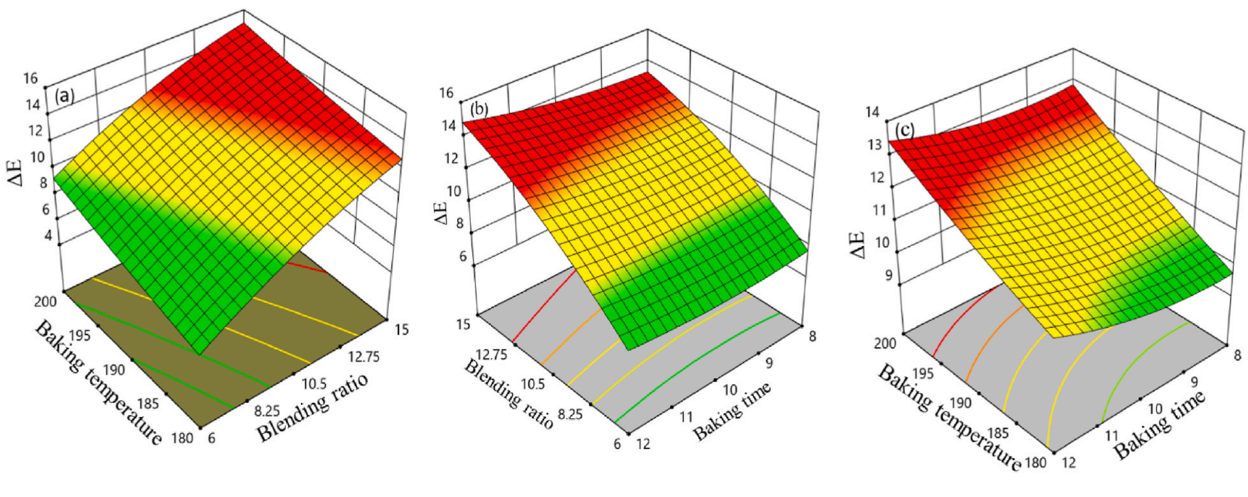

Fig. 2. Total color difference of cookies in terms of surface plot as functions of (a) blending ratio and baking temperature, (b) baking time and blending ratio and (c) baking time and temperature.

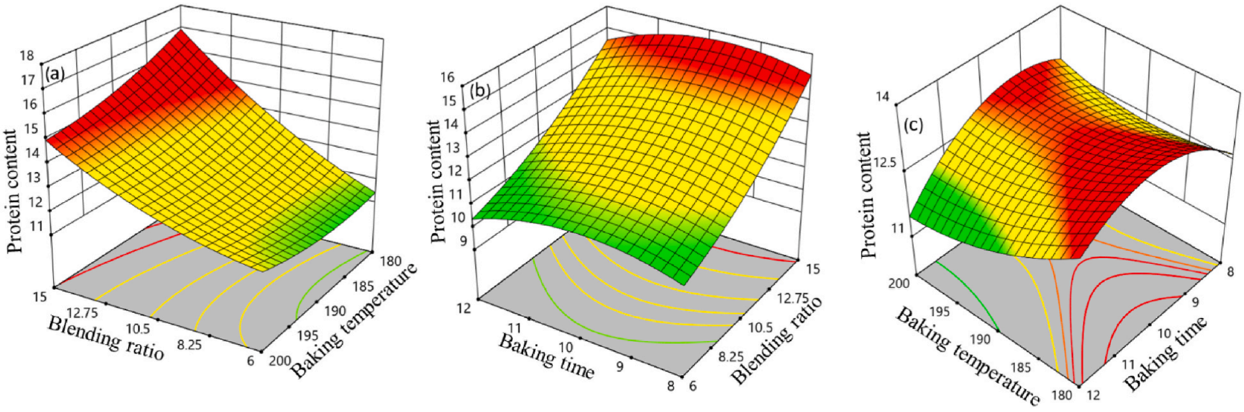

Fig. 3. Protein content of cookies in terms of surface plot as functions of (a) blending ratio and baking temperature, (b) baking time and blending ratio and (c) baking time and temperature.

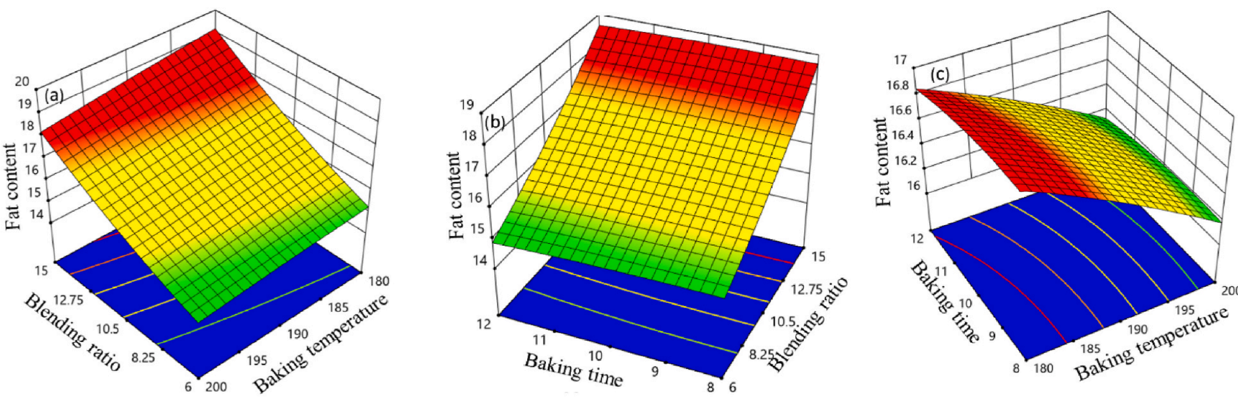

Fig. 4. Fat content of cookies in terms of surface plot as functions of (a) blending ratio and baking temperature, (b) baking time and blending ratio and (c) baking time and temperature.

ranges.

Likewise, the ash content of the enriched cookies also was more likely influenced by the variation to the amount of pumpkin seed added to the wheat flour. Consequently, the ash content varied up to 2.5 % when the blending ratio changed from 6 to 15 %, putting aside the other parameters. The variation of the ash content as function of blending ratio, baking temperature and time is depicted in Fig. 5 (a), (b) and (c).

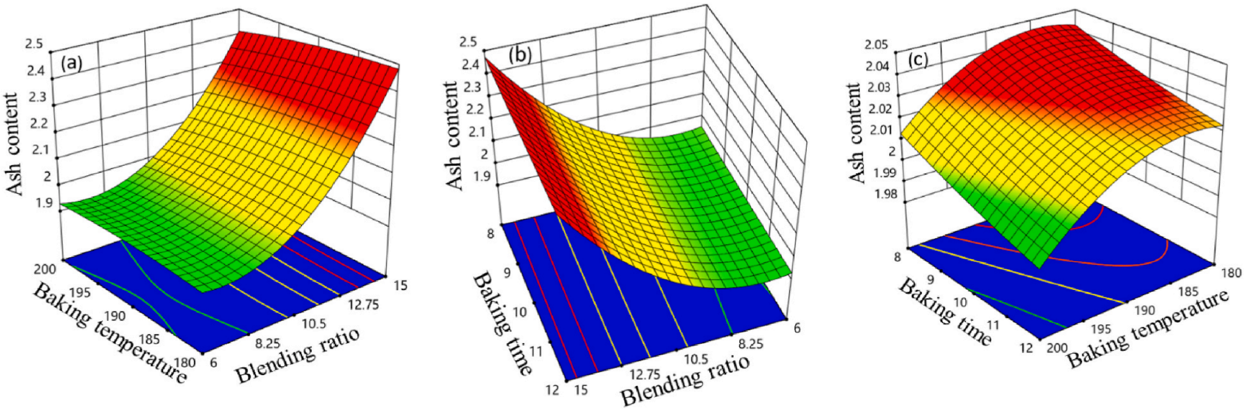

Fig. 5. Ash content of cookies in terms of surface plot as functions of (a) blending ratio and baking temperature, (b) baking time and blending ratio and (c) baking time and temperature.

As shown in Fig. 6 (a, b and c), the moisture content of the enriched cookies baked at different conditions, particularly at baking temperature ranges of 180–200 °C, Blending ratio of 6–12 % and baking time of 8–12 % come out to change from 2 to 3%. Out of the three variables considered to influence the moisture content of the product, blending ratio, i.e the amount of pumpkin seed added to the wheat flour, affects more relatively. This was occurred because of the presence of moisture content in the pumpkin seed might be less than the presence of moisture content in the wheat flour.

The fiber content of product slightly varies from 2 to 2.6 % as blending ratio of pumpkin seed to wheat increases from 6 to 15 %, baking temperature varies from 180 to 200 °C and baking time increase from 8 to 12 min. Fig. 7 (a, b and c) unfolds that the fiber content was more dependent on the ratio of pumpkin seed flour to wheat flour relative to the other two variables baking time and temperature.

3.4. Process optimization and model validation

While optimizing the independent variables, it was computed that the optimized values for blending ratio, baking temperature and baking time were 12.87, 186 °C, and 9.5 min, respectively. This yields overall acceptability of 8, protein content of 14.53 %, fat content of 17.85 %, ash content of 2.23 %, moisture content of 2.46 %, fiber content of 2.38 %, total color difference of (ΔE) 12.01 and the desirability of 0.78. Since the established model was found to be significant and reliable to the predicted responses, result of the optimization process can be considered for statistically appropriate and the same has been summarized in Table 7.

3.5. Characteristics of optimized and control cookies

The best cookies samples from the optimization of overall acceptability, proximate analysis, and total color difference were (12.87 % pumpkin seed flour and 87.13 % wheat flour), compared to the control cookies (100 % wheat flour) which baked at the temperature and time for 186 °C and 9.5 min, respectively. Fig. 8(a) dipicts the control product without pumpkin seed powder and Fig. 8(b) depicts the optimized cookies prepared with 12.87 % pumpkin seed powder.

3.6. Proximate and mineral composition of optimized and control cookies

The result of proximate composition and energy value of optimized and control cookies is shown in Table 8. The crude protein content of cookies was found to be appreciably increased from 11.49 to 14.28 % due to the optimal supplementation of pumpkin seed flour with wheat flour. The protein content of pumpkin seed flour (33.66 %) is higher than wheat flour (11.85 %). This study agreed with the outcome of Dixit et al. who stated an increase in protein content of akara with substitution of soybean, higher than that of wheat-mango kernel cookies (9.74%–11.93 %) [35].

The fat content of control and enriched cookies significantly ($P < 0.05$) increased from 13.99 % to 17.73 % due to addition of pumpkin seed flour in the cookies. This may be due to high fat content of pumpkin seed flour. The same observation was reported for African walnut which was employed as composite flour in the cookies production and the cookies fat content was increased [36].

The result of ash content of control and optimized cookies followed same trend with protein and fat content. It was observed that ash content of cookies significantly ($P < 0.05$) increased (1.77 %–2.19 %) with supplementation of pumpkin seed flour. The same trend was observed by Kumari et al. [37], who reported that ash content increased from 0 to 30 % in the biscuits with addition of pumpkin seed flour.

The moisture content of optimized cookies (2.52 %) was lower than the control (3.05 %). This may be resulted in increase of dry matter content when pumpkin seed flour was substituted. The higher moisture content improves the growth of microorganisms, and this reduces the quality and stability of foods. Moisture content is an indicator of shelf life stability [38]. Fiber content of cookies significantly ($P < 0.05$) increased from 0.93 % to 2.41 %. Pumpkin seed flour contributed to the enhancement of fiber content in optimized cookies. The finding of research agreed with Florence Abolaji Bello et al. (2021) who reported fiber content from 2.16 % to

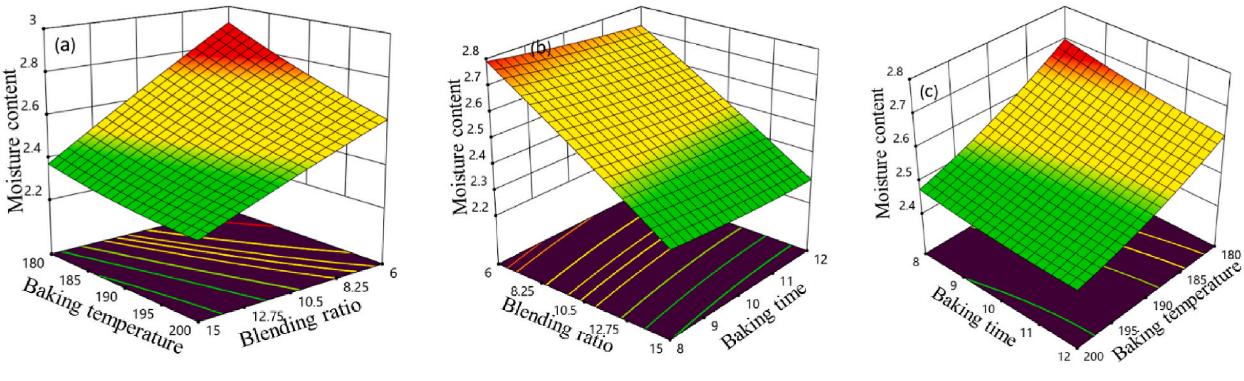

Fig. 6. Moisture content of cookies in terms of surface plot as functions of (a) blending ratio and baking temperature, (b) baking time and blending ratio and (c) baking time and temperature.

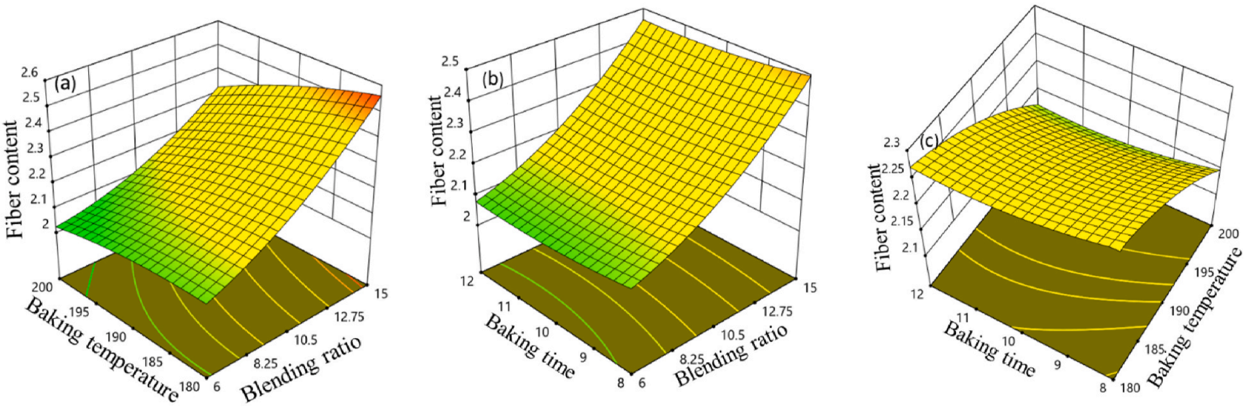

Fig. 7. Fiber content of cookies in terms of surface plot as function of (a) blending ratio and baking temperature, (a) baking time and blending ratio and (c) baking time and temperature.

Table 7
Model prediction and experimental responses at optimum conditions.

Value	Variable			Response							
	BR (%)	T (°C)	TD (min)	OAA	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Fiber (%)	ΔE	TCHO (%)
Predicted	12.87	186	9.5	8	14.53	17.85	2.23	2.46	2.38	12.01	62.94
Actual	12.87	186	9.5	7.85 ± 0.32	14.28 ± 0.01	17.73 ± 0.01	2.19 ± 0.02	2.52 ± 0.01	2.41 ± 0.02	11.76 ± 0.01	63.28 ± 0.03

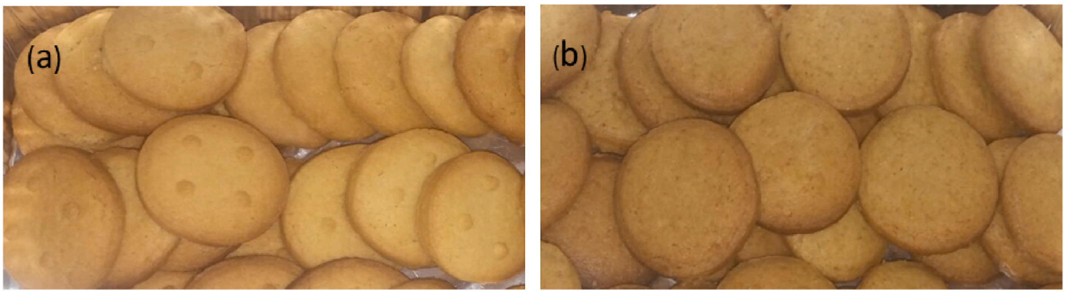

Fig. 8. (a) represents the control product without pumpkin seed powder and (b) optimized cookies prepared with 12.87 % pumpkin seed powder.

Table 8
Proximate and energy value of optimized and control cookies.

Sample code	Parameters						
	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Fiber (%)	TCHO (%)	Energy (cal)
C	11.49 ± 0.12^{b}	13.99 ± 0.19^{b}	1.77 ± 0.02^{b}	3.05 ± 0.02^{a}	0.93 ± 0.03^{b}	70.11 ± 0.24^{a}	452.00 ± 0.46^{b}
OCs	14.28 ± 0.01^{a}	17.73 ± 0.01^{a}	2.19 ± 0.02^{a}	2.52 ± 0.01^{a}	2.41 ± 0.02^{a}	63.28 ± 0.03^{b}	469.81 ± 0.01^{a}

^{ab}Mean (n = 3) values in the same column with different superscript letters indicate significant difference at 5 % level of significance, C: control cookies and OCs: optimized cookies.

3.13 % for biscuits incorporated with pineapple pomace powder.

The carbohydrate contents of optimized and control cookies were 63.28 % and 70.11 %, respectively. It significantly ($P < 0.05$) decreased with addition of pumpkin seed flour due to the high amount of protein and fat content in pumpkin seed flour. The result agrees with earlier studies that presented reduction of carbohydrate content when wheat flour was supplemented with coconut flour [37,. The energy content of optimized cookies was significantly ($P < 0.05$) enhanced (469 kcal) as compared to the control cookies (452 kcal). The energy value indicates that the optimized cookies contain a reasonable amount of energy since wheat flour comprised mainly of carbohydrate.

The mineral content of optimized and control cookies samples is presented in Table 9. Generally, the optimized cookies have higher Ca, Mg, Zn and Fe content as compared to control cookies. Magnesium was the most abundant element in the cookies samples. Pumpkin seed supplemented cookies (optimized cookies) have highest magnesium content (51.33 mg/100 g) than control cookies (40.12 mg/100 g). Abulude et al. [39], stated that magnesium is an essential micronutrient for nervous system health. Similarly, adding pumpkin seed to the cookies increases the calcium content from 32.23 mg/100 g to 46.25 mg/100 g. In human nutrition, calcium is an important bone-related microelement [40]. Calcium, along with other microminerals and protein, can aid in bone formation, with calcium being the primary contributor [41]. Calcium is required for blood clotting, muscle contraction, and the activity of certain enzymes in metabolic processes.

Mineral content increases due to substitution of pumpkin seed in the wheat flour. Zinc and iron content ranged from 1.42 to 2.63 mg/100 g and 2.12–3.20 mg/100 g, respectively. Inadequate micronutrient intakes (Zinc and Iron) have been linked to severe malnutrition, increased disease conditions, and mental impairment [42]. Kaur & Sharma [43], studied the mineral contents of cake supplemented with 20 % roasted pumpkin seed flour and found that the cake contained 1.96 and 0.63 mg/100 g of iron and zinc, respectively, which was higher than the control biscuits made from refined flour i.e. iron (1.24 mg/100 g) and zinc (0.20 mg/100 g). Prepared nutritional biscuit by replacing 15 % pumpkin seeds flour with wheat flour and observed a significant increase Fe and Zn content from 2.44 to 4.23 and 1.45–4.37 mg/100 g [44].

3.7. Functional group analysis using FTIR

The FTIR spectra of optimized and control cookies are shown in Fig. 9. It can be observed from the graph that the two spectra are similar in their pattern and are different in the quantity of the functional groups available. The optimized cookies (red) have peaks at 3274.46, 2921.75, 2853.27, 1743.01, 1648.09, 1458.86, 1149.31, 991.89, 855.76 and 521.65, and the control cookies (blue) at 3286.41, 2920.74, 2852.80, 1742.48, 1648.31, 1458.86, 1150.46, 994.37, 857.11, and 521.53 wavenumbers (cm^{-1}). The broad peak of the control at 3274.46 wavenumbers (cm^{-1}) may show the existence of trace amount of water and it is evident that the optimized cookies contain less amount of water compared to the control due to supplementation and optimization processes. The peaks at 2921.75 and 2853.27 may attribute to the presence of C–H stretching group, probably alkane compound. Besides, the strong peak at 991.89 might show the presence of C═C bending group. It can be seen from the work of Kotsiou et al. [45] that the functional group of fortified wheat bread is more or less similar with the findings of this study.

3.8. Water activity, browning index and texture properties of cookies

As shown in Table 10, the water activity (a_w) of optimized cookies was significantly ($P < 0.05$) lower than the control cookies and the values are 0.19 and 0.29, respectively. It could be associated with the higher WAC of the wheat flour. According to Chowdhury et al. [46], microbial spoilage can be prevented by reducing the water activity below 0.7 [47].

The browning index value of control (C) cookies were compared with pumpkin seed enrich cookies (OCs) (Table 10). The effect of addition of pumpkin seed flour can be seen by observing the values of browning index (BI) values of control cookies and pumpkin seed flour added cookies. Browning index of cookies was increased significantly from 84.61 to 101.84 with the addition 12.78 % of pumpkin seed flour. This increase in BI was due to formation of Millard browning reactions. Millard browning reactions during cooking of pumpkin seed flour led to considerably darker and browner appearance of cookies with pumpkin seed flour addition. P. Kaur et al., 2019 [48] in their study reported that cookies prepared from supplemented with flaxseed flour browning index increased from 47.39 to 82.11.

The maximum force needed to break the cookies in the mouth is known as hardness, which is a significant characteristic property for product acceptance. In addition, the hardness was noticed along with the distance the results are presented in Table 10. The analysis on texture seemed to be significantly improved hardness since it resulted due to higher Newton force in cookies which prepared from composite pumpkin seed flour when compared with control. The cookies samples experienced a force ranged from 13.32 N to 13.72 N forces per sample with a distance ranged from 2.71 to 3.11 mm. The penetration curve for the texture analysis of the control cookies and optimized cookies have been presented in Fig. 10(a) and (b). Texture of optimized cookies becomes harder since pumpkin seed flour contains water absorbing components such as fiber and protein which can contribute to the sticky nature of dough which reduces the extensibility of dough. The result agrees with Kaur et al. [49], who have reported in their study that incorporation of flaxseed flour increased hardness.

Table 9
Mineral content of optimized and control cookies.

Sample code	Ca (mg/100g)	Mg (mg/100g)	Zn (mg/100g)	Fe (mg/100g)
C	32.23 ± 0.12^b	40.12 ± 0.01^b	1.42 ± 0.01^b	2.12 ± 0.04^b
OCs	46.25 ± 0.08^a	51.33 ± 0.03^a	2.63 ± 0.00^a	3.20 ± 0.01^a

[ab]Mean (n = 3) values in the same column with different superscript letters indicate significant difference at 5 % level of significance, C: control cookies and OCs: optimized cookies.

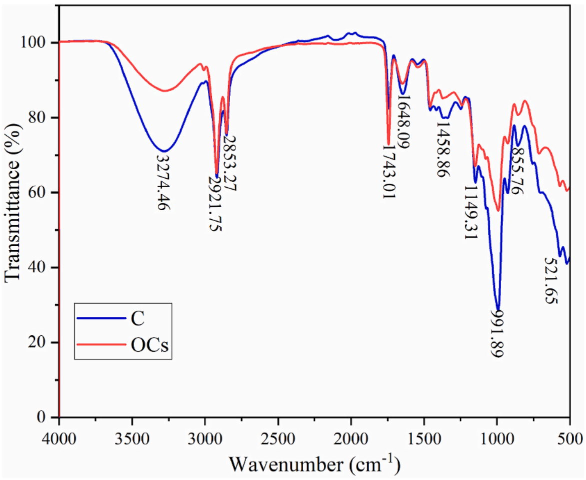

Fig. 9. FTIR spectra of optimized (OCs) and control cookies (C).

Table 10
Water activity and texture properties of cookies.

Sample code	Water activity (a_w)	Browning index	Texture profile
			Toughness (N × mm)
C	0.29 ± 0.00^a	84.61 ± 1.64^b	412.34 ± 0.02^b
OCs	0.19 ± 0.01^b	101.84 ± 1.48^a	483.72 ± 0.01^a

^{ab}Mean (n = 3) values in the same column with different superscript letters indicate significant difference at 5 % level of significance, C: control cookies and OCs: optimized cookies.

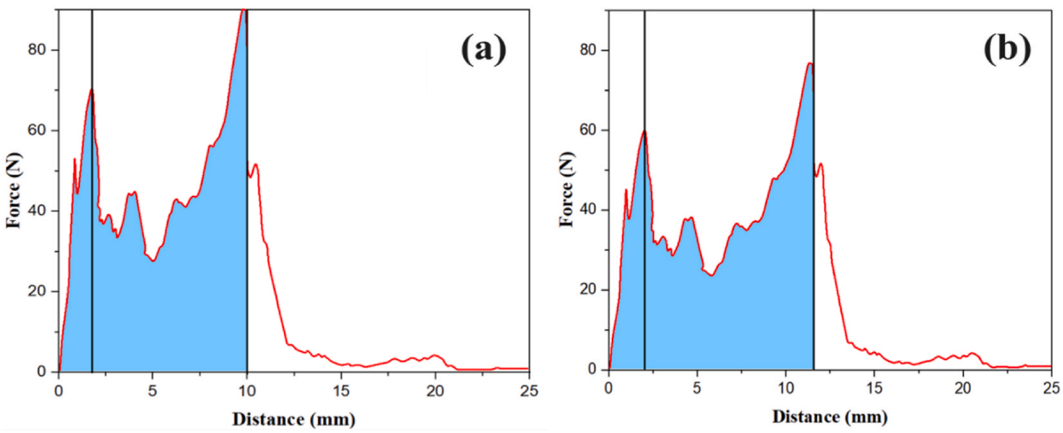

Fig. 10. The penetration curve for the texture analysis of the control (a) and the optimized cookies (b).

4. Thermal properties of optimized and control cookies

Fig. 11 depicts DSC curves for the cookies, where the sample undergone both endothermic and exothermic processes. The optimized and control cookies sample peak temperatures were around 97.4 °C and 103.3 °C, respectively. The onset and offset temperatures of optimized cookies were 31.2 °C and 131.3 °C and for control cookies were 42.3 and 128.3 °C, reactively. Optimized cookies have lower onset and peak temperature values as compared with the control cookies. This is probably due to the nature of pumpkin seed flour and their interaction with other hydrophilic components.

The result agreed with Paciulli et al. [21] who reported biscuits containing chestnut flour with lowest onset and offset temperature of 67.3 °C and 74.0 °C, correspondingly.

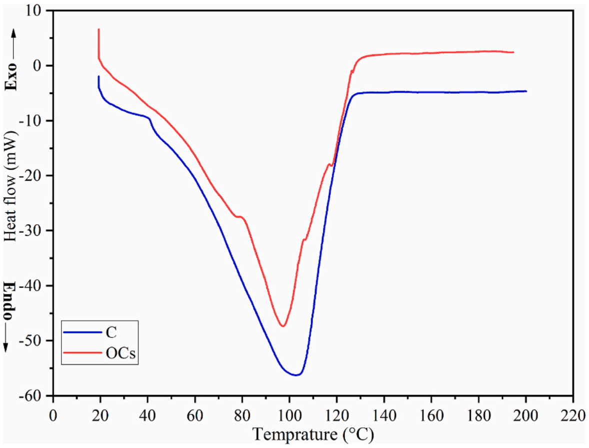

Fig. 11. Differential scanning calorimeter analysis of optimized cookies (OCs) and control cookies (C).

4.1. Antioxidant activity and oxidative stability of cookies

Antioxidant activity and oxidative stability of cookies illustrated in Table 11. There were significant ($P < 0.05$) differences in total antioxidant activity between optimized and control cookies. The optimized cookies had higher total antioxidant activity (43.1860 %) than the control (38.7158 %) cookies. Thus, the higher total antioxidant activity in product supplemented with roasted pumpkin seed flour may be due to the higher antioxidant activity of pumpkin seed. The result agreed with Kaur & Sharma [43], who reported that the DPPH radical scavenging activity of cake supplemented with 20 % roasted pumpkin seed flour has 57.00 % antioxidant activity. Moreover, bread supplemented with 5 % pumpkin seed showed a 37.99 % increase in total antioxidant activity as compared to control bread [50].

Oxidation stability characterizes the resistance of oils and fats and of fat-containing foods to oxidation. It is a standard parameter of quality control in the production of oils and fats in the food industry or for the incoming goods inspection in processing facilities. The oxidative stability measurement showed that induction period of cookies sample was 28.61 h for control cookies and 51.24 h for optimized cookies. The IP value of cookies supplemented with pumpkin seed flour has significantly higher than the control cookies, this may be due to antioxidant content of pumpkin seed. Similar result can be seen from the work of Paciulli et al. [21], in which case they claimed about 25 h induction period of biscuits using oxitest method at 100 °C.

4.2. Phytochemical composition of optimized and control cookies

The result of antinutritional composition of wheat and pumpkin seed blend cookies is shown in Table 12. Optimized and control cookies had not significant ($P > 0.05$) difference between phytate content whose values were 19.20 mg/100 g and 18.17 mg/100 mg, respectively. The phytate content binds some essential mineral nutrients in the digestive tract and can result in mineral deficiencies. According to Florence A Bello [51], the lethal point is 25 mg/100 g.

Tannins content had no significant ($P > 0.05$) difference between the control (1.62 mg/100 g) and optimized (1.72 mg/100 g) cookies. Ifie & Emeruwa [52], stated that the lethal point of tannins is 90 mg/100 g. The lower tannins content in the cookies made it safe for consumption according to its lethal point. The optimized cookies had significant ($P < 0.05$) increase in oxalate content when compared with control cookies with values of 0.25 mg/100 g and 0.39 mg/100 g, respectively.

Generally, the phytochemical composition of optimized and control cookies are lower as compared to raw flours. According to Ochanda et al. [53], fermentation may improve the quality in terms of nutritional for cereals and legumes by influencing substantial modifications in chemical composition that may results in removal of anti-nutritional factors.

5. Conclusions

In this study, with aiming to produce the better quality cookies, an optimized processing strategy was developed. In compliance

Table 11
Antioxidant activity and oxidative stability of cookies.

Sample code	Total Antioxidant activity (%)	Induction period (IP) (h)
C	38.7158 ± 0.06^b	28.61 ± 0.01^b
OCs	43.1860 ± 0.01^a	51.24 ± 0.01^a

[ab]Mean (n = 3) values in the same column with different superscript letters indicate significant difference at 5 % level of significance, C: control cookies and OCs: optimized cookies.

Table 12
Phytochemical composition (mg/100g) of optimized and control cookies.

Sample code	Anti-nutritional factor		
	Phytates	Tannins	Oxalates
C	18.17 ± 0.41[a]	1.62 ± 0.06[a]	0.25 ± 0.00[b]
OCs	19.20 ± 0.37[a]	1.72 ± 0.04[a]	0.39 ± 0.02[a]

[ab]Mean (n = 3) values in the same column with different superscript letters indicate significant difference at 5 % level of significance, C: control cookies and OCs: optimized cookies.

with, baking process of enriched cookies production was optimized using different limiting factors, such as blending ratio (BR) of wheat flour and pumpkin seed flour, baking time (TD), and temperature (T) to enhance the nutritional values of the cookies. It was found that the cookies product at optimal condition contains more nutritional value as compared to the control cookies that made from wheat flour alone. Besides, it was found that the blending of pumpkin seed flour with wheat would improve induction period (oxidation stability) of the product.

The study revealed that the supplementing the cookies with pumpkin seed appears an easily and cheap available option that every bakery has access to it. Moreover, the blending of pumpkin seed with wheat flour in baking cookies would reduce the demand of wheat. Furthermore, the enriched cookies can be an alternative source of nutrients to children and women to eradicate malnutrition.

Data availability statement

Data will be provided on request to corresponding author.

Funding

No funding was obtained for this study.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Feriehiwote Weldeyohanis Gebremariam: Writing – original draft, Formal analysis. **Eneyew Tadesse Melaku:** Project administration. **Venkatesa Prabhu Sundramurthy:** Writing – review & editing, Software. **Henock Woldemichael Woldemariam:** Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Addis Ababa Science and Technology University, Department of Food Science and Applied nutrition for their support during this study.

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