DOI: https://doi.org/10.21323/2618-9771-2024-7-1-165-178

Received 13.08.2023
Accepted in revised 15.09.2023
Accepted for publication 20.09.2023

Available online at https://www.fsjour.com/jour
Original scientific article
Open access

BISCUITS ENRICHED WITH THE EDIBLE POWDER OF ANGOUMOIS GRAIN MOTH (*SITOTROGA CEREALELLA*): OPTIMIZATION, CHARACTERIZATION AND CONSUMER PERCEPTION ASSESSMENT

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KEY WORDS:
insects, flour, evaluation, protein

ABSTRACT

During this study, the dried powder of Angoumois grain moth (*Sitotroga cerealella*) (AGM) was used as an excellent source of protein to produce nutritional biscuits. Physical, chemical, rheological and microbiological characteristics of biscuits with the optimum AGM percentage (5%) were evaluated compared to the control. The response surface methodology (RSM) with optimal (custom) design was first employed for optimizing the percentage of the AGM powder in biscuits. The optimum selected percentage of the AGM powder was 5% and a quadratic model was found to yield the best fit. The evaluation of parameters of biscuits (ash, protein, fiber and fat content) showed that most of the values, except carbohydrate, significantly increased with AGM inclusion (5%) compared to the control. Also, an increase in diameter, spread ratio and weight values and a decrease in the thickness and break strength of the biscuits were observed with AGM powder inclusion. The presence of the 5% AGM powder slightly increased water absorption, and dough development time also increased compared to the control sample. However, stability of dough decreased. The sanitary conditions of the AGM biscuits were satisfactory as the analytical results were within the established values specified in the regulation. The study revealed that Angoumois grain moth is an excellent source of nutrients necessary for combating protein-energy-malnutrition rampant in our world today.

ACKNOWLEDGEMENTS: The authors would like to thank the Food Science Department Faculty of Agriculture Cairo University for support this work.

Поступила 13.08.2023
Поступила после рецензирования 15.09.2023
Принята в печать 20.09.2023

https://www.fsjour.com/jour
Научная статья
Open access

ПЕЧЕНЬЕ, ОБОГАЩЕННОЕ ПИЩЕВЫМ ПОРОШКОМ АНГУМУАЗСКОЙ МОЛИ (SITOTROGA CEREALELLA): ОПТИМИЗАЦИЯ, ХАРАКТЕРИСТИКА И ОЦЕНКА ПОТРЕБИТЕЛЬСКОГО ВОСПРИЯТИЯ

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КЛЮЧЕВЫЕ СЛОВА:
домашние фильтры воды, фильтрованная вода, химическое и микробиологическое качество, безвредность воды

АННОТАЦИЯ

В данном исследовании использовали сухой порошок Ангумуазской моли (*Sitotroga cerealella*) (AGM) как превосходный источник белка для производства питательного печенья. Оценивали физические, химические, реологические и микробиологические характеристики печенья с оптимальным процентом AGM (5%) по сравнению с контролем. Сначала был применен метод поверхности отклика для оптимизации процента порошка AGM в печенье. Оптимальным выбранным процентом порошка AGM было 5%, и было установлено, что квадратичная модель дает наибольшее соответствие. Оценка показателей печенья (содержание золы, белка, клетчатки и жира) показала, что большинство значений, за исключением углеводов, существенно увеличилось при включении 5% AGM по сравнению с контролем. Кроме того, наблюдали увеличение диаметра, отношения диаметра к толщине и веса, и снижение толщины и разрывного усилия печенья при включении порошка AGM. Присутствие 5% порошка AGM несколько увеличивало абсорбцию воды, также увеличивалось время образования теста по сравнению с контрольным образцом. Однако стабильность теста снижалась. Санитарное состояние печенья с AGM было удовлетворительным, так как результаты исследования были в пределах установленных уровней, указанных в нормативных документах. Данное исследование продемонстрировало, что зерновая моль является превосходным источником нутриентов, необходимых для борьбы с неполноценным питанием и недостатком белка и энергии, распространенных в нашем мире в настоящее время.

БЛАГОДАРНОСТИ: Авторы выражают благодарность кафедре науки о пище, сельскохозяйственного факультета, Каирского университета за поддержку данной работы.

1. Introduction

Food security, quality and nutritional value are the world's most pressing concerns to meet an increase in population and shortage of food sources. Malnutrition has been a problem for most of consumers caused by insufficient diet. For centuries, wheat has been a central component of the typical diet of the country's inhabitants. Egypt is the most populous country in the Arab World, and the largest importer of wheat globally [1].

There is a big shortage of wheat, which is considered the main ingredient in bread and other bakery products. The present state of food security in Africa makes it pertinent to search for new alternatives for wheat that could enrich the basic diet. There are an increase in population worldwide and, consequently, an increasing demand for additional sources of proteins. Many studies were conducted using different plant protein sources like beans and soy to improve the quality of wheat flour

FOR CITATION: **Mohsen, S. M., Ashraf, A., Ahmed, S. S., Abedelmaksoud, T. G.** (2024). Biscuits enriched with the edible powder of Angoumois grain moth (*Sitotroga cerealella*): Optimization, characterization and consumer perception assessment. *Food Systems,* 7(1), 165-178. https://doi.org/10.21323/2618-9771-2024-7-1-165-178

ДЛЯ ЦИТИРОВАНИЯ: **Мохсен, С. М., Ашраф, А., Ахмед, С. С., Абедельмаксуд, Т. Г.** (2024). Печенье, обогащенное пищевым порошком Ангумуазской моли (*Sitotroga cerealella*): оптимизация, характеристика и оценка потребительского восприятия. *Пищевые системы*, 7(1), 165-178. https://doi.org/10.21323/2618-9771-2024-7-1-165-178

dough and bakery products. Also, due to the shortage in the traditional sources, different trials are focused on the non-traditional sources such as insects and algae [2].

Recently, the high nutritional value of edible insects has attracted the attention of researchers and the food industry for their potential use in foods. Edible insects contain around 35–61% proteins, 15–40% fats, and 3–10% minerals (e. g., iron, selenium, and zinc) and vitamins (mainly those of the B-group) [3]. Therefore, they can be used as good sources for these nutrients to improve health and avoid starvation. Edible insects are part of the diet for at least two billion people all over Asia, Africa, and South America. In Europe, Asia, and Africa, consumers' interest in insect-based foods is quickly increasing. Therefore, the new novel foods Regulation (Regulation 2015/2283) entered into force in January 2018 has given the green light for insect-based foods which safety has been assessed by the European Food Safety Authority (EFSA) upon the request of the European Commission and which have been authorized by the Commission for placing on the market within the Union to be legally sold in all EU member states [4].

Certain insect species such as *Hermetia illucens*, *Acheta domestica*, *Tenebrio molitor*, *Macrotermes nigeriensis*, *Macrotermes bellicosus*, *Brachytrupes megacephalu*s, *Ephestia cautella*, *Spodoptera littoralis*, *Agrotis ipsilon*, *Ephestia kuehniella* and *Schistocerca gregaria*, as well as *Syntermes* soldiers, can be targeted for being incorporated as a powder in food products providing additional nutrients.

There were several studies that used edible insects as a supplement in bakery products to increase the nutritional value and improve quality characteristics of the bakery products such as studies by Ogidi et al. [5] who added *Brachytrupes membranaceus*, *Macrotermes nigeriensis* and *Rhynchophorus ferrugineus* in cookies, Pérez-Rodríguez et al. [6] who added *Tenebrio molitor* and *Sphenarium purpurascens* in bread and Kowalski et al. [7] who added *Tenebrio molitor* in sponge cakes.

De Oliveira et al. [8] made a conclusion about the safety of cinereous cockroach (*Nauphoeta cinerea*) in bread. In their study, the insect flour showed satisfactory sanitary conditions since the results of the microbiological analysis of the flour with cinereous cockroach were within the range of the established values specified in the regulation.

There is no reported data available on utilization of the Angoumois grain moth (AGM) powder in food. Therefore, the aim of this study was the use of Angoumois grain moth (*Sitotroga cerealella*) as a functional food supplement with the high protein concentration for different consumer categories, especially children. The percentage of the AGM powder was optimized; then, physical, chemical, rheological and microbiological properties were studied.

2. Materials and methods

2.1. Materials

Angoumois grain moth (*Sitotroga cerealella*) (AGM) was provided by the Agricultural Research Center, Department of Biological Control, Giza, Egypt. Other ingredients such as wheat flour, sugar, butter, baking powder, and eggs were purchased from the local market in Giza, Egypt. All chemicals, solvents, and reagents were provided by PioChem Company (Egypt).

2.2. Selection of insect variety

Different varieties of insects, i. e. Mediterranean flour moth (*Ephestia kueniella*), almond moth (*Ephestia cautella),* Angoumois grain moth (AGM) (*Sitotroga cerealella*), cotton leafworm *(Spodoptera littoralis*) and palm weevil (*Rhynchophorus ferrugineus*) were evaluated for their productivity.

2.3. Rearing of Angoumois grain moth, Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae)

The wheat grains were boiled, strained, spread over trays and left to dry. Then, the wire trays were filled with the dried wheat grains (6 kg/tray). The dried wheat grains in trays were infested with the grain moth eggs (1g egg moth/1kg wheat grains). The wire trays were placed in a horizontal position. After ten days, *S. cerealella* eggs hatched and the wire trays were placed vertically in the cages and maintained until emergence and falling of the moths in the plastic jars fitted under each cage. The plastic jars were replaced daily. After that, the collected moths were emptied into the wire cylinders (wire screen allows *Sitotroga* eggs to fall through and prevent the moths to escape).

2.4. Production of the AGM powder

AGMs were dried using an oven (Shel-lab, Cornelius, OR, USA) at different temperatures (from 50 °C to 160 °C) at different times (2hrs and 4hrs). The dried AGMs were ground by an analytical mill (Cole-Parmer, Vernon Hills, IL, USA), sieved up to 50 mesh and stored at −20 °C (Blocean, Egyptian Electrical Appliances Co., Egypt) in brown laboratory bottles until further analysis.

2.5. Preparation of biscuits using the AGM powder

Five formulations of biscuits were made using the method of Oluwamukomi et al. [9] with some modifications as follow. The AGM powder was individually added to wheat flour, mixed and then water, sugar (22.5%), butter (40%), eggs (0.3%) and baking powder (2.8%) were added. The ingredients were mixed and the dough was continuously mixed until a smooth consistency was obtained. The dough was then kneaded, and rolled out thinly on a cutting board where it was cut out into uniform shapes and sizes. The cut dough was placed in a greased baking tray and transferred into the oven (Zanussi, Electrolux, Italy). The biscuits were baked at 180 °C for 20–30 minutes, cooled, and packed. The weight of each biscuit was determined (10–15g).

2.6. Optimization of the AGM powder percentage

The response surface methodology (RSM) was used to examine the effects of the AGM powder percent (independent variable) on the overall acceptability, texture, and color values (*L, a,* and *b*) (responses) of biscuits. An optimal (custom) design was used: AGM powder (0–15%) as shown in Table 3. Analysis of variance (ANOVA) found the significant terms in the model for the responses (overall acceptability, texture and color values (*L, a,* and *b*)) and model ANOVA statistics investigated the validation of the equation. Statistical calculations were made using the regression coefficients in order to generate a response surface plot from the model (Trial version of Design Expert Version 10.0.6 software).

The generalized second-order polynomial model was used in the response surface analysis, which is described by (Equation 1):

$$Y = a_0 + a_1 x_1 + a_{11} x_1^2, \tag{1}$$

where

Y is the response variable;

X_1 (AGM powder%) is the independent variable.

Regression coefficients are:

a_0 is for intercept;

a_1 is for linear term;

a_{11} is for quadratic term.

The experimental data were fitted to a second-order polynomial model (Equation 1) to obtain the regression coefficients. Using the R^2, adjusted-R^2, predicted-R^2 (should be over 0.90), and prediction error sum of squares (PRESS) values, the adequacy of the model was checked. Good model fitting is shown by a high predicted R^2 and a low PRESS [10]. The perturbation plot was also used to compare the effects of the factor at a particular point in the design space. Response surface and contour plots were then generated. The optimization of percentage of the AGM powder for overall acceptability maximization, assigned texture and color values (*L, a,* and *b*) in the range was done by using a desirability function. Desirability function d(y) ranges from 0 to 1 for each response (y), and depends totally on closeness to the lower and upper limits. The range of the desirability value is from 0 (which represents a totally undesirable value of y) to 1 (which represents an ideal or completely desirable value of y). Many desirability functions can be used depending on whether a certain response is to be maximized, decreased, or allocated to a target value [11].

In this study, the main objective of optimization is maximizing the overall acceptability and assigned color values (*L, a* and *b*) and texture to a target value to be in the range (response, y). Equations 2, 3 and 4 describe the desirability function as follows:

$$d(y) = 1, y \leqslant L \tag{2}$$

$$d(y) = \frac{(y-L)}{(U-L)}, L < y < U \tag{3}$$

$$d(y) = 0, y \geqslant U \tag{4}$$

where *L* and *U* are the response's lower and upper limits, respectively.

By the desirability function method, maximization of polynomials was carried out using a trial version of design expert version 10.0.6 software.

2.7. Consumer perception

Participants were given a link to the online questionnaire by email and social media, using the free online survey tool Google Form (WhatsApp and Facebook). A succinct explanation of the current study was provided, and participants were encouraged to contact us with any questions by email as well as by typing, calling from a cell phone, or speaking directly during the contact. The current study examines consumers' perception of biscuits, including AGM biscuits in general, and their desire to purchase such types of biscuits but does not represent a particular market segment or the total market population.

There were 261 respondents to the Google form questionnaire: 54.4% female and 45.6% male, the age range was 14 to 79. The educational level of the respondents was as follows: high school (19.9%), a college degree (32.6%), a master's degree (23.8%), and a doctoral degree (23.8%) The respondents were asked to rate how much they agreed or disagreed with the statement "Consumers consider biscuits (AGM biscuits) as a source of nutritional ingredients" as described by Torres et al. [12]. The survey was divided into three parts.

The first three questions in Part 1 focused on demographic information such age, gender, and educational attainment. The remaining questions focused on the objective, which was to determine the level of consumer perception of biscuits in general and the AGM biscuits in particular. To recognize and understand what the AGM biscuit product is, an image of the product, a small voice and a call were introduced in this section [13]. The respondents were asked the following questions:

- ❑ Which type do you find most comfortable to eat during work or as a snack in between meals?
- ❑ This is Angoumois grain moth (AGM). Did you hear about it and its nutritional value before?
- ❑ How many servings of biscuits do you consume daily in total?
- ❑ Do you know functional food?
- ❑ Instead of using commercial food, would you buy functional food?
- ❑ Which consumer groups eat high-protein foods the most?
- ❑ If AGM biscuits is a new product, will you buy them provided that you know that they are an excellent source of protein, minerals, and phenolic compounds?
- ❑ What degree of your demands is met with AGM biscuits?
- ❑ Which aspects of our product would you modify, if anything?
- ❑ What do you think about the three-month shelf life of this product?
- ❑ When compared to other biscuit varieties you've tried, the nutritional value of our product is...............

Part 2: The stimulus dialogue was used in the completion task. Three stimuli were used as follows (Figure 1): The first stimulus –"Oh! The AGM biscuits is a brand-new type of biscuits, I love biscuits so much that I normally buy them as......... "; the second stimulus — "I like them very much, but I won't buy them if........... "; the third stimulus –"If..........., I will buy them " [14].

The obtained data were analyzed as described by Torres et al. [12]. The triangulation method was employed to choose particular words or phrases that participants had submitted. Three knowledgeable researchers then assessed the responses and organized them into groups based on their individual interpretations. These groupings were joined to create the categories, which were then broken down into each category based on how frequently each group was referenced. The categories that accounted for more than 5% of the responses were the focus of the analysis. This was done in order to avoid the loss of a large amount of information [15].

2.8. Chemical evaluation

The chemical composition of the samples, i. e. moisture, ash, protein, fat, fiber, and carbohydrates, was determined according to AOAC [16].

The total phenolic content (TPC) of the AGM powder samples was determined using the Folin–Ciocalteu assay and expressed as mg Gallic acid equivalents (GAE) per gram of powder [10]. Phenolic compounds in the AGM powder samples were analyzed using HPLC following the protocol described by Elsayed et al. [17].

Fatty acids were identified using gas chromatography–mass spectrometry analysis (GC–MS). The GC–MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt.

The mineral content of the AGM powder samples was determined using inductively coupled plasma-atomic emission spectroscopy (ICP-OES: Icap6000 serious, Serial No. Icp-20080614, England). Each sample was measured twice to determine the macroelements, such as Ca and Mg as well as the microelements, such as Zn and Fe. The amino acid profile was determined by high-performance liquid chromatography (HPLC) (Agilent Technologies Inc., Santa Clara, CA, USA) at Central Laboratories Network, National Research Centre, Cairo, Egypt.

2.9. Physical evaluation

Thickness and diameter (using a vernier caliper) and weight (using a balance, KERN, Kern& Sohn, Germany) of the biscuits were determined [18]. The spread ratio of the biscuits was calculated by Equation 5.

$$\text{Spread ratio} = \text{Diameter}/\text{Thickness} \quad (5)$$

The break strength was determined according to Okpala and Egwu [19]. A biscuit of known thickness was placed between two parallel wooden bars, weights were added until the biscuit snapped. The least weight that caused the breaking of the biscuit was regarded as the break strength.

The biscuits' color parameters (L^*, a^*, b^*, and ΔE) were measured by a Minolta colorimeter (Model CR400, Konica Minolta, INC, Tokyo, Japan). The L^* value representing lightness, the a^* value representing redness (with + values) and greenness (with — values), the b^* value representing yellowness (with + values) and blueness (with — values), and ΔE (total color differences) were measured using methods described by Abedelmaksoud et al. [10].

The hardness of biscuits was measured by using a three point test on a Universal testing machine (Cometech, B type, Taiwan). The compression strength of biscuits was measured at a 20% level of compression with a cross-head speed of 100 mm/min and a flat ended probe (2.5 mm thickness). All measurements were performed at an ambient temperature of 25 °C. The hardness was expressed in Newton (N).

2.10. Microbiological evaluation

The total viable count, mold and yeast counts and *Staphylococcus aureus* were determined according to the international standards ISO 4833–1:2013 [20], ISO 21527–1:2008 [21] and ISO 6888–1:2021 [22].

Detection of thermo-tolerant coliforms at 45 °C was performed according to the protocol described by Food and Drug Administration.2002/updated 2017 [23]. The samples were also subjected to *Salmonella* analysis based on the international standard ISO 6579:2002/Cor.3:2014(E) [24].

Figure 1. **Three (X, Y, Z) stimulus dialogues in the completion task**
Рисунок 1. **Диалоги с тремя (X, Y, Z) стимулами в задаче завершения**

2.11. Rheological characteristics

The mixing behavior of dough obtained from wheat flour as a control and with the 5% AGM powder was studied using Mixolab (Chopin, Tripette et al, Renaud, Paris, France). It measured in real-time the torque (expressed in Nm) produced by the passage of dough between the two kneading arms, hence allowing the study of its rheological behavior [25]. Parameters recorded from the Mixolab curve included: water absorption (the amount of water required to reach the consistency of 1.1 Nm expressed as milliliters per 100g of flour at 14.0% mass fraction moisture content), dough development time, and stability of the dough during mixing that indicated the elapsed time, at which dough kept the maximum consistency.

Rheological characteristics of all samples were determined by a Chopin MA 82 alveograph according to (ICC Standard 55 30–3) [26]. Each alveograph chart was analyzed for four factors: P — the maximum over pressure needed to blow the dough bubble (expresses dough elasticity), L — the average abscissa at bubble rupture (expresses dough elasticity), P/L — alveograph ratio, W — the baking strength and Le%– the flexibility index. The variables represent the average of five curves from five dough samples [27].

2.12. Statistical Analysis

Data were statistically analyzed using analysis of variance (ANOVA), and the means were compared by the Turkey test, with 95% statistical significance (α) and p <0.05, using XL STAT 2014.5.03 software (Addinsoft, New York, USA).

3. Results and discussion

3.1. Selection of insect variety

Table 1 represents the quantity of powder produced for each insect variety i. e. *Ephestia kuehniella, Spodoptera littoralis, Rhynchophorus ferrugineus, Ephestia cautella*, and Angoumois grain moth (*Sitotroga cerealella*) (AGM) as follow.

Table 1. **Yield of different insect varieties**

Таблица 1. **Выход различных видов насекомых**

Variety	Insect powder/ 100 eggs (g)
Ephestia kuehniella	10
Spodoptera littoralis	16
Rhynchophorus ferrugineus	5
Ephestia cautella	12
Sitotroga cerealella (AGM)	150

The above table shows that the selected insect was Angoumois grain moth (*Sitotroga cerealella*) (AGM) according to higher yield compared to other studied varieties. Therefore, the AGM powder was selected, evaluated and used with wheat flour in biscuit making at different ratios.

3.2. Evaluation of the AGM powder

The chemical composition (moisture, ash, protein, fat, fiber, carbohydrates and total phenolic content) was determined in the samples of the AGM powder and wheat flour as shown in Table 2. The results demonstrated that the AGM powder was rich in protein, fat, minerals, total phenolic content and fiber compared to wheat flour. The use of the AGM powder mixed with wheat flour will lead to an increase in the contents of these components. The protein content of the AGM powder (54.26%) was similar to that presented by de Oliveira et al. [8] for *Periplaneta americana* L (65.60%) and *Periplaneta australasiae* (62.40%) showing a high content of the component that was desired to elevate in the tested biscuits. The use of the AGM powder mixed with wheat flour increased the protein content by 2% at 5% AGM powder substitution, respectively (Table 2). The protein quality refers to its ability to meet the nutritional requirements of humans for essential amino acids and non-essential nitrogen for protein synthesis purposes. Considering the average contents of the different orders of insects, the main components of insects are protein and fat followed by fiber and ash.

In regard to the amino acid profile of the AGM powder (Figure 2A), it can be seen that those classified as essentials are present in large quantities. Among them, histidine (13.17 mg/g), threonine (16.18 mg/g), valine (15.56 mg/g), methionine (9.29 mg/g), phenylalanine (13.22 mg/g), leucine (31.55 mg/g) and isoleucine (14.93 mg/g) are worth mentioning, since they are of great importance due to their daily intake necessity for humans. It is known that wheat flour has a shortage of the essential amino acids such as lysine, histidine, leucine and valine.

Figure 2B shows the fatty acid profile of the AGM powder. Oleic acid (ω9) with 45.16%, linoleic acid, the essential omega-6 fatty acid, with 12.01% and linolenic acid with 8.30% were present as unsaturated fat. The omega-9 (ω9) fatty acid showed a good concentration in the AGM powder accounting for approximately 45.16% of total fatty acids compared with beef marbling, which typically contains 43% SFA, 50% MUFA, and 7% PUFA [28]. This high concentration is a positive factor, since the ω9 fatty acids have a hypocholesterolemic effect [29].

Table 2. **Chemical composition of wheat flour, AGM powder and 5% AGM powder substitution**

Таблица 2. **Химический состав пшеничной муки, порошка AGM и с 5% замены порошком AGM**

Parameter	Wheat flour	AGM powder	5% AGM powder substitution
Moisture, %	9.87 ± 0.08	19.89 ± 0.13	10.36 ± 0.01
Ash, %	0.63 ± 0.09	2.77 ± 0.07	0.73 ± 0.05
Protein, %	13.70 ± 0.11	54.26 ± 0.15	15.72 ± 0.04
Fat, %	2.66 ± 0.10	6.88 ± 0.10	2.87 ± 0.02
Carbohydrates, %	72.6 ± 0.05	15.68 ± 0.10	69.75 ± 0.08
Fiber, %	0.54 ± 0.02	11.25 ± 0.12	1.07 ± 0.10
Total phenolic content (mg GAE/g of dry weight)	0.25 ± 0.02	4.36 ± 0.09	0.448 ± 0.10

Data are presented as means ± standard deviation of triplicate determinations.

According to Hautrive et al. [30], the saturated fatty acid content in ostrich meat was 27.34%, similar to that of pork ham (28.02%) and beef rump (29%), while chicken (thigh and drumstick) was the one with the least percentage of 19.73%. When comparing these data with those found in the AGM powder, we found a low percentage of saturated fat, which reached less than half the value obtained for the chicken, and thus, the AGM powder showed the lowest percentage compared to other meats. This indicates that the addition of the AGM powder to biscuits can increase the percentage of unsaturated fats (beneficial) and reduce harmful ones (saturated fats). Saturated fat can contribute in the medium term and long term to the onset of obesity and cardiovascular disease; therefore, the low percentage in the AGM powder is a positive point. The saturated fatty acids increased the level of cholesterol in the blood by reducing the LDL-cholesterol receptor activity and the clearance of LDL in the bloodstream [31].

Figure 2C shows that iron, magnesium, zinc and calcium were present in different amounts in the AGM powder being 5373.58mg/L, 5718.97mg/L, 237.60 mg/L and 510.11mg/L for iron, Mg, Ca and Zn, respectively. This indicates that the AGM powder was rich in Ca, Mg, Fe and Zn. Akullo et al. [32] reported that the consumption of 25 g of crackers enriched with 5% winged termites contributed 19.10% and 34.58% of the recommended dietary intake (RDI) of iron and zinc in pre-school children. However, the consumption of 25 g of biscuits enriched with the 5% AGM powder contributes 100%, 100%, 89% and 0.4% of RDI of iron, zinc, magnesium and calcium in pre-school children.

The results in Figure 2D present the concentration and identification of phenolic compounds of the AGM powder. Vanillic acid (564.7 mg/kg), p-coumaric acid (39.79 mg/kg) and quinol (12.06 mg/kg) were the major phenolic compounds in the AGM powder extract. From a human physiological standpoint, phenolic compounds are vital in defense responses, such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities. Vanillic acid is a very important compound because it exerts diverse bioactivity against cancer, diabetes, obesity, neurodegenerative, cardiovascular and hepatic diseases by inhibition of the associated molecular pathways [33].

Microbiological evaluation of the AGM powder showed that it had a satisfactory safety condition. The results of the microbial analysis did not exceed the established values specified in the regulation [34,35]. Additionally, with increasing the heat treatment temperature of the AGM powder up to 120 °C, the total plate count, mold and yeast count, *Staphylococcus aureus* and thermo-tolerant coliforms decreased and became to be within the acceptable limit. This indicates that the heat treatment of the AGM powder at 120 °C for 2 hours inhibited microorganisms and the AGM powder became safe according to the regulation [34,35] and Table S1.

3.2. Effect of the AGM powder percent on the overall acceptability, texture and color values (L, a, and b) of biscuits

The AGM powder percentage was chosen (Table 3) for RSM to evaluate the impact of an AGM powder percentage on overall acceptability, texture, and color values (*L, a,* and *b*) of biscuits. It was observed during a pretest experiment that AGM powder percentages greater than 15% resulted in dark color changes in the biscuits.

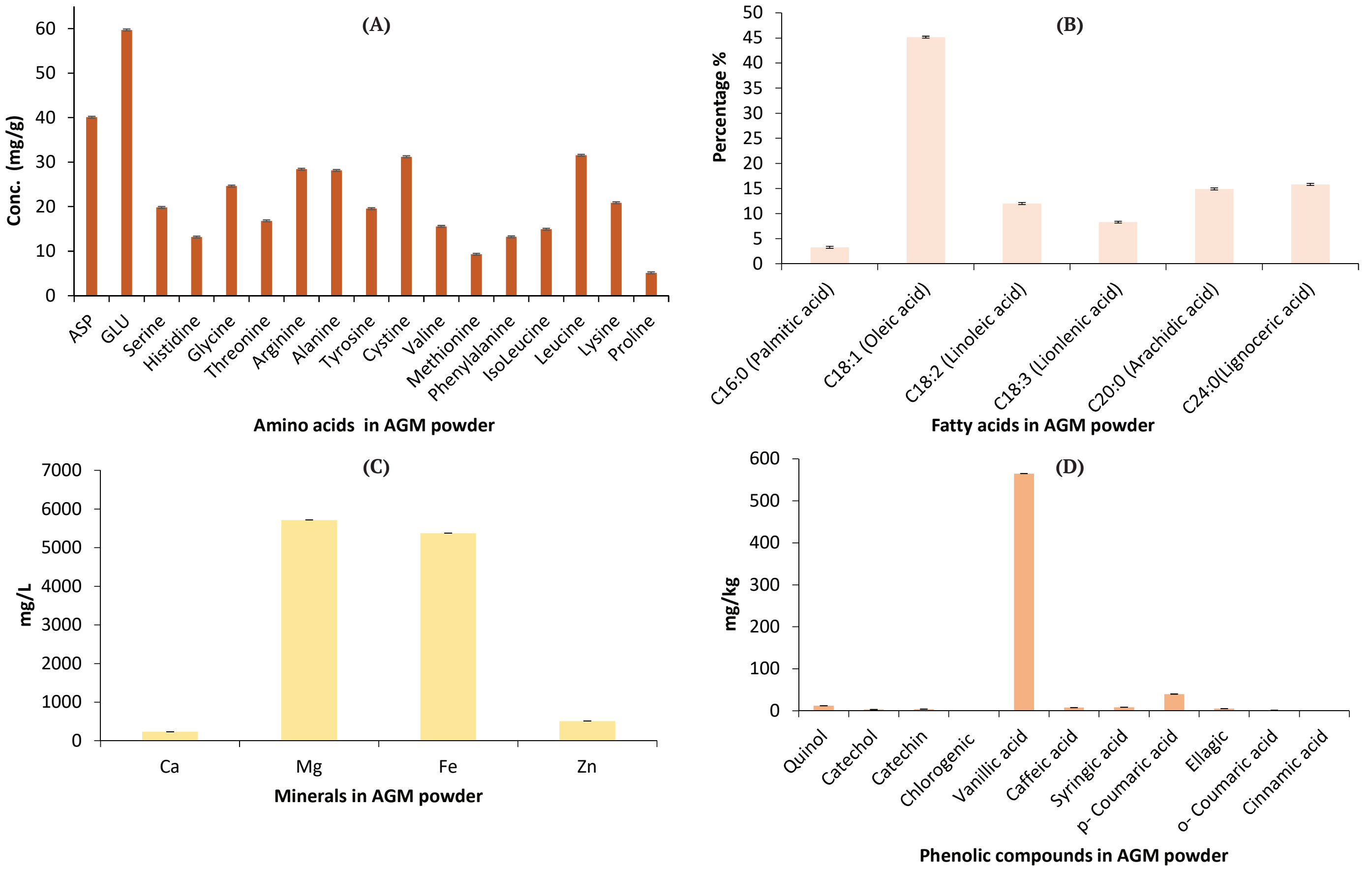


Figure 2. **Evaluation of the AGM powder:**
(A) amino acid profile; (B) fatty acid profile; (C) mineral content; (D) total phenolic compounds
Рисунок 2. **Оценка порошка AGM: (A) аминокислотный профиль; (B) жирнокислотный профиль; (C) содержание минеральных веществ; (D) общие фенольные соединения**

Table 3. **An optimal (custom) design with experimental values of responses (overall acceptability, texture and color values (*L*, *a*, and *b*) of biscuits)**
Таблица 3. **Оптимальный (индивидуальный) дизайн с экспериментальными уровнями откликов (общая приемлемость, текстура и уровни цвета (*L*, *a*, и *b*) печенья)**

Run order	AGM powder% (X_1)	Overall acceptability	*L*	*A*	*b*	Texture (N)
1	13.8	4.51	43.92	11.91	27.26	5.41
2	8.35	5.14	47.18	13.08	31.86	6.63
3	5	6	51.51	13.32	33.38	7.29
4	10	5.33	45.6	12.84	29.58	6.15
5	6.65	5.84	49.33	13.13	32.11	7.01
6	0	7.5	53.7	14.39	38.42	7.31
7	5	6.28	51.8	13.28	33.28	7.08
8	10	5.28	45.53	12.71	29.41	6.09
9	15	4.3	43.65	11.75	26.12	5.56
10	10	5.16	45.42	12.62	29.32	5.92
11	15	4.11	43.81	11.63	26.01	5.42
12	12.5	4.82	44.51	12.11	28.12	5.63
13	11.3	5.03	45.06	12.51	28.31	5.88

Table 3 represents the measured overall acceptability, texture and color values (*L, a,* and *b*) of biscuits. The second order polynomial equation (Equation 1) was used for optimization of the AGM powder percentage and multiple regression analysis determined the regression coefficients for independent variables. The effects of the AGM powder percent on the overall acceptability, texture, and color values (*L, a,* and *b)* of biscuits are shown in Tables S.2, S.3, S.4, S.5 and S.6 with a 95% confidence interval using ANOVA analysis.

The model displays an excellent fit with the measured overall acceptability, texture and color values (*L, a,* and *b*) of biscuits with a high level of significance and less variance around the mean. The fitted model could explain 97.7% of the response variability according to R^2= 0.9779 of the overall acceptability response. For *L* response, R^2 = 0.9987, which means that 99.8% of the response variability could be explained by the fitted model. For (*a*) response, R^2 = 0.9931, i. e., 99.3% of the response variability could be explained by the fitted model. For (*b*) response, R^2 = 0.9875, i. e., 98.7% of the response variability could be explained by the fitted model. Finally, for texture response, R^2 = 0.9751, i. e., 97.5% of the response variability could be explained by the fitted model. The adjusted R^2 values for the models of overall acceptability, color values (*L, a,* and *b*), and texture responses were 0.9735, 0.9980, 0.9896, 0.9850, and 0.9627, respectively, and did not significantly differ from R^2 values. The lack-of-fit was not significant (p>0.05). Based on these results, the model was satisfactory for predicting the overall acceptability, texture and color values (*L, a,* and *b*) of biscuits in the experimental ranges.

The negative linear effect of the AGM powder percent (x_1) was significant for the response variables (the overall acceptability, texture and color values (*L, a,* and *b*) of biscuits). The quadratic effect of the AGM powder percent (x_1^2) on color values (*L, a* and *b)* of biscuits was also found to be significant (p<0.05). However, the quadratic effect of the AGM powder percent (x_1^2) on overall acceptability and texture of biscuits was found to be insignificant (p>0.05). The non-significant variables were removed and the fitted second order polynomial equations (Equations 6, 7, 8, 9 and 10) are shown as follows:

$$\text{Overall acceptability} = +5.70-1.60x_1 + 0.1829x_1^2 \quad (6)$$

$$L = +48.15-9.60x_1 + 4.87x_1^2 + 4.60x_1^3-4.32x_1^4 \quad (7)$$

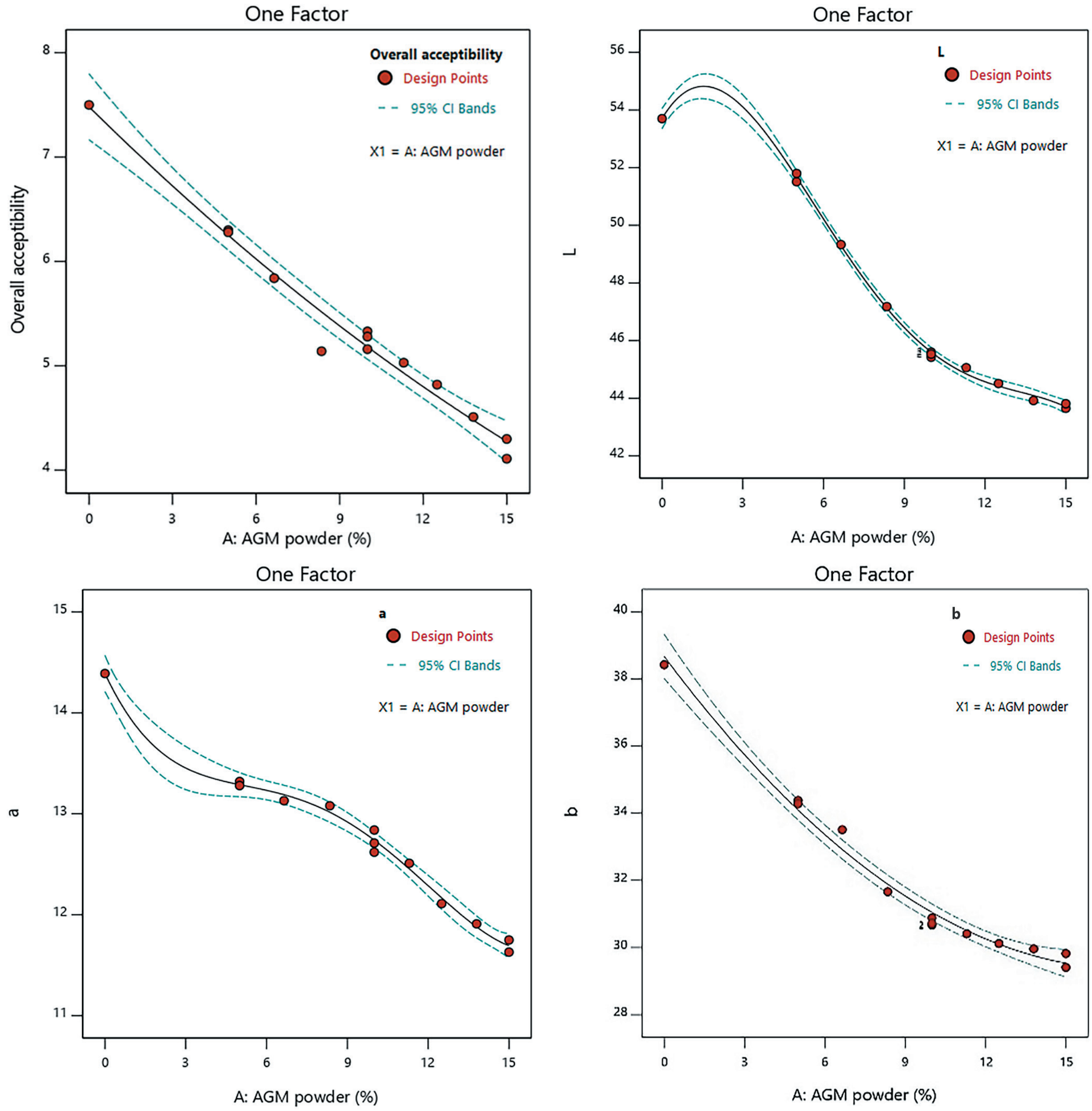
$$a = +13.12-0.7594x_1-1.02x_1^2-0.5895x_1^3 + 0.9460x_1^4 \quad (8)$$

$$b = +32.838-4.57x_1 + 1.72x_1^2 \quad (9)$$

$$\text{Texture} = +6.80-1.62x_1 + 1.55x_1^2 + 0.6939x_1^3 + 1.13x^4 \quad (10)$$

Where x_1: AGM powder% is the coded value.

To determine the optimum percentage of the AGM powder, second order polynomial models were used for the responses (overall acceptability, color values (*L, a,* and *b*) and texture of biscuits). Perturbation plots of the response surface methodology (RSM)) and the model equation demon-



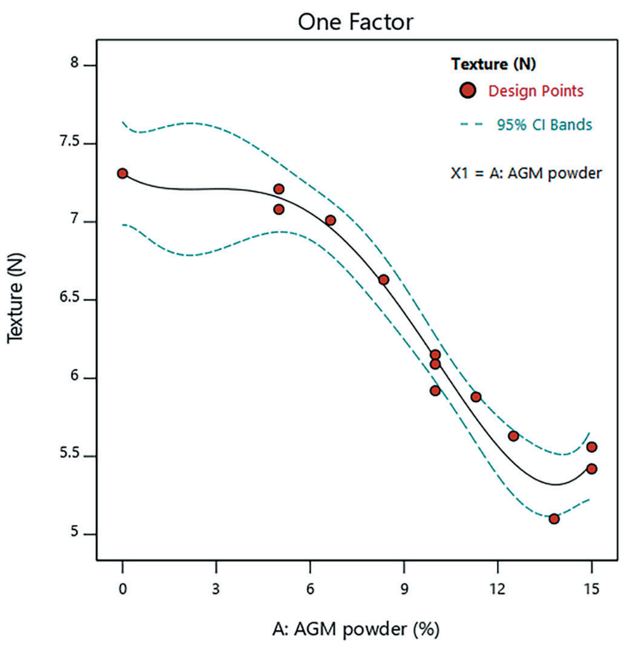


Figure 3. **Perturbation plot showing the relative significance of the AGM powder percent for the overall acceptability, color values (*L*, *a*, and *b*) and texture of biscuits**

Рисунок 3. **График возмущений, показывающий относительную значимость процента порошка AGM для общей приемлемости, уровней цвета (*L*, *a*, и *b*) и текстуры печенья** strate the significant influence of factor A (AGM powder%) on the overall acceptability, color values (*L, a,* and *b*), and texture of the biscuits. The effect of factor A (AGM powder%) is shown in Figure 3. The perturbation plot (Figure 3) shows the following hierarchy of the relative effect of the AGM powder percentage on the target responses: with increasing the percentage of the AGM powder, overall acceptability, color values (*L, a,* and *b*) and texture of biscuits decreased.

By using the desirability function, the ideal AGM powder percentage in biscuits was discovered at the highest levels of overall acceptability and target texture and color values (*L, a,* and *b*). The 5% AGM powder was selected as an optimum addition for maximization of overall acceptability and target color values (*L, a,* and *b*) and texture of biscuits, which gave the best (predicted) result for maximization of overall acceptability (6.16), target color values (L= 51.76, a=13.33 and b=34.43) and texture (7.22N). The observed values of responses (overall acceptability, color values (*L, a,* and *b*) and texture of biscuits) were 6.28, 51.66, 13.28, 34.37 and 7.14, respectively.

3.4. Consumer perception of AGM biscuits

The most promising food category in the world right now appears to be functional foods. Markets in Egypt today sell a variety of functional food items, such as candies, milk products, juice, bars, biscuits, and cookies. Based on customer knowledge and expectations, this study sought to create a novel and healthful AGM biscuits. The questionnaire was split into two sections, and the results are shown in Figure S1 and Figure 4.

Figure S1 displays the questions and respondents' responses to them from Part 1 of the questionnaire. As can be seen from the figure, the most comfortable types of snacks for the respondents were bread items (43.3%), fruits (11.1%), vegetables (10.7%), crackers (8.8%), nuts (8.8%), and potato chips (7.3%). As for the knowledge of Angoumois grain moth (AGM) and its nutritional value, 35.6% of the respondents knew about them, 35.2% did not know, while 29.1% of respondents answered "maybe". About 7% of the respondents did not eat biscuits, 11.9% ate one serving of biscuits a day, 18.8% ate two servings a day, 22.6% ate three servings a day, and 39.8% ate ten servings a day. Answering the ques-

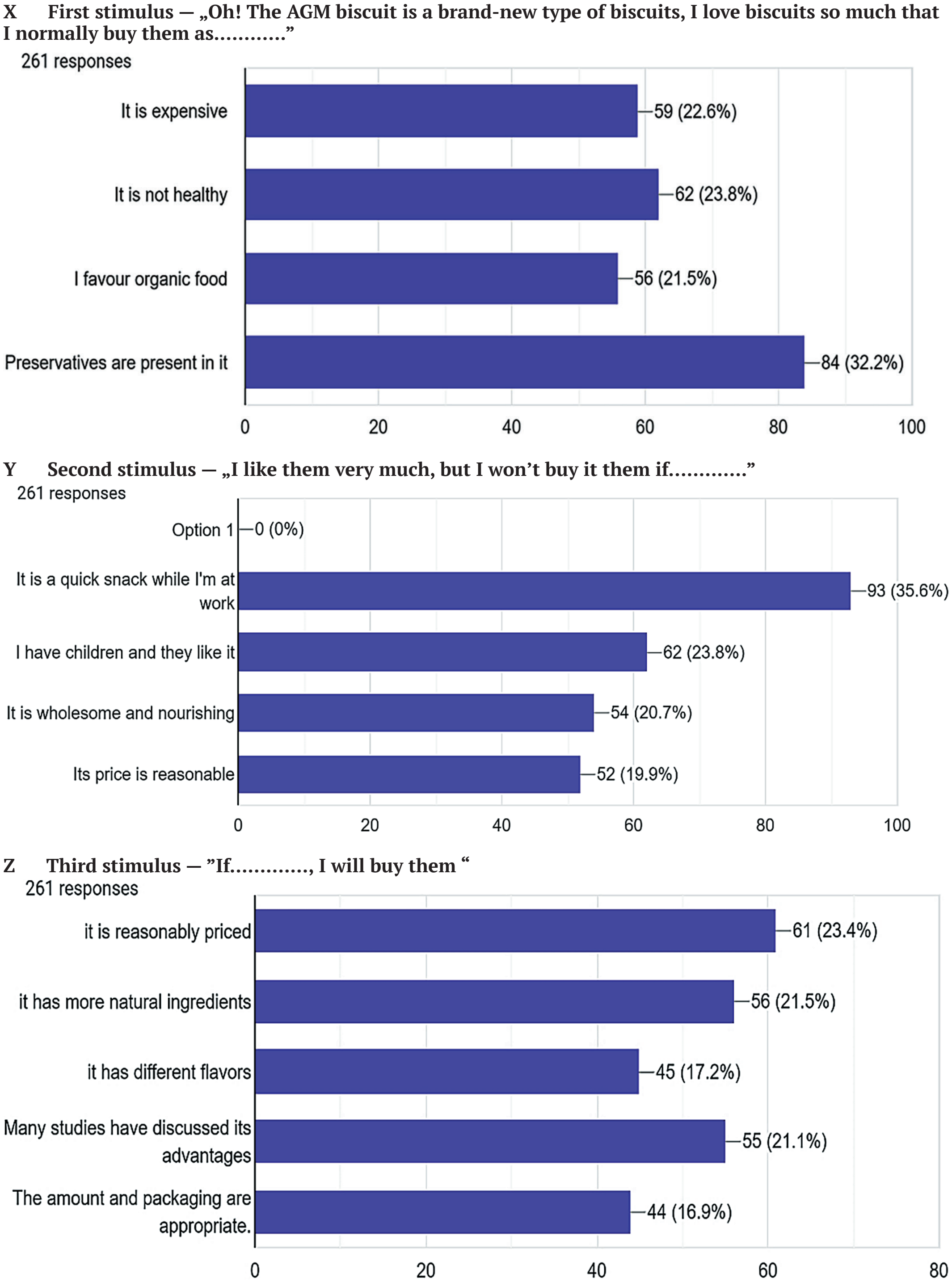


Figure 4. **Results of the online questionnaire about three (X, Y, Z) stimulus dialogues used in the completion task**

Рисунок 4. **Результаты онлайн анкеты о диалогах с тремя (X, Y, Z) стимулами, использованными в выполнении задачи** tion "Do you know functional food?", 47.1% of the respondents selected "yes" and 52.9% selected "no". The next question was "Instead of using commercial food, would you buy functional food?". The responses to this question were "yes of course" (36.2%), "absolutely, but only if the cost is reasonable" (35.4%) and "no, I am not interested" (28.5%). The responses to the question "Which consumer groups eat high-protein foods the most?" were "all consumer groups" (36%), "people having mental disability" (19.9%), "children" (16.5%), "old people" (15.3%), and "young people" (12.3%). As regards the question "If AGM biscuits is a new product, will you buy them provided that you know that they are an excellent source of protein, minerals, and phenolic compounds?", 44% of the respondents answered "yes", 19.6% "maybe", and 36.4% "never". The high percent (37.2%) of the respondents answered that AGM biscuits met the consumer demands very well, followed by those who answered "fine" (32.2%), "well" (24.9%) and "badly" (5.7%). About 40% of the respondents would not modify any aspect of our product, 21.1% would use various colors to attract children, 18.4% would change the flavor, 14.6% would add other components like fruits or nuts, and 5.3% would change price. Among the respondents, 47.1% believed that the shelf life of this product was very good, 24.5% thought that it was fine, 22.6% thought that it was good, and 5.8% answered that the shelf life was not good. Approximately 50% of the respondents concluded that the nutritional value of our product was better than that of other biscuit varieties they tried, 40.6% answered that it was the same, while 9.2% thought that it was worse. Figure 4 shows the percent of responses for three (X, Y, Z) stimulus dialogues used in the completion task.

The information concerning consumer opinions and knowledge of AGM biscuits that was acquired through the online survey was crucial. Regarding the purchase intention (Figure S1), the samples of biscuits with the Angoumois grain moth (AGM) powder received a positive attitude toward purchase, with 44% of the judges stating that they would "of course, buy" and 19.6% "possibly buy", totaling a 63.6% positive response. Since biscuits enriched with the AGM powder is an unknown product for consumers, being new with respect to the culture of the judges, purchase intention of 63.6% is considered a good result.

3.5. Evaluation of the wheat flour-AGM powder mixture

The chemical composition (moisture, ash, protein, fat, fiber, carbohydrates and total phenolic content) of the AGM powder, wheat flour and 5% AGM powder substitution was evaluated as shown in Table 1. The results demonstrated that the addition of the AGM powder at 5% to wheat flour in the processing of biscuits caused an increase in protein, fat, ash, fiber and total phenolic content by 2%, 0.2%, 0.1%, 0.53% and 0.2%, respectively. However, total carbohydrates reduced from 72.6% in wheat flour to 69.75% (Table 1).

3.6. Evaluation of biscuit quality (physical, chemical and microbial properties of biscuits)

The results of the chemical composition of different processed biscuits are shown in Table 4. Biscuits made from the 5% AGM powder and wheat flour were chemically analyzed for moisture, ash, protein, fat, fiber and carbohydrates (Table 4). The results demonstrated that the addition of the AGM powder to wheat flour affected the chemical composition of the baked biscuits and caused an increase in the protein, fat, ash and fiber content of the biscuits with the 5% AGM powder by 1.39%, 3%, 0.4% and 0.58% compared to the control. This indicates that the addition of the AGM powder increased the nutritional value of the baked biscuits compared to the control.

The physical properties of different processed biscuits are shown in Table 4. Physical characteristics of the biscuits made from wheat flour changed with the incorporation of the AGM powder. In general, an increase in weight, diameter and spread ratio values with the inclusion of AGM flour and a decrease in both height and breaking strength were recorded. This was in agreement with the study by Baljeet et al. [36] where buckwheat flour was added to wheat flour for biscuit making.

The spread ratio of biscuits, which is the ratio between the diameter and the thickness, increased in a range from 8.56 to 9.05 compared with the control (having the lowest value). The spread ratio increased with 5% AGM powder substitution. The spread ratio is the most important parameter to assess the quality of biscuits as the high values of spread ratio were considered the best according to Awobusuyi et al. [37] who reported that the incorporation of termite flour in wheat flour reduced gluten content and hence, reduced the elasticity of the pasta as well as the thickness of biscuits which might be responsible for an increase in the spread ratio with an increase in the level of substitution of termite flour.

The AGM powder had a high and positive effect on the spread ratio since its inclusion enhanced the spread ability of the biscuit samples. The results also showed that the breaking strength of biscuits containing the AGM powder decreased from 283 g to 277 g for the control and 5% AGM, respectively. There is also a relationship between the spread ability, height (thickness) and the breaking strength. The thinner a biscuit, the lesser its ability to withstand stress/load. The inclusion of the AGM powder in the biscuits produced affected the ability of the biscuits to withstand stress. The use of composite flour for biscuit making in order to reduce the breaking strength had been reported by Amjid et al. [38].

Table 4. Physical, chemical and microbial properties of the biscuit samples

Таблица 4. **Физические, химические и микробиологические свойства образцов печенья**

Sample	Control	5% AGM
Moisture, %	3.13±0.06	1.6±0.10
Ash, %	1.47±0.05	1.7±0.14
Protein, %	4.99±0.10	5.69±0.13
Fat, %	30±0.11	31.27±0.12
Fiber, %	0.84±0.06	1.24±0.07
Carbohydrates, %	59.6±0.11	59.37±0.10
Weight (g)	12.58±0.02	13.40±0.02
Diameter (cm)	5.39±0.01	5.42±0.02
Height (cm)	0.63±0.01	0.60±0.02
Spread Ratio	8.56±0.02	9.05±0.03
Breaking Strength (g)	283±0.05	277±0.08
L	66.69±0.02	51.50±1.08
A	10.33±0.10	13.32±0.11
B	38.84±0.11	33.38±0.18
C	40.19±0.08	35.94±0.06
H	75.10±0.11	68.34±0.08
ΔE	0	16.42
Hardness (N)	7.54±0.06	7.29±0.08
Total viable count (log CFU/g)	2.4	2.5
M and Y count (log CFU/g)	Absence	Absence
Thermo-tolerant coliforms (log CFU/g)	Absence	Absence
Salmonella spp.	Absence	Absence

Estimated value. CFU: colony-forming units, *M* is Mold and *Y* is Yeast

The color parameters and hardness of different processed biscuits are shown in Table 4. The whole wheat biscuits had no significant difference from the biscuits with the 5% AGM powder. The results agree with the findings of de Oliveira et al. [8] who reported that with increasing concentrations of cinereous cockroach (*Nauphoeta cinerea*) flour meal, the hue (h_{ab}) angle in the crumb and crust of bread decreased, while a distance to the desired value (close to 90°) increased. With the addition of the AGM powder to the whole wheat flour biscuits, the change in color of the biscuits (ΔE) increased. The biscuits with the addition of the AGM powder became darker than the control because of the color of the AGM powder. The values of hardness found for standard biscuits and for biscuits enriched with the 5% AGM powder did not present a significant difference.

Regarding the microbiological evaluation of biscuits, Table 4 demonstrates that the samples i. e., the wheat flour biscuits and biscuits made from wheat flour with the AGM powder, showed low microbial load and the absence of pathogenic microorganisms. The results were within the range of the established values as specified in the regulation [34,35].

Dough rheological properties

The rheological properties of dough made from wheat flour and wheat flour with the 5% AGM powder were evaluated by using the mixolab and alveograph. Figure 5 illustrates the plots recorded during mixing in the mixolab. It can be noticed that addition of the AGM powder to wheat flour affected the rheological characteristics, i. e. water absorption, dough development time and stability of dough. The presence of the 5% AGM powder slightly increased water absorption compared to the control sample (wheat flour). Generally, the addition of flours with a high amount of hydrophilic proteins increased the hydration and water absorption of dough [39]. Dough development time increased with adding the 5% AGM powder compared to the control sample. However, stability of dough decreased. A decrease in stability might be attributed to a decrease in the percentage of wheat flour proteins (gliadin and glutenin) responsible for the formation of the gluten network [40].

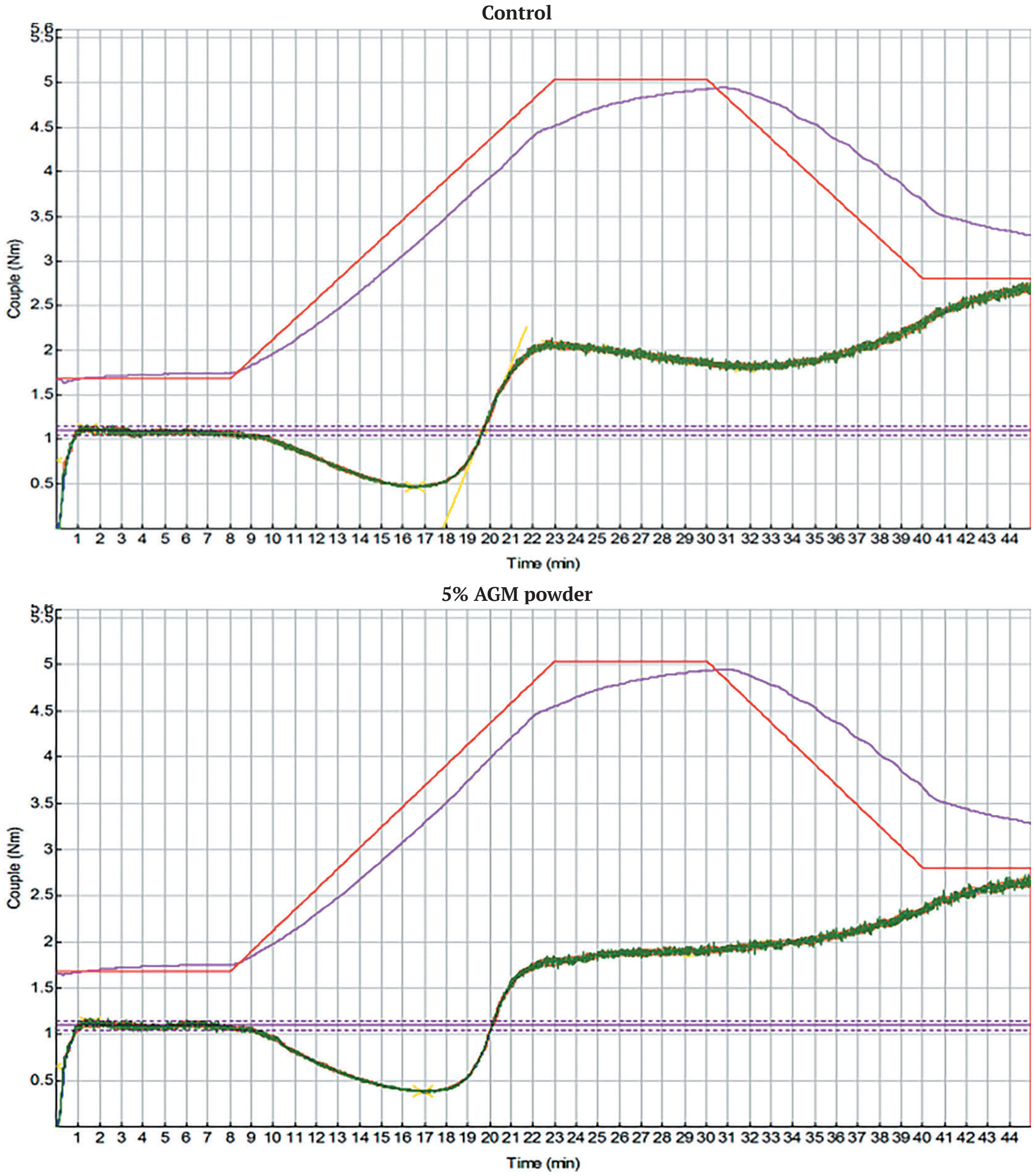


Figure 5. **Mixolab curves of the control and the 5% AGM powder**
Рисунок 5. **Кривые Mixolab контрольных образцов и с 5% порошка AGM**

The results obtained from alveographic measurements of dough are presented in Table 5. Dough prepared from the AGM powder at 5% did not demonstrate optimal viscoelastic behavior as shown by the higher ratio P/L. The presence of the 5% AGM powder increased the P value (dough elasticity) and P/L (Alveograph ratio) compared to the control sample, while L (dough elasticity), W (10^{-4} J) (baking strength) and flexibility index Le% decreased. This means that the addition of the AGM powder decreased the strength of the dough. This could be attributed to the reduction of wheat flour proteins (gliadin and glutenin).

Table 5. **Alveographic measurement of dough**
Таблица 5. **Альвеографическое испытание теста**

Parameter	Control	5%
P (mm)	95	83
L (mm)	117	77
P/L	0.81	1.08
W (10^{-4}J)	350	203
Le %	56.6	46.9

4. Conclusion

Chemical and physical characteristics of the wheat-AGM biscuits observed in this study prove that Angoumois grain moth (*Sitotroga cerealella*) have the potential to contribute to the nutritional and physical quality of the biscuits. It can be used as a partial substitute for wheat in biscuit production without affecting the quality of biscuits made from it. The biscuits produced from wheat flour-AGM powder in this study generally had higher content of protein, fat, ash, fiber and total phenols and improved physical properties. Based on RSM, the optimum selected percentage of the AGM powder in biscuits was 5%. Therefore, this study confirmed the possibility of Angoumois grain moth inclusion in carbohydrate-rich foods to obtain products of acceptable quality. This study has revealed that Angoumois grain moth is an excellent source of nutrients and can be used as a supplement in biscuit production since it enhanced crude fiber and protein that are necessary for combating malnutrition rampant in our world today.

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Conflict of interest	**Конфликт интересов**
The authors declare no conflict of interest.	Авторы заявляют об отсутствии конфликта интересов.

SUPPLEMENTARY DATA

Table S1. **Microbiological evaluation of the AGM powder (*Sitotroga cerealella*)**

Таблица S1. **Микробиологическая оценка порошка AGM (*Sitotroga cerealella*)**

Samples	Total viable count (log CFU/g)	Mold and yeast count (log CFU/g)	*Staphylococcus aureus* (log CFU/g)	Thermo-tolerant coliforms at 45 °C (log CFU/g)	*Salmonella*
Control	10.9	4.3	3.8	5.1	Absence
Heat treated AGM powder at 50 °C					
1 hr	8.5	4.3	3.8	5.8	Absence
3 hrs	8.4	4.3	3.7	5.4	Absence
6 hrs	8.4	4.3	3.7	5.8	Absence
Heat treated AGM powder at 70 °C					
2 hrs	7.4	4.3	4.6	5.4	Absence
4 hrs	7.4	4.3	4.4	5.2	Absence
Heat treated AGM powder at 100 °C					
2 hrs	7.1	4.1	4.1	5.1	Absence
4 hrs	7	4	3.9	4.9	Absence
Heat treated AGM powder at 120 °C					
2 hrs	3.4	2.9	3.2	—	Absence
4 hrs	3.3	—	3.1	—	Absence
Heat treated AGM powder at 140 °C					
2 hrs	3.3	2.8	2	—	Absence
4 hrs	2.7	—	2	—	Absence
Heat treated AGM powder at 160 °C					
2 hrs	2.4	2.6	—	—	Absence
4 hrs	—	—	—	—	Absence

Estimated value. CFU is colony-forming units.

Table S2. **Analysis of variance (ANOVA) and significant coefficient for overall acceptability of biscuits**

Таблица S2. **Дисперсионный анализ (ANOVA) и значимый коэффициент для общей приемлемости печенья**

Source	Coefficient Estimate	Sum of Squares	Df	p-value
Model	5.70	10.21	2	< 0.0001
X_1	−1.60	9.80	1	< 0.0001
X_1^2	0.1829	0.0591	1	0.1405
Residual		0.2305	10	
Lack of Fit		0.1969	6	0.1036
DF = 12				
R^2	0.9779			
Adj-R^2	0.9735			
Pred-R^2	0.9657			
C.V. %	2.84			
PRESS	0.3583			

p-value is significant at $p < 0.05$, x_1 is coded AGM powder%

Table S3. **Analysis of variance (ANOVA) and significant coefficient for color value (L) of biscuits**

Таблица S3. **Дисперсионный анализ (ANOVA) и значимый коэффициент для уровней цвета (L) печенья**

Source	Coefficient Estimate	Sum of Squares	Df	p-value
Model	48.15	141.01	4	< 0.0001
X_1	−9.60	43.91	1	< 0.0001
X_1^2	4.87	1.18	1	< 0.0001
X_1^3	4.60	8.34	1	< 0.0001
X_1^4	−4.32	1.06	1	0.0001
Residual		0.1854	8	
Lack of Fit		0.1141	4	0.3300
DF= 12				
R^2	0.9987			
Adj-R^2	0.9980			
Pred-R^2	0.9962			
C.V. %	0.3239			
PRESS	0.5358			

p-value is significant at $p < 0.05$, x_1 is coded AGM powder%

Table S4. **Analysis of variance (ANOVA) and significant coefficient for color value (a) of biscuits**

Таблица S4. **Дисперсионный анализ (ANOVA) и значимый коэффициент для уровней цвета (a) печенья**

Source	Coefficient Estimate	Sum of Squares	Df	p-value
Model	13.12	6.94	4	< 0.0001
X_1	−0.7594	0.2746	1	0.0001
X_1^2	−1.02	0.0520	1	0.0191
X_1^3	−0.5895	0.1368	1	0.0014
X_1^4	0.9460	0.0508	1	0.0201
Residual		0.0485	8	
Lack of Fit		0.0160	4	0.7441
DF= 12				
R^2	0.9931			
Adj-R^2	0.9896			
Pred-R^2	0.8222			
C.V.%	0.6125			
PRESS	1.24			

p-value is significant at $p < 0.05$, x_1 is coded AGM powder%

Table S5. Analysis of variance (ANOVA) and significant coefficient for color value (b) of biscuits

Таблица S5. Дисперсионный анализ (ANOVA) и значимый коэффициент для уровней цвета (b) печенья

Source	Coefficient Estimate	Sum of Squares	Df	p-value
Model	32.38	79.58	2	< 0.0001
X_1	−4.57	79.55	1	< 0.0001
X_1^2	1.72	5.23	1	< 0.0001
Residual		1.01	10	
Lack of Fit		0.8922	6	0.0650
DF= 12				
R^2	0.9875			
Adj-R^2	0.9850			
Pred-R^2	0.9370			
C.V.%	0.9951			
PRESS	5.08			

p-value is significant at $p < 0.05$, x_1 is coded AGM powder%

Table S6. Analysis of variance (ANOVA) and significant coefficient for texture of biscuits

Таблица S6. Дисперсионный анализ (ANOVA) и значимый коэффициент для текстуры печенья

Source	Coefficient Estimate	Sum of Squares	Df	p-value
Model	6.80	6.44	4	< 0.0001
X_1	−1.62	1.25	1	< 0.0001
X_1^2	1.55	0.1199	1	0.0421
X_1^3	0.6939	0.1896	1	0.0161
X_1^4	1.13	0.0728	1	0.0965
Residual		0.1643	8	
Lack of Fit		0.1176	4	0.1966
DF= 12				
R^2	0.9751			
Adj-R^2	0.9627			
Pred-R^2	0.9303			
C.V.%	2.30			
PRESS	0.4601			

p-value is significant at $p < 0.05$, x_1 is coded AGM powder%

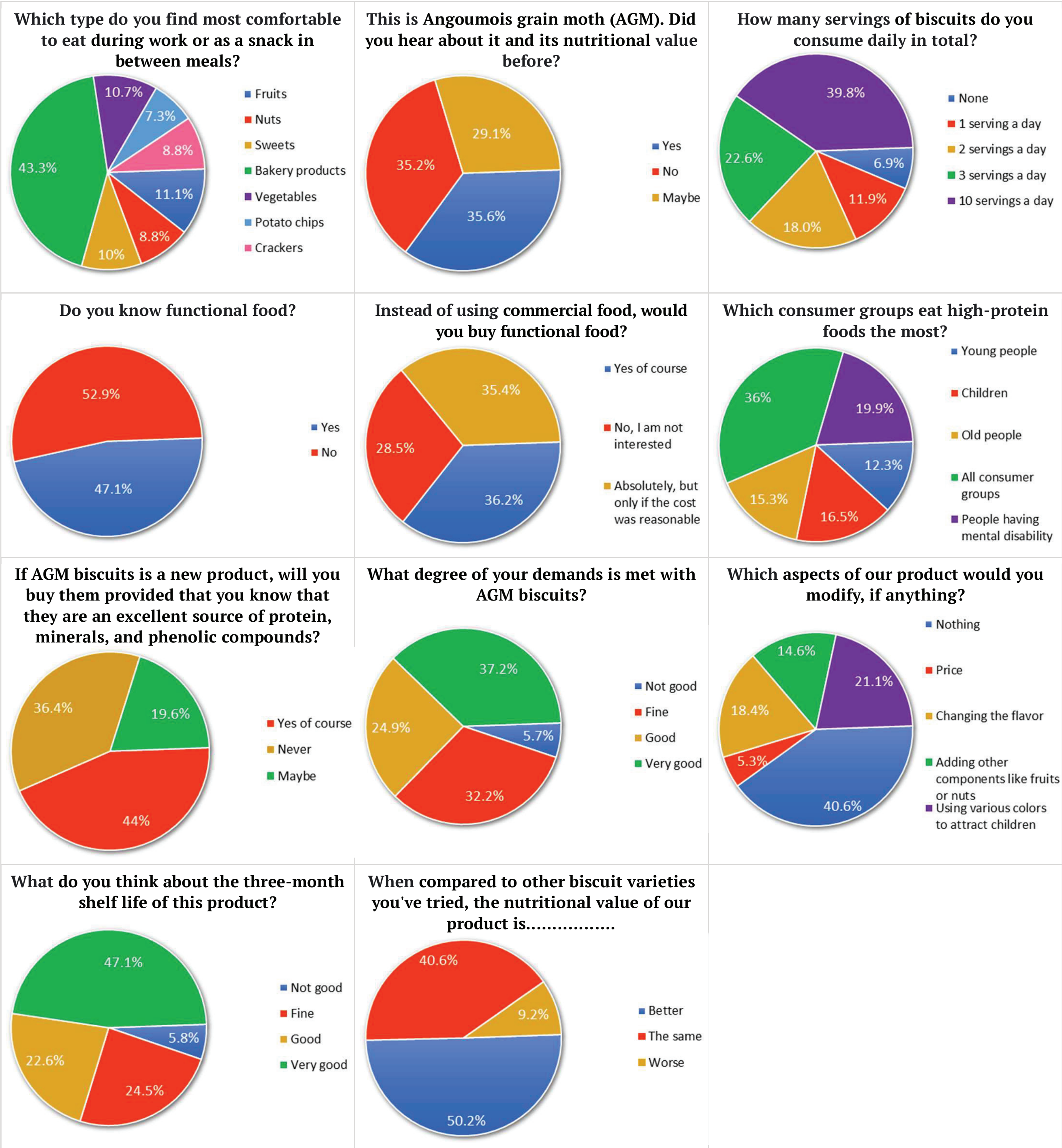

Figure S1. **Results of Part 1 questions that concentrated on the objective, which was to see a degree of consumer perception about biscuits in general and AGM biscuit in particular**

Рисунок S1. **Результаты вопросов части 1, сконцентрированные на цели, которая заключалась в установлении степени потребительского восприятия печенья в целом и печенья AGM в частности**

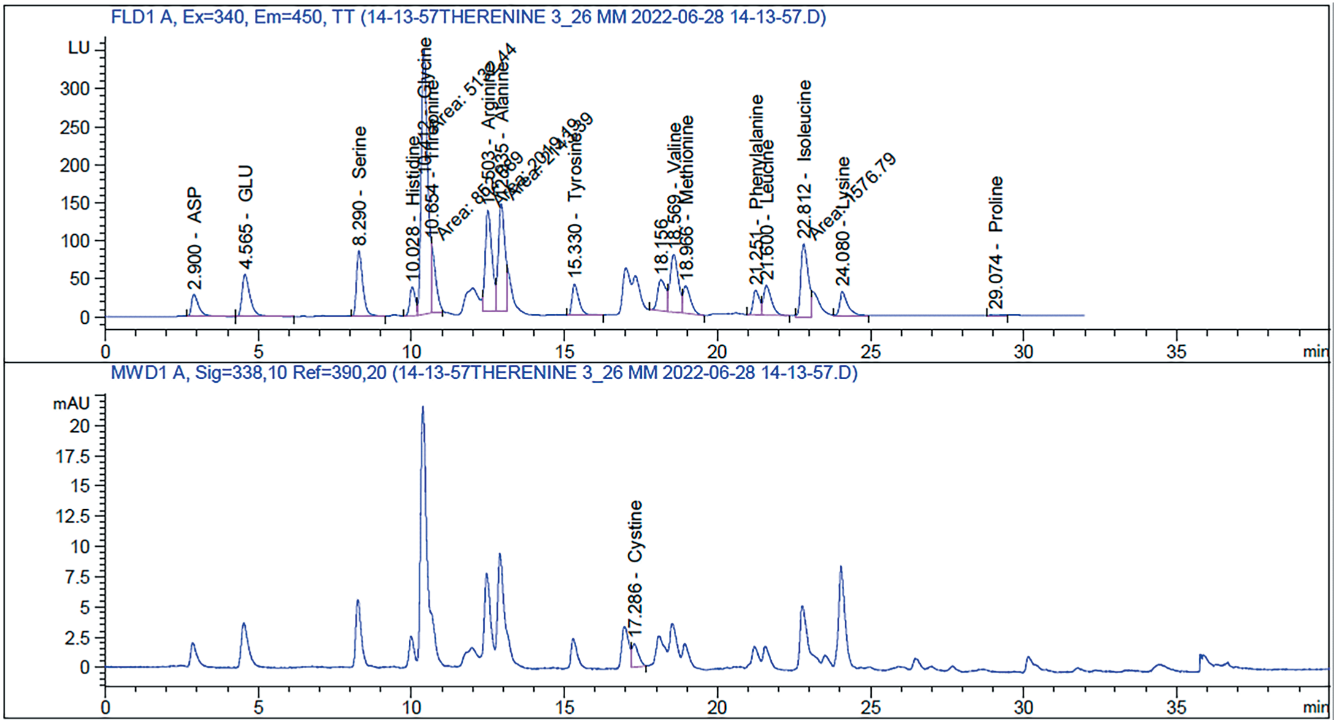

Figure S2. **Amino acid profile of the AGM powder**
Рисунок S2. **Аминокислотный профиль порошка AGM**

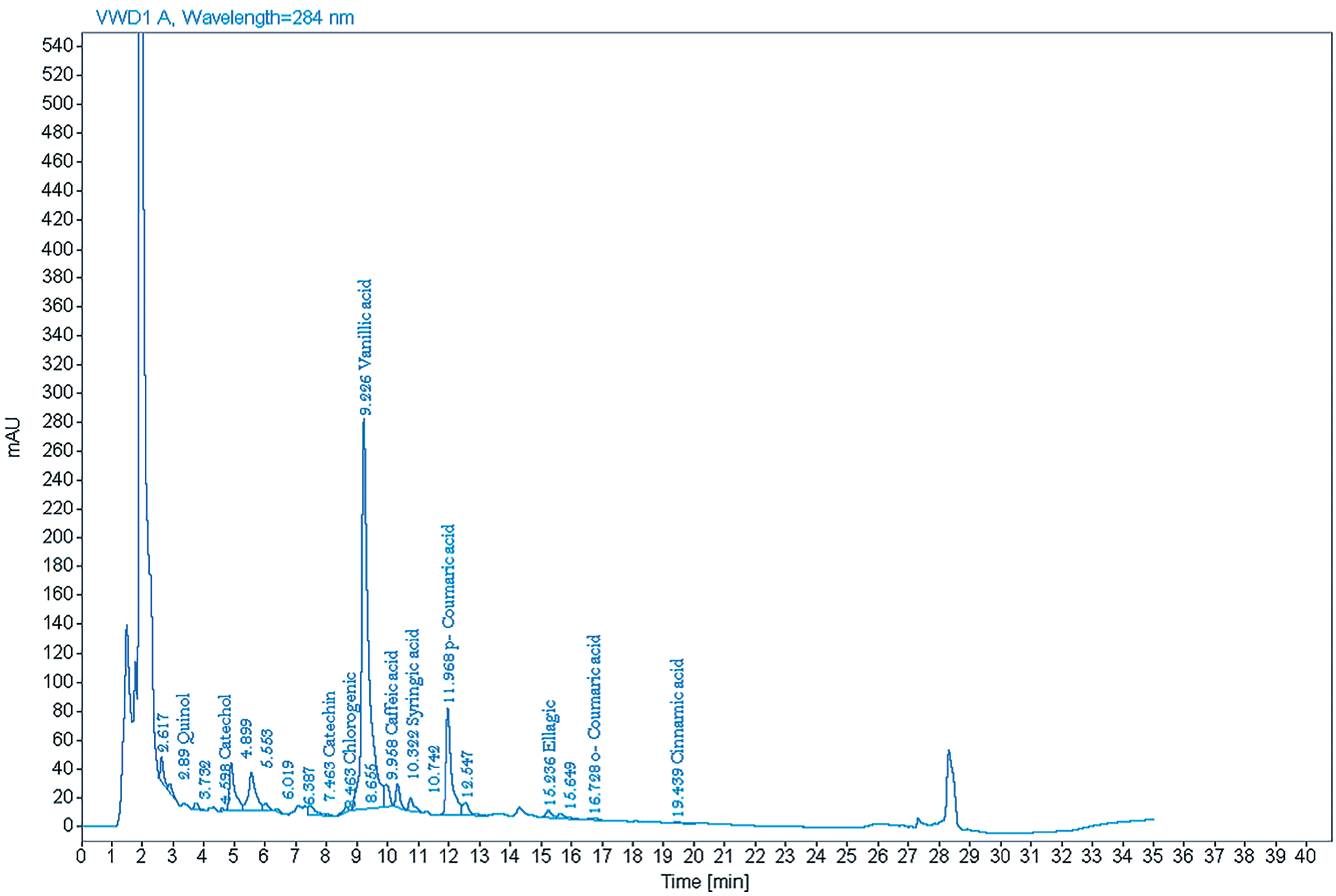

Figure S3. **Total phenolic compounds in the AGM powder**
Рисунок S3. **Общие фенольные соединения порошка AGM**