Simultaneous inhibition of acrylamide formation and fat oxidation in quinoa cakes using gum Arabic supplementation coupled with fat reduction

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**ABSTRACT**

The current work aimed to study the effect of adding gum Arabic and varying fat (butter) contents on the physicochemical characteristics of gluten-free quinoa cakes and compared it to cakes made from 100% wheat flour and control quinoa flour. Gum Arabic (GA) was supplemented in three levels (0.1, 0.5, and 1%, w/w) while fat was simultaneously reduced to 25, 50, and 75% (w/v) during the preparation of 100% gluten-free quinoa cakes (GFQCs). A coupling effect of 1% GA and 50% lower fat was successfully applied to inhibit AA up to 83%, fat oxidation up to 64%, and browning intensity up to 59.1% without impairing the baking performance. Moreover, supplementation of 1% GA produced softer and lighter QFQCs, which was similar to 100% wheat cakes. The odor intensity detected by electronic nose technique was also lower in QFQCs. Consequently, the current work serves the worldwide demand for gluten-free bakery products having healthier profile with low fat oxidation and AA formation.

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**Introduction**

Demand for gluten-free (GF) food products has markedly increased in the last decade. Globally, GF product sales reached 4.63 billion USD in 2017 and are expected to reach 6.47 billion USD by 2023.\cite{1} GF diets are currently the only effective means of treating the individuals with gluten sensitivity, gluten allergy, and celiac disease. Some American studies revealed that the population of Americans suffering from these diseases is 1 in every 14 people.\cite{1} Many GF products are put into production including bread, pasta, noodles, biscuits, and cakes. Of the different types of GF foods, cakes are of great importance from a commercial standpoint due to their ease of preparation and their relatively low selling price.\cite{2} Nonetheless, one of the most problems hampering the production of GF cakes is quality defects and an unbalanced nutritional profile resulting from the lower content of several important nutrients, such as minerals, dietary fiber, and vitamins.\cite{3}

Consumers adhered to gluten-free products are increasingly demanding gluten-free foods equivalent to the traditional gluten ones. As consequences, in recent years, pseudo-cereals such as buckwheat, quinoa and amaranth were considered as GF seeds and be suitable for incorporation into the GF products to solve their nutritional drawbacks.\cite{4} Quinoa is known as a treasure trove of nutrients in the last decade with high protein content (16-18%), fiber content (7-9%), and a wide range of vitamins and minerals.\cite{5} Quinoa flour (QF) is suitable for partial or complete replacement of wheat flour (WF) in bakery products, because of its high yield, low cost of production, and the unique functional properties of its flour. QF was successfully applied to replace WF in cakes at level as high as 100% without...
adversely affecting its baking performance. However, gluten-free quinoa cakes (GFQC)s failed to gain higher consumer acceptability due to its darker color. In other QF bakery products, authors also observed the color lightness decreased with increasing of quinoa levels. Generally, these observations could majorly attributed to occurring non-enzymatic browning in these bakery products, which accelerated by the higher content of amino acids in QF.

Non-enzymatic browning intensity (BI) is predominantly coming from Maillard reaction. Maillard reaction is a complex network of reactions involving a chemical reaction between amino acids (especially asparagine) and reducing sugars (especially glucose or fructose) when dough is being processed at high temperatures. Acrylamide (AA) is unfavorable, considered a contaminant and classified by the International Agency of Research on Cancer (IARC) as a potential carcinogenic (class 2A). Recently, EU commission regulation, 2017/2158 establishes mitigation measures and benchmark levels for the reduction of AA in foods. It is recommended that AA must be lower than 1044 μg/kg in bakery products. Another possibility for non-enzymatic browning is oxidation of fats. Fat oxidation occurs by the action of oxygen and reactive oxygen species on the fatty acids, especially unsaturated fatty acids, forming aldehydes and ketones, which then react with amino acids to form brown pigments. Moreover, a high value of animal fats in bakery products is not recommended which has become a health related issue. Therefore, an effort to reduce non-enzymatic browning intensity in quinoa cakes is majorly contributed in developing the consumer acceptability of these products.

Hydrocolloids are frequently used in GF products making to mimic the viscoelastic properties of gluten, thereby increasing gas retention during baking; and hence enhancing dough-specific volume. Moreover, hydrocolloids were widely used in bakery products to improve moisture retention, control water mobility, and maintain overall product quality during storage. It is well known that the non-enzymatic browning reaction is strongly influenced by water content in food products, where browning decreased with increasing water retention. Hydrocolloid gum Arabic (GA) was used to preserve the water content and inhibited AA formation in biscuits due to the formation of tight thin layer of GA during the initial stages of baking. Furthermore, fat molecules may also be combined with GA to form products with physico-chemical properties similar to full-fat products. It should be noted that there is limited data in literature about the effect of GA supplementation and fat reduction together on developing the quality of gluten-free quinoa cake products.

Acrylamide (AA) formation and fat oxidation (non-enzymatic browning products) could be markedly produced during the baking of gluten-free quinoa dough. Therefore, the objective was to create healthy gluten-free quinoa cakes (GFQC)s that have low AA and fat contents. The effect of GA supplementation on the inhibition of AA formation in GFQC was investigated. The amounts of GA were varied between 0.1 and 1 % (w/w). Simultaneously, animal fat in cakes was partially removed within the range of 25–75% (w/w).

Materials and methods

Materials

Commercial quinoa seeds (grown in Egypt in 2019 season) were purchased from The Ministry of Agriculture and were kept at 4°C until used. Quinoa seeds were washed three times (30 min each) with cold water (1:100 w/v) to remove saponins until there was no more foam in the washing water. Quinoa flour was prepared using Blendtec Kitchen Mill Model 91 at medium setting (Blendtec Inc., Wichita, KS, USA). Wheat flour (72% extraction) was obtained from the North Cairo Flour Mills Company, Egypt. Gum Arabic (GA, Acacia Senegal, 8.33% polysaccharide and 2.41% protein) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acrylamide (AA, ≥ 99.8%) was purchased from Merck (Steinheim, Germany). Powdered sucrose (Nile Sugar Company, Egypt, pure and melting point 186°C), unsalted cow butter (80% fat including 54% saturated and 26% unsaturated fats, 17% water
and 3% milk solid), salt, baking powder (sodium bicarbonate), skim milk (less than 0.5% fat and 34% protein including 86% casein) and egg white powder (fat free and 11% protein) were purchased from a local supermarket. Other chemicals were analytical grades. Millipore water system (Millipore, Billerica, MA) was used to prepare the deionized pure water.

**Preparation of cakes**

A cake formulation usually includes flour, sugar, baking powder, protein, fat, and salt. For the preparation of 100% wheat dough, 55 g butter (fat source) was creamed with 100 g powdered sucrose till light and fluffy, and 56 g egg white powder (protein source) was then added little by little mixing continuously for 3 min at 60 rpm using a dough mixer Artisan Kitchen Aid 5KSM150 (MI, USA). Then, 1.0 g salt, 5.5 g baking powder, and 20.5 mL water were added and stirred for 3 min at 150 rpm. After that, 100 g wheat flour was slowly added to the mixture. Then, 11.5 mL skim milk (protein source) and 0.5 g vanilla extract were added and again stirred for 2 min at 80 rpm to obtain homogenous dough (control wheat, CW sample). Skim milk was used as an additional protein source with low fat and high casein contents in order to improve the structure characteristics of cake dough.\[19,20\] The total mass per dough was preserved at 350 g including flour 29% (w/w), (fat + protein) mixture 35% (w/w) and other constituents 36% (w/w). The amount of skim milk was calculated based on the consideration that the density of its solution is about 1.0 g/mL. For the preparation of 100% gluten-free quinoa dough, all above ingredients were mixed by the same procedure except the use of 100% quinoa flour instead of wheat flour (control quinoa, CQ sample). For the preparation of treated quinoa dough samples (G1F1→G3F3 samples), variant concentrations of GA solutions (0.1, 0.5, and 1.0 %, w/v) were supplemented; at the same time, butter levels were reduced down to 25, 50, and 75% (w/v) concurrently with adding more amounts of skim milk as described in Table 1. Skim milk was added in order to save the mass content of fat and protein mixture at 35% (w/w) and the total mass of dough at 350 g among all studied samples. The obtained dough in each sample was divided into 15 g pieces and each peace was cut into round shape by a 60 mm diameter cutter. Finally, the obtained dough pieces were baked on an aluminum plate in an electric oven (Memmert UNE 400, Germany) at 180°C for 35 min. The baking cakes were rotated from front to back after 15 min. After baking, the cakes were removed and allowed to cool at the ambient temperature. Cake samples were packed in low-density polyethylene bags for further analyses.

**Proximate evaluation of flours and cakes**

Proximate analyses of wheat flour, quinoa flour, and all baked cakes were based on the standard method of AOAC\[21\] including moisture, crude fat, crude fiber, crude protein, and ash content. All

**Table 1.** The studied cake control samples and cake samples blended with variant percentages of GA and fat.

| Samples | Gum Arabic (GA) Concentration (%) | Usage levels (g/20.5 mL water) | Fat (butter) Concentration (%) | Usage levels (g) | Skim milk Added amount (mL) |
|---------|----------------------------------|-------------------------------|--------------------------------|-----------------|-----------------------------|
| CW      | 0.00                             | 0.00                          | 100                            | 55              | 0                           |
| CQ      | 0.00                             | 0.00                          | 100                            | 55              | 0                           |
| G1F1    | 0.1                              | 0.021                         | 75%                            | 41.25           | 13.75                       |
| G1F2    | 0.1                              | 0.021                         | 50%                            | 27.5            | 27.5                        |
| G1F3    | 0.1                              | 0.021                         | 25%                            | 13.75           | 41.25                       |
| G2F1    | 0.5                              | 0.103                         | 75%                            | 41.25           | 13.75                       |
| G2F2    | 0.5                              | 0.103                         | 50%                            | 27.5            | 27.5                        |
| G2F3    | 0.5                              | 0.103                         | 25%                            | 13.75           | 41.25                       |
| G3F1    | 1.0                              | 0.205                         | 75%                            | 41.25           | 13.75                       |
| G3F2    | 1.0                              | 0.205                         | 50%                            | 27.5            | 27.5                        |
| G3F3    | 1.0                              | 0.205                         | 25%                            | 13.75           | 41.25                       |
measurements were carried out in triplicate. Crude protein was analyzed using kjeldahl method with factor of 6.25 for conversion of nitrogen to crude protein. The carbohydrate content of the cakes was calculated by subtracting 100 g from the sum of moisture, ash, fat, fiber and protein expressed in g/100 g. The total energy value was calculated as follows:

\[
\text{Total energy value (kcal/100g)} = (\text{protein} \times 4 \text{ kcal/g}) + (\text{fat} \times 9 \text{ kcal/g}) + (\text{carbohydrate} \times 4 \text{ kcal/g})
\]

(1)

**Analysis of amino acids in flours**

The amino acid profile was performed following the published work.\[^7\] The quinoa and wheat flours were hydrolyzed in 6 N hydrochloric acid followed by pre-column derivation of free amino acids with phenylisothiocyanate (PITC). The separation of the derived phenylthiocarbamyl amino acids (PTC-AA) was performed by High Performance Liquid Chromatography (VARIAN, Waters 2690, California, USA). Detection was performed at 254 nm and 35°C with a flow rate of 1 mL/min.

**Physical characteristics of cakes**

All baked cake formulations were evaluated for the height, width, spread ratio, and spread factor. The spread ratio and spread factor were calculated according to Manohar and Rao\[^22\] using the following equations:

\[
\text{Spread ratio} = \frac{\text{width}}{\text{thickness}}
\]

(2)

\[
\text{Spread factor} = \frac{\text{spread ratio of sample}}{\text{spread ratio of control}}
\]

(3)

Moreover, volume of cakes was determined according to the following formula:

\[
V = 3.14r^2h
\]

(4)

where, \(r\) is radius and \(h\) is height.

**Water loss (WL) and browning intensity of cakes**

Water contents (WC) of all studied baked cakes were measured on the dried basis before and after baking process.\[^21\] In these experiments, samples were put in metal plates and then were dried in a forced-air oven at 180°C. The following equation was used for the calculation of water loss percent (%WL) as the following:

\[
\%\text{Water loss} (WL) = \left(\frac{WC_{\text{before baking}} - WC_{\text{after baking}}}{WC_{\text{before baking}}}\right) \times 100
\]

(5)

All results were expressed as mean values (w/w, %) after three replications. The % inhibition rate was calculated by the following equation:

\[
\%\text{Inhibition rate in WL} = \left(\frac{WL_{\text{CQ}} - WL_s}{WL_{\text{CQ}}}\right) \times 100
\]

(6)

WL\(_{\text{CQ}}\) and WL\(_s\) are the water loss of the control quinoa and the water loss of cakes treated with GA, respectively. The browning intensity (BI) in the studied cakes was measured by colorimeter (Konica Minolta Colorimeter BC-10). The scale ranged from 0 for the darkest to 5.25 for the lightest color. Color measurement was repeated three times in the middle of each cake. Then, the % inhibition rate was calculated by the following equation:

\[
\%\text{Inhibition rate in BI} = \left(\frac{BI_{\text{CQ}} - BI_s}{BI_{\text{CQ}}}\right) \times 100
\]

(7)
BI\textsubscript{CQ} and BI\textsubscript{s} are the browning intensity of the control quinoa and the browning intensity of cakes treated with GA, respectively.

**Determination of AA in cakes by LC/MS/MS**

AA was extracted from baked cakes by following the procedure as described elsewhere with little modifications.\textsuperscript{[11]} 1.0 g of cake was dissolved in a solution mixture containing 9.0 mL of 10 mmol/L formic acid, 0.5 mL of Carrez I and 0.5 mL of Carrez II. The supernatant of each sample solution was collected by centrifugation at 8000 rpm for 7 min. Then, the extractions were repeated two times using 5 mL of 10 mmol/L formic acid as extraction solvent followed by centrifugation to achieve a clear extract. Further cleaning up of the extract was carried out by passing 1.5 mL of the supernatant through a preconditioned Oasis MCX cartridge. Then, the eluent was filtered through 0.45 μm membrane filter and injected into LC/MS/MS. An Agilent 1100 HPLC system equipped with a binary pump and coupled to an Agilent 1100 MS detector (Waldbronn, Germany) was used to carry out the LC/MS/MS analysis. The mode atmospheric pressure chemical ionization (APCI) was chosen for the AA analysis. The pre-packed Sunfire\textsuperscript{TM} C18 column (250 × 4.6 mm, 5 μm, Waters Corporation, Ireland) was used as the stationary phase and an isotropic mixture of 0.01 mmol/L acetic acid in a 0.2% formic acid was used as the mobile phase at 0.8 mL/min flow rate. Data acquisition was carried out in the selected ion monitoring (SIM) mode using the following parameters: nitrogen drying gas (100 psi), nebulizer pressure of 60 psi, vaporizer temperature at 425°C, drying gas temperatures at 325°C, capillary voltage of 4 kV, corona current of 4 μA and fragmented voltage of 55 eV. The ions monitored for AA were m/z 55 and 72 and the m/z 72 was selected for its quantification. Working solutions of AA were prepared by diluting its stock solution (1 mg/mL) in water to the range between 0.5 and 300.0 μg/kg sample. Limit of detection (LOD) and limit of quantification (LOQ) of AA were 0.1 μg/kg and 0.4 μg/kg, respectively. All AA analyses were conducted in triplicate. The % inhibition rate was calculated by the following equation:

\[
%\text{Inhibition rate in AA} = \frac{(AACQ - AA_s)}{AACQ} \times 100
\]  

(8)

BI\textsubscript{CQ} and BI\textsubscript{s} are the browning intensity of the control quinoa and the browning intensity of cakes treated with GA, respectively.

**Measurement of fat oxidation in cakes**

The formation of malondialdehyde (MDA) was determined for evaluation of fat oxidation. Three samples were analyzed in each time. Malondialdehyde (MDA) stock solution was prepared by the acid hydrolysis of 17 mL of 1,1,3,3-tetramethoxypropane (TMP) in 10 mL of 0.1 mol/L HCl and incubated at 40°C for 60 min to convert TMP into MDA. The concentration of MDA was determined by measuring its absorbance at 245 nm (ε = 13700). This stock solution was freshly prepared per week and was stored at 4°C during that time. Moreover, 2-thiobarbituric acid (TBA) (0.6%, w/v) was prepared in water. The solution was stirred on a hot plate (50–55°C). After cooling down to room temperature the volume was adjusted to 100 mL with water. Orthophosphoric acid solution (3 mL) was added into cake sample, and the filtrate was diluted into 10 mL water. Then, the reaction mixture (1 mL) containing equal volumes of 15% TCA (trichloroacetic acid), 0.25 mol/L HCl and 0.375% TBA (thiobarbituric acid) as well as 0.1 mL of a 0.4% solution of BHT (butylated hydroxytoluene) in 96% ethanol were added with stirring. The upper phase was transferred to test tubes. The precipitate left was again homogenized with 1 mL of the reaction mixture. The combined upper phases were heated in an oven at 100°C for 1 h. Then, the reaction was stopped through rapid cooling in ice and centrifuged for 5 min at 894 x g. After that, the supernatant was subjected for HPLC analysis. The HPLC system consisted of Dionex P 580 pump, Dionex column thermostat, Rheodyne 7725i injector and spectrophotometric detector Spectra SYSTEM UV3000HR (Thermoseparation). The column Hypersil BDS C18 (250
x 4.6 mm, 5 µm) (Agilent Technologies) at temperature of 20°C together with Rheodyne 5 µL loop injector were used. The mobile phase was 5 mmol/L phosphate buffer pH 7.0:ACN, 85:15 (v:v). Using standard solutions of MDA, calibration curve was plotted for the relationship between chromato-

graphic peak area and a concentration of MDA ($\lambda_{\text{max}} = 534$ nm). The calibration equation was response ($y = 9583$ . concentration ($x + 230.0$ ($R^2 = 0.9981)$ with linearity from 1 to 400 µg MDA/ kg cake.

The peroxide value (PV) was also determined based on the International Dairy Federation method of Shantha and Decker. The baked treated cakes were extracted with chloroform and methanol (2:1, v/v). To the solvent extract (50 µL), 75% ethanol (2.35 mL), 30% ammonium thiocyanate (50 µL), and 20 mM ferrous chloride solution in 3.5% HCl (50 µL) was added. After 3 min, the absorbance of the solution was measured at 500 nm with a UV double beam spectrophotometer (M6850, Jenway, Staffordshire, UK). The PV was obtained by calibration curve of Fe (III) ($y = 0.031 x + 0.054$, $R^2 = 0.9994$). The PV was expressed as mEq. O₂/kg of fat. Each measurement was repeated three times ($n = 3$).

**Instrumental texture profile and microstructural analyses of cakes**

For TPA analysis, texture analyzer (TA1, LLOYD Instruments, a trademark of AMETEK S.A.S., France) was used. The baked cakes were placed on the platform of the Texture Analyzer, a cylinder length of 125 mm and a disc diameter of 45 mm was attached to a 50 N load cell, and a two-cycle compression test was performed up to 50% compression of the original portion height at a cross head speed of 2 mm/s with a 30 s delay between compressions. A texture profile analysis test replicates the effect of two bites on a sample. Lloyd Instruments texture analysis software captures force, distance, area and time during the test (TPA graph), allowing the calculation of texture critical parameters such as: hardness, cohesiveness and springiness. Hardness (N): the breaking force of the product at the first loading cycle. Cohesiveness: the ratio of storage work to total work in the second loading cycle. Springiness (%): the ratio of storage deformation to total deformation in the second loading cycle.

**Color measurement of cakes**

Color measurement was performed instrumentally using a Hunterlab model Precise Color Reader TCR 200 (BAMR Ltd., Claremont, South Africa). 8-mm-diameter circle and the specular component included (SCI) mode was used to measure. The colorimeter, using D65 as a standard daylight illuminant and a standard observer position of 10°, was standardized against a white calibration plate (lightness ($L^*$) = 97.79, redness ($a^*$) = −0.11, yellowness ($b^*$) = 2.69). Three replicate measurements were taken for each sample per treatment.

**Electronic nose detection**

Electronic nose detection was used to characterize the odor among the studied baked cakes. The baked cake samples were crushed and sealed in a 50-mL small beaker. Thereafter, samples were heated in a 45°C water bath for 30 min and detected by manual headspace injection with an electronic nose (PEN3, AIRSENSE Inc.). Test conditions were as follows: Carrier gas was clean and dry air, sampling time interval, 1 s; cleaning time, 100 s; zero adjustment time, 10 s; presampling time, 5 s; measuring time, 100 s; sensor chamber flow rate, 400 mL/min; measuring sample flow rate, 400 mL/min; maximum G/G0, 3.0; and sensor, 8.

**Statistical analysis**

All analyses were performed in triplicates at each experiment. Experimental data were presented as the means and standard error (mean±SE). Experimental data were analyzed statistically by using SPSS package program (SPSS version). Linear mixed model (LMM) procedure was carried out to analyze the effect of GA concentrations as fixed effects included along with the interaction between them and
replications were dealt as random effects. Duncan’s multiple comparison tests were used to compare between mean values. Differences among mean values were considered significant when \( P < .05 \).

**Results and discussion**

**Chemical composition and physical characterization of flours and/or cakes**

The chemical composition of quinoa flour and wheat flour were given in Table 2. It is clear to notice that QF had higher (\( P < .05 \)) protein (2.2 times), fat (4.5 times), fiber (18 times) and moisture (1.3 times) than those in WF, while carbohydrate in QF is lower than that in WF. The reason may be attributed to potentially higher water absorbing of QF, since QF recorded higher fiber and fat contents; these results are in agreement with those reported in literature.\[^{[2,6]}\] Moreover, QF and WF are considered rich sources of amino acids as shown in Table 2. It is obviously that asparagine (20.33 ± 0.31 g/100 g protein) is the highest amino acid in QF and is 14.7 times higher than that in WF. Asparagine is the main amino acid participated in Maillard reaction. These results are harmonized with the published work.\[^{[6]}\]

The data in Table 3 show that the values of moisture, crude protein, crude fiber, crude fat and ash are (\( P < .05 \)) higher in quinoa-baked cakes (CQ samples) than those in wheat-baked cakes (CW samples). However, carbohydrate and energy values in CQ are significantly (\( P < .05 \)) lower than those in CW. The same observations were reported in literature about biscuits substituted by quinoa flour.\[^{[8]}\] In the current work, when quinoa cakes were fortified with different concentrations of GA from 0.1% to 1.0% (w/v), the values of fiber, moisture and ash markedly (\( P < .05 \)) increased compared with those of control samples. Moreover, the moisture values within GA groups (G1F1→F3, G2F1→F3 and G3F1→F3) reduced significantly (\( P < .05 \)) when butter concentration at 25%; however, no significant changes (\( P > .05 \)) in moisture existed at 75% and 50% when data were compared with the CQ. This observation is in agreement with previous study, which revealed that the combination of hydrocolloid and low level of fat was found to produce a slightly moister biscuit.\[^{[25]}\] The highest moisture content

| Table 2. The chemical analysis of quinoa flour and wheat flour (mean value±SE, n = 3). |
|-------------------------|-------------------------|-------------------------|
| **Constituents**        | **Quinoa flour**         | **Wheat flour**          |
| Crude protein (g/100 g) | 17.83 ± 0.62\[^{a}\]     | 7.97 ± 0.82\[^{b}\]     |
| Crude fiber (g/100 g)  | 8.66 ± 0.75\[^{a}\]     | 0.48 ± 0.31\[^{b}\]     |
| Crude fat (g/100 g)    | 3.77 ± 0.63\[^{a}\]     | 0.83 ± 0.46\[^{b}\]     |
| Moisture (g/100 g)     | 11.75 ± 0.24\[^{a}\]    | 9.24 ± 0.51\[^{b}\]     |
| Ash (g/100 g)          | 2.37 ± 0.20\[^{a}\]     | 0.55 ± 0.15\[^{b}\]     |
| Carbohydrate (g/100 g) | 55.62 ± 0.41\[^{a}\]    | 90.93 ± 0.73\[^{b}\]    |
| **Amino acids (g/100 g proteins)** |                |                         |
| Asparagine             | 20.33 ± 0.31\[^{a}\]    | 1.38 ± 0.10\[^{b}\]    |
| Glutamine              | 14.82 ± 0.32\[^{a}\]    | 32.98 ± 0.57\[^{b}\]    |
| Arginine               | 8.52 ± 0.71\[^{a}\]     | 4.22 ± 0.30\[^{b}\]    |
| Lysine                 | 6.87 ± 0.16\[^{a}\]     | 5.54 ± 0.90\[^{b}\]    |
| Glycine                | 5.93 ± 0.36\[^{a}\]     | 3.65 ± 0.60\[^{b}\]    |
| Serine                 | 5.40 ± 0.53\[^{a}\]     | 4.34 ± 0.42\[^{b}\]    |
| Alanine                | 4.82 ± 0.08\[^{a}\]     | 4.37 ± 0.05\[^{b}\]    |
| Valine                 | 4.53 ± 0.08\[^{b}\]     | 3.43 ± 0.41\[^{b}\]    |
| Proline                | 4.43 ± 0.21\[^{b}\]     | 5.37 ± 0.28\[^{a}\]    |
| Tyrosine               | 4.09 ± 0.33\[^{a}\]     | 10.37 ± 0.17\[^{b}\]   |
| Methionine             | 3.63 ± 0.50\[^{a}\]     | 3.47 ± 0.11\[^{b}\]    |
| phenylalanine          | 3.52 ± 0.58\[^{a}\]     | 4.57 ± 0.73\[^{a}\]    |
| Threonine              | 3.50 ± 0.67\[^{a}\]     | 2.50 ± 0.20\[^{a}\]    |
| Tyrosine               | 2.83 ± 0.83\[^{a}\]     | 3.35 ± 0.20\[^{a}\]    |
| Histidine              | 2.56 ± 0.20\[^{a}\]     | 3.42 ± 0.77\[^{a}\]    |
| Cysteine               | 2.72 ± 0.13\[^{a}\]     | 2.47 ± 0.70\[^{a}\]    |
| Tryptophan             | 1.33 ± 0.31\[^{a}\]     | 2.78 ± 0.62\[^{a}\]    |
| Lysine                 | 0.17 ± 0.88\[^{b}\]     | 1.82 ± 0.49\[^{b}\]    |

Values which don’t share the same letter in each row are significantly different (\( P < 0.05 \))
was achieved in the G3F1 and G3F2 samples containing 1% (w/v) GA concentration and 75% or 50% butter amount. The reason could be due to that these samples contained the highest amounts of functional fibers GA compared to other treated samples, which have the ability to retain water in cakes during baking process. Perhaps a coupling effect of hydrocolloid and fat may have had an effect on the water-binding capacity in the cake dough. This result is in a good agreement with previous findings.\(^\text{[13]}\) Moreover, caloric values decreased as butter was progressively reduced, and GA concentration was progressively increased. G3F2 samples containing 1% GA and 50% butter presented lower energy value than that in G3F1 sample with 75% butter, which are significantly \((P < .05)\) different from control samples CW and CQ.

The mean values of physical characteristics of CW, CQ and all treated GFQCs were measured. It is obvious that there is an increment \((P < .05)\) in the spread ratio value of CQ compared to CW. This means that the full substitution of WF by QF enhanced the spread ratio of cakes. It is due to that the gluten network was weakened, which is responsible for retaining the leavening gases, and cookies having higher spread ratios are considered most desirable.\(^\text{[6]}\) On the contrary, there is no any observed difference \((P > .05)\) in the spread ratio between CW and other cakes fortified with variant GA concentrations. The same observation was obtained with mitigating butter amounts in treated quinoa cakes. However, these GA treated cakes presented more spread ratio (flatter) \((P < .05)\) than cakes made with wheat flour. GA should contain emulsifiers, and these ingredients may be responsible for weakening the structural framework in a baked product.\(^\text{[11]}\) Considering the spread factor, results indicated that the values of CQ were significantly \((P < .05)\) increased compared to CW. On the other hand, the fortification of quinoa cakes with GA and the replacement of butter recorded a slight change \((P > .05)\) in the spread factor compared to CQ. This result is in a good agreement with the previous findings.\(^\text{[25]}\) Furthermore, the volume of CQ was higher \((P < .05)\) than that of CW. This is due to that the presence of gluten-free quinoa flour weakening the gluten network. Although the volume increased proportionally with the addition of 0.1–1.0% GA, which was similar to the cake volume made with wheat flour. This indicated that the addition of GA could thicken the dough during the mixing of the ingredients, thereby improving its volume to a level comparable to that of cakes made with 100% wheat flour. The GA could be used as a thickening agent instead of gluten in the gluten-free quinoa cakes.

### Water loss and browning intensity

Changes in water loss (WL) and browning intensity were measured in the treated cakes and compared with the control wheat and quinoa cakes as shown in Table 4. The data revealed that the fortification with GA resulted in significant changes \((P < .05)\) of WL in a comparison with control samples. This
observation is in agreement with previous findings.\textsuperscript{[11]} Moreover, water loss (WL) values within samples containing the same GA concentration and different butter amounts appeared only significant changes ($P < .05$) in the presence of 25% butter when compared to 75% and 50% samples. Similar results were observed with moisture as declared in Table 3. The highest ($P < .05$) inhibition rate (47.9%) in WL was obtained in G3F1 and G3F2 samples containing 1% GA. Reasons of these achievements should be attributed to the presence of higher functional fiber GA contents in these samples, which should inhibit the loss of water content. In other words, GA could form a tight layer on the outer surface of cake with fewer voids preventing the escape of water from the porous surface, and the surface permeability was then reduced.\textsuperscript{[9]} Further study proved that hydrocolloids are able to form a three-dimensional continuous network which entraps and immobilizes water, thereby forming a rigid structure.\textsuperscript{[25]} Moreover, a coupling effect of 1% GA and 75% or 50% butter may have had an effect on the water-binding capacity in the cake dough.

The browning intensity (BI) was also measured among the studied cakes. It is interesting to observe higher BI value in control quinoa cake (2 times) than that in control wheat cake. The presence of high amounts of protein and fat in QF when compared to WF (Table 3) should promote the formation of non-enzymatic browning products in the studied cakes. The presence of high amounts of asparagine in QF (about 15 times higher than WF, Table 2) could interact with glucose/fructose at the baking temperature forming Maillard reaction. Furthermore, the existence of fat in QF (about 4.5 times higher than WF, Table 2) could be oxidized by oxygen and reactive oxygen species forming aldehydes and ketones, which then react with amino acids in QF to form brown pigments.\textsuperscript{[13,26]} By fortification with GA, the BI values decreased significantly ($P < .05$) with increasing GA concentrations from 0.1% to 1% compared with CQ. In addition, the use of 1% GA in cakes reduced significantly ($P < .05$) the BI values against those in control wheat cakes (CW) reflecting the advantages of GA as fortifier in both of wheat and quinoa cakes. GA tight layer could be formed on the external surface of cakes at the baking temperature reducing the WL and subsequently inhibiting the BI values. On the same hand, the reduction of butter amounts appeared significant inhibition ($P < .05$) in BI values. This observation is in agreement with previous study.\textsuperscript{[25]} The highest inhibition rate (59.1%) in BI was obtained in G3F2 and G3F3 samples as presented in Table 4. Therefore, the highest inhibition rates in both of WL (47.9%) and BI (59.1%) achieved in G3F2 sample containing 1% (w/v) GA and 50% (w/w) butter lower than full fat cake.

**Inhibition of acrylamide (AA)**

The AA content is very sensitive to the amount of asparagine (limiting factor) in quinoa flour (QF). Asparagine was found to be $20.33 \pm 0.31$ g asparagine/100 g protein (Table 2) in QF, which is 15 times

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**Table 4.** Water loss (WL), browning intensity (BI) and acrylamide (AA) determination (mean value±SE, n = 3) in the studied baked cake products.

| Sample | WL % (m/m) | Inhibition rate in WL (%) | BI % (m/m) | Inhibition rate in BI (%) | Acrylamide (μg/kg)±SD | Inhibition rate in AA (%) |
|--------|------------|--------------------------|------------|--------------------------|-----------------------|-------------------------|
| CW     | 40.5 ± 0.3\textsuperscript{a} | --                        | 32.5 ± 0.8\textsuperscript{a} | --                        | 934.0 ± 8.5\textsuperscript{a} | --                      |
| CQ     | 28.8 ± 0.2\textsuperscript{b} | --                        | 64.5 ± 1.3\textsuperscript{b} | --                        | 2147.2 ± 9.0\textsuperscript{b} | --                      |
| G1F1   | 22.6 ± 0.7\textsuperscript{c} | 21.5                     | 55.3 ± 0.7\textsuperscript{c} | 14.3                     | 1646.9 ± 9.5\textsuperscript{c} | 23.3                    |
| G1F2   | 22.5 ± 0.1\textsuperscript{c} | 21.9                     | 50.9 ± 1.0\textsuperscript{d} | 21.1                     | 1632.4 ± 7.1\textsuperscript{c} | 24.0                    |
| G1F3   | 25.2 ± 0.2\textsuperscript{e} | 12.5                     | 43.2 ± 0.3\textsuperscript{g} | 33.0                     | 1620.9 ± 5.0\textsuperscript{c} | 24.5                    |
| G2F1   | 19.3 ± 0.5\textsuperscript{f} | 33.0                     | 41.0 ± 1.0\textsuperscript{e} | 36.4                     | 927.1 ± 5.8\textsuperscript{a} | 56.8                    |
| G2F2   | 19.1 ± 0.8\textsuperscript{g} | 33.7                     | 38.2 ± 1.1\textsuperscript{g} | 40.8                     | 925.1 ± 9.2\textsuperscript{a} | 56.9                    |
| G2F3   | 20.8 ± 0.6\textsuperscript{h} | 27.8                     | 32.2 ± 0.7\textsuperscript{g} | 50.1                     | 919.7 ± 3.7\textsuperscript{a} | 57.2                    |
| G3F1   | 15.0 ± 0.9\textsuperscript{g} | 47.9                     | 31.0 ± 0.4\textsuperscript{h} | 51.9                     | 367.0 ± 7.3\textsuperscript{d} | 82.9                    |
| G3F2   | 15.0 ± 0.5\textsuperscript{h} | 47.9                     | 26.4 ± 1.3\textsuperscript{g} | 59.1                     | 358.4 ± 6.3\textsuperscript{d} | 83.3                    |
| G3F3   | 16.2 ± 0.7\textsuperscript{h} | 43.8                     | 26.4 ± 1.0\textsuperscript{g} | 59.1                     | 356.8 ± 6.5\textsuperscript{d} | 83.4                    |

Different letters within a column indicate significantly different values ($P < 0.05$).

Inhibition rates were calculated by the equations as described in experimental section.
higher than WF. On the contrary, asparagine was very small amount lower than 0.2 g in other protein sources (egg protein and skim milk protein) which could be considered as negligible value compared with that in QF (100 times). Prior to the baking of cakes, no AA was detected in the dough. These results also corroborated with the previous study in which AA was detected after 10 min of baking.[26] In the current work, results in Table 4 showed that the amount of AA in control quinoa cake (CQ) after baking at 180°C was 2147.2 ± 9.0 μg/kg, which is 2.3 times higher than AA in wheat cake (CW). As well, this AA amount in CQ is higher than the allowable amount of AA (1044 μg/kg) in bakery products.[12] The addition of GA to the cake caused a significant inhibition (P < .05) in the formation of AA. The use of 0.021 g GA/20.5 mL water (samples from G1F1 to G1F3) decreased AA content down to about 1620.9 μg/kg dry cake (about 24.5% inhibition) compared to CQ sample without any significant change (P > .05) by variation of butter amount. Unfortunately, AA content in quinoa cakes supported by this level of GA (0.1%) is still higher than the allowable recommended amount and, further addition of GA should be studied. In the case of 0.103 g GA/20.5 mL water (samples from G2F1 to G2F3), AA was significantly reduced down to about 919.7 μg/kg dry cake (about 57.2% inhibition compared to CQ), which is lower than the EU-recommended level. Further increment of GA up to 0.205 g GA/20.5 mL water (samples from G3F1 to G3F3), AA decreased significantly (P < .05) down to about 356.8 μg/kg dry cake. The highest inhibition rate (about 83%) was achieved in G3F2 and G3F3 samples compared to CQ. The reasons for this behavior could be due to the gelling or thickening effect of GA on the texture modification of proposed cakes which consequently could interfere with the molecular interactions between glucose/fructose and asparagine as precursors of AA formation. Moreover, the acidic pH value of GA solution (pH = 4.9)[9] could be another factor to facilitate the reduction of AA formation in cakes. Therefore, the addition of 1% GA was significantly (P < .05) inhibited the AA content (inhibition rate 83%, about 356.8 μg/kg dry baked cake) which is lower than the EU-recommended level in bakery products.

**Oxidation of fats**

Fat oxidation is one of the main chemical changes, which is responsible for the loss in palatability and quality characteristics of food products. The malondialdehyde (MDA) contents of all studied samples as an indication of fat oxidation in cakes were presented in Figure 1. It was observed that the MDA.

![Figure 1](MDA values in control samples CW, CQ and other cakes treated with GA. Different distinct letters differed significantly one from another, P < .05.)
values in CQ is \( (P < .05) \) higher than that in CW. This is probably due to the presence of fat in quinoa flour which is about 4.5 times higher than that in wheat flour as indicated in Table 2. This could be oxidized by oxygen and reactive oxygen species forming aldehydes and ketones.\(^{[10]}\) It was also observed that the reduction of fat content decreased markedly the formation of MDA in all studied treated cakes (G1F1→G1F3; G2F1→G1F3; G3F1→G1F3). On the same hand, the presence of 50% lower butter in GFQCs (G3F2 sample) appeared the highest \( (P < .05) \) mitigation in fat oxidation (about 128 \( \mu \)g MDA/kg sample) compared to CQ (about 196 \( \mu \)g MDA/kg sample) as presented in Figure 1. The same observation was achieved with 25% butter amount (G3F3 sample) without any significant change \( (P > .05) \) compared to 50% butter amount (G3F2 sample). This situation may be associated with fat reduction without deteriorating characteristics of quinoa cakes due to the preservation of their moisture function by gum Arabic network formation. These data support the previous trends of fat contents (Table 3) and BI values (Table 4) in GFQCs supplemented with GA.

For greater evidence, the peroxide value (PV) is usually used as oxidation index of the primary oxidation stage. CQ showed higher value of peroxides (2.5 times) than CW. These data confirmed that higher oxidation state was obtained when high oxidized raw ingredients were used in the recipe. In fact, quinoa flour is more oxidized than wheat flour, and this was also reflected in the cakes formulated with quinoa. By the fat reduction in the other treated quinoa cakes, the PV values were decreased gradually from 7.8 mEq. \( \text{O}_2 / \text{kg of fat} \) (G3F1 sample) to 3.6 mEq. \( \text{O}_2 / \text{kg of fat} \) (G3F3 sample). However, all studied cakes reported PVs lower than 10 mEq. \( \text{O}_2 / \text{kg of fat} \), that was reported as the highest limit before considering these kinds of products sensorially unacceptable by the consumers.\(^{[27]}\) Similar observation was detected by Verardo et al.\(^{[28]}\) in biscuits, where oxidized fatty acids (OFA) noticed the same trend of PV.

**Texture profile and microstructural analyses**

Texture profile analysis (TPA) of proposed baked cakes was demonstrated in Table 5. Of these parameters, hardness is the most crucial since the lower hardness value, the softer food product. QF\(^a\) presented positive effect on hardness of the cakes. Previous studies reported that flour with high protein and fiber amounts could increase the hardness of food product.\(^{[18]}\) Considering the fiber and protein content (Table 2) existed in QF composition, when compared to WF, both of them might have influenced the hardness of CQ. The same observation was obtained in cohesiveness and springiness of CQ compared to CW as indicated in Table 5. On the other hand, GA supplementation from 0.1 to 1.0% (w/v) in cakes lowered \( (P < .05) \) hardness in comparable with CQ. The lowest hardness was achieved at 1% GA. A reduction in hardness is an inevitable result, since it is well documented that the dilution effect of polysaccharides or functional fibers on food system is due to its water-binding properties giving a softer texture.\(^{[25]}\) The same trend for cohesiveness and springiness in treated cakes was observed. Even though hardness, cohesiveness and springiness increased as butter levels decreased in GA

| Sample | Hardness (N) | Cohesiveness | Springiness (%) | \( L^* \) | \( a^* \) | \( b^* \) |
|--------|--------------|--------------|----------------|--------|--------|--------|
| CW     | 36.20 ± 0.11\(^a\) | 0.60 ± 1.40\(^c\) | 61.63 ± 1.85\(^c\) | 50.20 ± 1.25\(^a\) | 11.72 ± 0.02\(^a\) | 12.98 ± 0.58\(^a\) |
| CQ     | 53.14 ± 0.14\(^d\) | 0.71 ± 1.41\(^b\) | 72.54 ± 1.31\(^e\) | 34.85 ± 1.15\(^c\) | 11.17 ± 0.22\(^b\) | 15.97 ± 0.56\(^b\) |
| G1F1   | 51.49 ± 0.43\(^d\) | 0.70 ± 1.31\(^b\) | 70.40 ± 1.24\(^d\) | 40.50 ± 1.05\(^c\) | 11.18 ± 0.12\(^b\) | 15.97 ± 0.43\(^b\) |
| G1F2   | 52.32 ± 0.27\(^d\) | 0.71 ± 1.29\(^b\) | 71.05 ± 1.53\(^d\) | 44.80 ± 0.75\(^f\) | 11.17 ± 0.15\(^b\) | 15.97 ± 0.35\(^b\) |
| G1F3   | 53.49 ± 0.03\(^d\) | 0.71 ± 1.46\(^b\) | 72.27 ± 1.52\(^d\) | 47.57 ± 0.23\(^b\) | 11.18 ± 0.52\(^b\) | 15.97 ± 0.63\(^b\) |
| G2F1   | 46.47 ± 0.21\(^d\) | 0.65 ± 1.26\(^a\) | 64.47 ± 1.31\(^e\) | 46.67 ± 0.63\(^b\) | 11.18 ± 0.65\(^b\) | 15.97 ± 0.75\(^b\) |
| G2F2   | 48.20 ± 0.80\(^b\) | 0.67 ± 1.52\(^d\) | 67.99 ± 1.09\(^d\) | 48.35 ± 1.12\(^g\) | 11.19 ± 0.43\(^b\) | 15.97 ± 0.36\(^b\) |
| G2F3   | 50.62 ± 0.53\(^c\) | 0.69 ± 1.81\(^f\) | 70.66 ± 1.37\(^d\) | 50.84 ± 1.42\(^d\) | 11.17 ± 0.65\(^b\) | 15.97 ± 0.58\(^b\) |
| G3F1   | 36.08 ± 0.81\(^f\) | 0.59 ± 1.21\(^h\) | 60.32 ± 1.64\(^d\) | 51.72 ± 1.22\(^d\) | 11.16 ± 0.32\(^b\) | 15.97 ± 0.56\(^b\) |
| G3F2   | 36.22 ± 0.54\(^f\) | 0.59 ± 1.56\(^h\) | 60.91 ± 1.02\(^h\) | 52.18 ± 0.71\(^h\) | 11.15 ± 0.63\(^b\) | 15.97 ± 0.76\(^b\) |
| G3F3   | 39.87 ± 0.57\(^f\) | 0.60 ± 1.61\(^c\) | 61.47 ± 1.74\(^c\) | 52.30 ± 1.20\(^h\) | 11.17 ± 0.37\(^b\) | 15.97 ± 0.59\(^b\) |

Averages followed by distinct letters, on the column, differed significantly one from another \( (P < 0.05) \).
treated cakes, G3F1 and G3F2 samples containing 75/50% lower butter and 1% GA produced significantly ($P < .05$) softer cakes when compared with CQ samples and also with no significant ($P > .05$) changes compared to CW. These observations are correlated with other findings of WL (Table 4) and moisture content (Table 3) in G3F1 and G3F2 samples. Reasons of these achievements should be attributed to the coupling effect of GA and fat, which have had an effect on the water-binding capacity in the cake dough.\textsuperscript{[26]} GA could form a tight layer on the outer surface of cake with fewer voids preventing the escape of water from the porous surface, and the surface permeability was then reduced. Further study proved that hydrocolloids are able to form a three-dimensional continuous network which entraps and immobilizes water, thereby forming a rigid structure.\textsuperscript{[25]}

**Color and electron nose analyses**

Color characteristic is a major criterion that affects the quality of the final product. Table 5 shows values of whiteness ($L^*$), redness ($a^*$) and Yellowness ($b^*$) in cakes crust. The replacement of wheat flour (CW) by quinoa flour (CQ) showed a difference in color. The CQ samples were darker and yellower ($P < .05$) than CW. The increase in $b^*$ was due to that the color of QF was yellowish white, and it contained higher content of fiber. Moreover, as explained in previous studies, high protein content in QF may have contributed to decrease the $L^*$ value of the cookies due to the Maillard reaction resulting in a darkening of the product.\textsuperscript{[29]} In the current work, it was confirmed by measuring higher BI and AA values (Table 4) in CQ than that in CW. By fortification with GA in GFQCs, the $L^*$ values increased significantly ($P < .05$) with increasing GA concentrations from 0.1% to 1% compared with CQ. In addition, the use of 1% GA in G3F1, G3F2, and G3F3 samples enhanced significantly ($P < .05$) the $L^*$ values more than that in CW. On the same hand, the mitigation of butter amounts enhanced significantly ($P < .05$) in the $L^*$ values. These values were further complemented by a decrease in BI values (Table 4). Therefore, degree of browning was affected by the level of fat, as well as the presence of GA. GA may also have affected the rate of browning in cakes. This observation is in agreement with previous study.\textsuperscript{[25]} The highest $L^*$ values were obtained in G3F2 and G3F3 samples as presented in Table 5. The values of $a^*$ and $b^*$ ranged among the cakes formulated with higher GA and lower fat, but these variations were not significant, indicating an absence of correlation between $a^*$ and $b^*$ parameters and GA proportions in cakes.

The intensity curves of the electronic nose sensor can be used to characterize the odor intensity profiles of the studied cakes.\textsuperscript{[30]} The variation trend of the signal values of the 10 sensors was consistent with the intensity difference of the volatile components in the samples. Figure 2 was established based on the corresponding values between the sensors connected to each other for each of the studied cakes. It was obviously the odor intensity of quinoa cakes (CQ) is significantly higher than wheat cakes (CW), that is, the volatile components in CQ are higher ($P < .05$) than those in CW. This observation is in a good agreement with previous studies.\textsuperscript{[2,31]} It was reported that about 20 volatile compounds were detected in high amounts using QF in cookies. The volatile components were mainly aromatics, nitrogen oxides, sulfur compounds, ammonia, and alkanes. Aldehydes, which are well-known fat oxidation products, were detected in quinoa products.\textsuperscript{[31]} A further three compounds, 2-methyl-butanal (fruity, almond), benzaldehyde (bitter almond), and benzene acetaldehyde (honey, floral), which are attributable to Maillard reaction, are present at higher amounts in quinoa-based products. Thus, the use of 100% QF in CQ cakes resulted in an increase in volatile compounds that are nearly always characterized by positive odor intensity. On the other hand, hydrocolloid GA supplementation from 0.1 to 1.0% (w/v) in cakes lowered ($P < .05$) odor intensity in comparable with CQ samples. The lowest odor intensity ($P < .05$) was achieved at 1% GA. Moreover, intensity decreased ($P > .05$) as butter levels decreased in GA treated cakes as shown in Figure 2. Reasons of these achievements should be attributed to the combined effect of GA and fat, which have had an effect on the water-binding capacity in the cake dough.\textsuperscript{[26]} In addition, the
The supplementation with hydrocolloid GA developed successfully the quality of gluten-free quinoa cakes by reducing the formation of non-enzymatic browning acrylamide (AA) product. The addition of 1% GA significantly \( (P < .05) \) inhibited AA content up to 83% giving an amount of 356.8 μg/kg dry cake, which is lower than the EU-recommended level in bakery products. Moreover, the fat oxidation was significantly \( (P < .05) \) mitigated up to 64% in the presence of 50% lower amount of fat without impairing the baking performance of quinoa cakes. The highest inhibition rate (59.1%) in browning intensity was achieved with 1% GA and 50% lower fat. Data from the current study could serve as a resource to the researcher to further explore the utilization of hydrocolloids in protein-rich bakery products.

**Disclosure statement**

The author declares that I do not have any conflict of interest.

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**Figure 2.** Odor intensity values in control samples CW, CQ and other cakes treated with GA. Different distinct letters differed significantly one from another, \( P < .05 \).
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