2024 Т. 54 № 1 / Техника и технология пищевых производств / Food Processing: Techniques and Technology ISSN 2074-9414 (Print) ISSN 2313-1748 (Online)

https://doi.org/10.21603/2074-9414-2024-1-2490
https://elibrary.ru/LDFMSG

Original article
Available online at https://fptt.ru/en

Gliadin Proteins in Muffins with Quinoa Flour

Vesna S. Gojković Cvjetković[1,*], Dragana M. Škuletić[1], Željka R. Marjanović-Balaban[2], Dragan P. Vujadinović[1], Danijela Z. Rajić[1], Vladimir M. Tomović[3]

[1] University of East Sarajevo, Lukavica, Bosnia and Herzegovina

[2] University of Banja Luka, Banja Luka, Bosnia and Herzegovina

[3] University of Novi Sad, Novi Sad, Serbia

Received: 30.06.2023
Revised: 11.09.2023
Accepted: 03.10.2023

**Vesna S. Gojković Cvjetković: vesna.gojkovic@tfzv.ues.rs.ba, https://orcid.org/0000-0003-1118-4565*
Dragan P. Vujadinović: https://orcid.org/0000-0002-3809-4415
Danijela Z. Rajić: https://orcid.org/0000-0002-1169-5755
Vladimir M. Tomović: https://orcid.org/0000-0001-5055-1781

Abstract.
Partial replacement of wheat flour in foodstuffs is of great importance in the food industry. Muffins are a type of semi-sweet cake that is traditionally made from wheat flour. They are especially favored by children and senior citizens. Muffins have a long shelf life, which also contributes to their popularity. However, gluten, the main protein in wheat flour, is commonly associated with celiac disease. Gluten consists of two fractions: gliadins and glutenins.

In this experiment, the original muffins contained 100% wheat flour. Then, we replaced a portion of wheat flour with 25, 50, and 75% quinoa flour. The samples were stored for 0, 2, and 4 weeks. After that, gliadin proteins were extracted with 70% (v/v) ethanol. We separated gliadin using a high-performance liquid chromatograph (Agilent Technologies 1260 Infinity, USA) and measured the total amount of gliadin protein and the amount of gliadin proteins per fraction. The absorbance tests were conducted at 210 nm.

The gliadin protein content was significantly reduced to the wheat vs. quinoa ratio of 50:50 because quinoa is gluten-free, even though it is rich in protein. During the storage time of 0, 2, and 4 weeks, the protein content fell down in the samples with the wheat vs. quinoa ratios of 100:0, 75:25, and 50:50. However, the muffins with 25% wheat flour and 75% quinoa demonstrated an increase in gliadin content.

The results obtained could be a good starting point for the development of high-fiber, gluten-free, and more nutritionally valuable muffins.

Keywords. Muffins, wheat flour, quinoa flour, gluten, gliadins, HPLC

Funding. This work is a part of the research project "Examination of the effect of replacing wheat flour with pseudocereals on gluten proteins", financed by the Ministry of Science and Technology Development and Higher Education of the Republic of Srpska.

For citation: Gojković Cvjetković VS, Škuletić DM, Marjanović-Balaban ŽR, Vujadinović DP, Rajić DZ, Tomović VM. Gliadin Proteins in Muffins with Quinoa Flour. Food Processing: Techniques and Technology. 2024;54(1):82–92. https://doi.org/10.21603/2074-9414-2024-1-2490

https://doi.org/10.21603/2074-9414-2024-1-2490
https://elibrary.ru/LDFMSG

Оригинальная статья
https://fptt.ru

Влияние на белки глиадина частичной замены пшеничной муки в кексах мукой из киноа

В. С. Гойкович Цветкович[1,*], Д. М. Шкулетич[1], Ж. Р. Марьянович-Балабан[2], Д. П. Вуядинович[1], Д. З. Раич[1], В. М. Томович[3]

[1] Восточно-Сараевский университет, Лукавица, Босния и Герцеговина

[2] Баня-Лукский университет, Баня-Лука, Босния и Герцеговина

[3] Нови-Садский университет, Нови-Сад, Сербия

Поступила в редакцию: 30.06.2023
Принята после рецензирования: 11.09.2023
Принята к публикации: 03.10.2023

**В. С. Гойкович Цветкович: vesna.gojkovic@tfzv.ues.rs.ba, https://orcid.org/0000-0003-1118-4565*
Д. П. Вуядинович: https://orcid.org/0000-0002-3809-4415
Д. З. Раич: https://orcid.org/0000-0002-1169-5755
В. М. Томович: https://orcid.org/0000-0001-5055-1781

Аннотация.
Кексы относятся к мучным кондитерским изделиям, которые имеют длительный срок хранения, что влияет на их популярность. Благодаря вкусовым характеристикам кексы любят дети и пожилые люди. Главным рецептурным компонентом кексов является пшеничная мука. Глютен – основной белок в составе пшеничной муки. Именно его связывают с возникновением заболевания целиакия. Глютен состоит из двух фракций: глиадинов и глютенинов. Цель исследования – изучение возможности замены части пшеничной муки в кексах мукой киноа.
Заменили часть пшеничной муки в традиционных кексах на муку киноа – 25, 50 и 75 %. Образцы хранили в течение 0, 2 и 4 недель. После этого белки глиадина экстрагировали 70 % этанолом. Глиадин отделяли с помощью высокоэффективного жидкостного хроматографа (Agilent Technologies 1260 Infinity, США), чтобы измерить его общее количество белка и количество белков в каждой фракции. Тесты на поглощение проводились при длине волны 210 нм.
Содержание белка глиадина снижалось до соотношения пшеницы и киноа 50:50, поскольку мука киноа не содержит глютена, хотя богата белком. За 0, 2 и 4 недели хранения содержание белка снижалось в образцах, где соотношение муки пшеницы и киноа было 100:0, 75:25 и 50:50. Однако в кексах с 25 % пшеничной муки и 75 % муки киноа наблюдалось увеличение содержания глиадина.
Полученные результаты могут стать точкой для разработки кексов с высоким содержанием клетчатки, без глютена и повышенной питательностью.

Ключевые слова. Кексы, пшеничная мука, мука из киноа, глютен, глиадины, ВЭЖХ

Финансирование. Работа является частью исследовательского проекта «Воздействие замещения пшеничной муки псевдозернами на белки клейковины», финансируемого Министерством развития науки, технологий и высшего образования Республики Сербской.

Для цитирования: Влияние на белки глиадина частичной замены пшеничной муки в кексах мукой из киноа / В. С. Гойкович Цветкович [и др.] // Техника и технология пищевых производств. 2024. Т. 54. № 1. С. 82–92. (На англ.). https://doi.org/10.21603/2074-9414-2024-1-2490

Introduction

By replacing wheat flour with its safer alternatives, food producers both fortify their products and contribute to preventing certain chronic diseases [1, 2]. Muffins are one of the most popular cakes, largely consumed by such population categories as children and senior citizens. Muffins are semi-sweet cakes prepared from wheat flour. They owe their popularity to their extended shelf life [3–5].

Wheat flour is the basic ingredient in muffins, gluten being its major protein. Gliadins represent one of the gluten fractions [6]. They are soluble in aqueous alcohol. Gliadins can be divided into four fractions marked as $\omega 5$, $\omega 1,2$, $\alpha + \beta$, and γ [7–9].

Pseudocereals do not belong to cereals per se: their seeds resemble cereals and, therefore, can be used similarly. The most important pseudocereals are amaranth (*Amaranthus* spp.), quinoa (*Chenopodium quinoa* Willd.), and buckwheat (*Fagopyrum esculentum* Moench. and *Fagopyrum tartaricum* (L.) Gaertn) [10].

Although it is not a cereal, quinoa is often called a super cereal. It is a rich source of protein. Unlike cereals proper, quinoa is a complete source of protein because it contains all nine essential amino acids, for instance, lysine. This amino acid is essential for tissue growth and recovery [11, 12]. In addition to protein, quinoa also contains iron, potassium, and vitamin B_2, as well as other vitamins from the B group, e.g., B_6, B_3, and B_1. It is a good source of magnesium, zinc, copper, manganese, as well as fibers [12].

Quinoa does not contain gluten, which makes it suitable for people suffering from celiac disease. It is good for digestion because it contains insoluble fibers [13, 14]. Quinoa is also known to prevent cardiovascular diseases and diabetes because it contains healthy fats and antioxidants [14].

The Food and Agriculture Organization (FAO) has recognized the importance and value of quinoa due to its various positive characteristics. Its genetic variability is quite impressive and opens possibilities of developing superior species [15]. Quinoa is resistant to different agricultural and climatic conditions as it grows at different altitudes and temperatures. Due to its high nutritional value and ability to provide food for the world's population, the year of 2013 was declared the International Year of Quinoa [16].

Considering that quinoa is a gluten-free grain rich in proteins and other nutrients, and that the number of people who suffer from celiac disease is increasing every day, this work examines how replacing part of wheat flour with quinoa flour and storage time (0, 2, and 4 weeks) affect gliadin proteins in muffins.

Study objects and methods

Preparing muffins. The muffins were prepared according to four different formulations, which differed in the amount of wheat flour (30 g). We replaced wheat flour with quinoa flour (Karlsruhe, Germany) in the proportions that ranged from 0, 25, 50, and 75% (by weight). The quinoa flour used in the experiment contained (as declared by the producer) the following macronutrients and nutrients per 100 g: 12.0 g proteins, 6.6 g fats, 67 g carbohydrates, 5.5 g fibers, < 0.01 g salt. The formulations for the muffins included other ingredients as well, i.e., sugar powder (10 g), milk (3.2% fat, 12 mL), butter (9 g), baking powder (0.6 g), and beaten whole eggs (7.6 g). The muffins were prepared as described by Bialek *et al.* [17].

Preparing gliadin. Gliadins were extracted from 100 g muffin samples with 70% (v/v) ethanol (96% v/v ethyl alcohol, Srbobran). The extraction was carried out according to the modified methods proposed by Wieser *et al.* and Gojković Cvjetković *et al.* [18, 19].

Separating gliadin separation with HPLC. The gliadin separation was performed on a high-performance liquid chromatograph (Agilent Technologies 1260 Infinity, USA). After separating the proteins, we measured the total amount of proteins and the amount of proteins in each fraction. The total amount of proteins was obtained as a sum of individual proteins. The method of gliadin separation followed the protocol described by Gojković Cvjetković *et al.* [19].

The **statistical data analysis** involved the IBM SPSS Statistics 26 software. We calculated the average amount of proteins (Xav), standard deviation, and standard error, as well as minimal and maximal values. We used the analysis of variance (ANOVA) of different groups to evaluate the effect of storage time (0, 2, and 4 weeks) and different shares of quinoa flour on the average amount of proteins. The significance of the differences between the average amount at the $p = 0.05$ level of significance was evaluated by the subsequent Tukey's HSD test of real difference.

Results and discussion

Table 1 shows the total amount of gliadin proteins and the amount of protein in each fraction in muffins with 100% wheat flour.

The highest amount of protein within the $\omega5$ gliadin fraction was isolated from the fresh samples (Xav = 4.00) while the lowest belonged to those stored for 2 and 4 weeks (Xav = 2.00). Within the $\omega1,2$ gliadin fraction, however, the highest amount of protein was isolated from the samples after 2 weeks of storage (Xav = 3.67) whereas the lowest amount was detected in the fresh samples (Xav = 2.67). The highest amount of protein within the $\alpha + \beta$ was obtained from the samples stored for 2 weeks (Xav = 6.00), and the lowest was registered in the samples stored for 4 weeks (Xav = 3.33). Within the γ gliadin fraction, the highest amount of protein belonged to the fresh samples (Xav = 15.00) while the lowest amount was revealed in the samples that underwent 2 weeks of storage (Xav = 8.67). The ANOVA confirmed a statistical difference: $F(2.6) = 16.35$, Sig. = 0.004 < 0.05. The Tukey's HSD test showed that the samples stored for 0 and 2 weeks and 0 and 4 weeks were significantly different, while the samples stored for 2 and 4 weeks did not differ significantly.

The highest total amount of protein was extracted from the fresh muffin sample (Xav = 26.00), and the lowest total amount of protein came from the samples subjected to 4 weeks of storage (Xav = 18.00). The ANOVA revealed a statistical difference: $F(2.6) = 13.44$, Sig. = 0.006 < 0.05. The Tukey's HSD test demonstrated that the samples stored for 0 and 2 weeks, as well as those stored for 0 and 4 weeks, had a statistically significant difference whereas the samples stored for 2 and 4 weeks were statistically the same.

Table 2 shows the total amount of gliadin proteins and the amount of protein for each fraction in the muffin samples made from 75% wheat flour and 25% quinoa flour.

Table 1. Total gliadin and gliadin by fractions in muffins with 100% wheat flour, with absorbance measurement at 210 nm

Таблица 1. Общий глиадин и глиадин по фракциям в кексах из 100 % пшеничной муки (измерение оптической плотности проводилось при 210 нм)

Proteins	Storage time (weeks)	N	Average protein (Xav)	Standard deviation	Standard error	Minimal value	Maximal value
ω5 gliadins	0	3	4.00	1.00	0.58	3.00	5.00
	2	3	2.00	1.00	0.58	1.00	3.00
	4	3	2.00	1.00	0.58	1.00	3.00
ω1,2 gliadins	0	3	2.67	0.58	0.33	2.00	3.00
	2	3	3.67	0.58	0.33	3.00	4.00
	4	3	3.33	0.58	0.33	3.00	4.00
$\alpha + \beta$ gliadins	0	3	4.33	1.15	0.67	3.00	5.00
	2	3	6.00	1.00	0.58	5.00	7.00
	4	3	3.33	1.15	0.67	2.00	4.00
γ gliadins	0	3	15.00	1.73	1.00	14.00	17.00
	2	3	8.67	1.15	0.67	8.00	10.00
	4	3	9.33	1.53	0.88	8.00	11.00
Total proteins	0	3	26.00	1.73	1.00	25.00	28.00
	2	3	20.33	2.08	1.20	18.00	22.00
	4	3	18.00	2.00	1.15	16.00	20.00
ANOVA (ω5)	F(2.6) = 4.00, Sig. = 0.08 > 0.05, eta square = 8/14 = 0.57						
ANOVA (ω1,2)	F(2.6) = 2.33, Sig. = 0.18 > 0.05, eta square = 1.55/3.55 = 0.44						
ANOVA ($\alpha + \beta$)	F(2.6) = 4.45, Sig. = 0.06 > 0.05, eta square = 10.89/18.22 = 0.60						
ANOVA (γ)	F(2.6) = 16.35, Sig. = 0.004 < 0.05, eta square = 72.67/86.00 = 0.84						
ANOVA (TAP)	F(2.6) = 13.44, Sig. = 0.006 < 0.05, eta square = 101.55/124.22 = 0.82						

Table 2. Total gliadin and gliadin by fractions in muffins made from 75% wheat flour and 25% quinoa flour, with absorbance measurement at 210 nm

Таблица 2. Общий глиадин и глиадин по фракциям в кексах с 75 % пшеничной муки и 25 % муки киноа (измерение оптической плотности проводилось при 210 нм)

Proteins	Storage time (weeks)	N	Average protein (Xav)	Standard deviation	Standard error	Minimal value	Maximal value
ω5 gliadins	0	3	2.67	0.58	0.33	2.00	3.00
	2	3	1.67	0.58	0.33	1.00	2.00
	4	3	2.33	0.58	0.33	2.00	3.00
ω1,2 gliadins	0	3	3.00	1.00	0.58	2.00	4.00
	2	3	3.67	0.58	0.33	3.00	4.00
	4	3	3.67	0.58	0.33	3.00	4.00
$\alpha + \beta$ gliadins	0	3	5.00	1.00	0.58	4.00	6.00
	2	3	4.00	1.00	0.58	3.00	5.00
	4	3	3.67	1.15	0.67	3.00	5.00
γ gliadins	0	3	8.33	1.15	0.67	7.00	9.00
	2	3	7.67	0.58	0.33	7.00	8.00
	4	3	8.33	0.58	0.33	8.00	9.00
Total proteins	0	3	19.67	1.53	0.88	18.00	21.00
	2	3	17.33	1.53	0.88	16.00	19.00
	4	3	17.67	1.15	0.67	17.00	19.00
ANOVA (ω5)	F(2.6) = 2.33, Sig. = 0.18 > 0.05, eta square = 1.55/3.55 = 0.44						
ANOVA (ω1,2)	F(2.6) = 0.80, Sig. = 0.49 > 0.05, eta square = 0.89/4.22 = 0.21						
ANOVA ($\alpha + \beta$)	F(2.6) = 1.30, Sig. = 0.34 > 0.05, eta square = 2.89/9.55 = 0.30						
ANOVA (γ)	F(2.6) = 0.67, Sig. = 0.55 > 0.05. eta square = 0.89/4.89 = 0.18						
ANOVA (TAP)	F(2.6) = 2.39, Sig. = 0.17 > 0.05, eta square = 9.55/21.55 = 0.44						

In this experiment, gliadin protein was extracted from the muffin samples made from 75% wheat flour and 25% quinoa flour. The chromatographic separation showed that the highest amount of protein in the ω5 gliadin fraction belonged to the samples stored for 0 weeks (Xav = 2.67). The lowest amount of protein was detected in the fresh samples (Xav = 1.67). As for the ω1,2 gliadin fraction, the highest amount of protein was found in the samples stored for 2 and 4 weeks (Xav = 3.67) whereas the fresh samples showed the lowest one (Xav = 3.00). The highest amount of protein belonged to the $\alpha + \beta$ fraction and was isolated from the fresh samples (Xav = 5.00), as well as to the γ gliadin fraction isolated from the four-week sample (Xav = 8.33). In these fractions, the samples stored for 4 and 2 weeks contained the lowest amount of protein, which amounted to Xav = 3.67 and Xav = 7.67, respectively. The highest total amount of protein was isolated from the fresh samples (Xav = 19.67) while the samples stored for 2 weeks had the lowest total amount (Xav = 17.33). The ANOVA revealed no statistically significant difference neither for any particular protein nor for the total.

Table 3 shows the total amount of gliadin proteins and the amount of protein by fractions in the muffin samples made from 50% wheat flour and 50% quinoa flour.

The gliadin proteins extracted from the muffin samples with 50% wheat flour and 50% quinoa flour were separated and tested for total amount and the amount of gliadin per fraction. As for gliadin fractions ω5 and ω1,2, the highest amounts of protein were extracted from the samples that were stored for 0 (Xav = 3.00) and 4 weeks (Xav = 3.67). The samples that underwent 2 and 0 weeks of storage demonstrated the lowest amounts of Xav = 1.00 and Xav = 1.67, respectively. The highest amount of protein within the $\alpha + \beta$ and γ gliadin fractions belonged to the fresh samples and equaled Xav = 3.67 and Xav = 8.67, respectively. The lowest volumes came from the samples that were stored for 2 (Xav = 3.00) and 4 weeks (Xav = 6.67). The ANOVA showed a statistical difference within the γ gliadin fraction: $F(2.6) = 9.33$, Sig = $0.01 < 0.05$. A subsequent comparison by Tukey's HSD test demonstrated that the samples stored for 0 and 4 weeks differed significantly while the samples stored for 0 and 2 weeks, as well as those stored for 2 and 4 weeks, were statistically the same.

The highest total amount of protein belonged to the fresh samples (Xav = 17.00) while the lowest data were obtained from the two-week samples (Xav = 14.33).

Table 4 shows the total amount of gliadin proteins and the amount of protein per fraction in the muffin samples made from 25% wheat flour and 75% quinoa flour.

The highest amount of protein within the ω5 gliadin fraction was obtained from the fresh samples (Xav = 2.67) while the two-week samples yielded the lowest amount (Xav = 1.00). As for the ω1,2 gliadin fraction, the highest

Table 3. Total gliadin and gliadin by fractions in muffins made from 50% wheat flour and 50% quinoa flour, with absorbance measurement at 210 nm

Таблица 3. Общий глиадин и глиадин по фракциям в кексах с 50 % пшеничной муки и 50 % муки киноа (измерение оптической плотности проводилось при 210 нм)

Proteins	Storage time (weeks)	N	Average protein (Xav)	Standard deviation	Standard error	Minimal value	Maximal value
ω5 gliadins	0	3	3.00	0	0	3.00	3.00
	2	3	1.00	0	0	1.00	1.00
	4	3	2.00	0	0	2.00	2.00
ω1,2 gliadins	0	3	1.67	1.15	0.67	1.00	3.00
	2	3	2.67	0.58	0.33	2.00	3.00
	4	3	3.67	0.58	0.33	3.00	4.00
$\alpha + \beta$ gliadins	0	3	3.67	0.58	0.33	3.00	4.00
	2	3	3.00	0	0	3.00	3.00
	4	3	3.00	1.00	0.58	2.00	4.00
γ gliadins	0	3	8.67	0.58	0.33	8.00	9.00
	2	3	7.33	0.58	0.33	7.00	8.00
	4	3	6.67	0.58	0.33	6.00	7.00
Total proteins	0	3	17.00	2.65	1.53	14.00	19.00
	2	3	14.33	1.15	0.67	13.00	15.00
	4	3	15.33	0.58	0.33	15.00	16.00
ANOVA (ω5)	$F(2.6)$ = inf.						
ANOVA (ω1,2)	$F(2.6) = 4.50$, Sig. = $0.06 > 0.05$, eta square = 6.00/10.00 = 0.60						
ANOVA ($\alpha + \beta$)	$F(2.6) = 1.00$, Sig. = $0.42 > 0.05$, eta square = 0.89/3.55 = 0.25						
ANOVA (γ)	$F(2.6) = 9.33$, Sig. = $0.01 < 0.05$, eta square = 6.22/8.22 = 0.76						
ANOVA (TAP)	$F(2.6) = 1.88$, Sig. = $0.23 > 0.05$, eta square = 10.89/28.22 = 0.39						

amount of proteins belonged to the four-week samples (Xav = 4.33) whereas the lowest one came from the two-week samples (Xav = 1.67). The highest amount of protein was observed in the $\alpha + \beta$ gliadin fraction in the fresh samples (Xav = 4.00) and in the γ gliadin fraction in the samples stored for 4 weeks (Xav = 7.00). The lowest amount was registered in the $\alpha + \beta$ gliadin fraction in the two-week samples (Xav = 3.00), as well as in the γ gliadin fraction in the four-week samples (Xav = 5.67). The highest total amount of proteins was obtained from the muffins after 4 weeks of storage (Xav = 16.33), and the lowest was detected in the two-week samples (Xav = 12.33). The ANOVA detected a significant difference: $F(2.6) = 12.00$, Sig. = 0.008 < 0.05. The Tukey's HSD test showed that the statistical difference concerned the samples stored for 2 and 4 weeks.

Table 5 shows the total amount of gliadin proteins and the amount of protein per fraction after the extraction from the muffin samples made from wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) after 0 weeks of storage.

The highest amount of protein within the ω5 gliadin fraction was isolated from the samples that contained no quinoa flour (Xav = 4.00) while the lowest one came from those with the wheat vs. quinoa ratios of 75:25 and 25:75 (Xav = 2.67). Within the ω1,2 gliadin fraction, the highest amount of protein belonged to the samples with 75% wheat flour and 25% quinoa flour (Xav = 3.00) whereas the lowest was registered in the samples with equal shares of wheat and quinoa (Xav = 1.67). As for $\alpha + \beta$ gliadin, the samples with 75% wheat flour and 25% quinoa flour had the highest amount of protein (Xav = 5.00). The lowest amounts in this fraction came from the samples with equal shares of wheat and quinoa (Xav = 3.67). Within the γ gliadin fraction, the highest amount of protein was isolated from the samples without quinoa (Xav = 15.00), while the lowest belonged to the samples with 25% wheat flour and 75% quinoa flour Xav = 5.67). The ANOVA detected a statistical difference: $F(3.8) = 37.58$, Sig. = 0.000 < 0.05. According to the Tukey's test, the significant difference was found between the samples with the following wheat vs. quinoa ratios: 100:0 and 75:25; 100:0 and 50:50; 100:0 and 25:75; 50:50 and 25:75. No significant difference was detected between the samples with the ratios of 75:25 and 50:50; 75:25 and 25:75. The highest total amount of proteins was extracted from the muffin sample that contained no quinoa and amounted to Xav = 26.00 while the lowest belonged to the samples with 25% wheat flour and 75% quinoa (Xav = 14.33). The ANOVA detected a statistical difference: $F(3.8) = 23.67$, Sig. = 0.000 < 0.05. A subsequent comparison by Tukey's test revealed a statistically significant difference in the samples with the following wheat vs. quinoa ratios: 100:0 and 75:25; 100:0 and 50:50; 100:0 and 25:75; 75:25 and 25:75.

Table 4. Total gliadin and gliadin by fractions in muffins made from 25% wheat flour and 75% quinoa flour, with absorbance measurement at 210 nm

Таблица 4. Общий глиадин и глиадин по фракциям в кексах с 25 % пшеничной муки и 75 % муки киноа (измерение оптической плотности проводилось при 210 нм)

Proteins	Storage time (weeks)	N	Average protein (Xav)	Standard deviation	Standard error	Minima value	Maximal value
ω5 gliadins	0	3	2.67	1.15	0.67	2.00	4.00
	2	3	1.00	0	0	1.00	1.00
	4	3	2.00	0	0	2.00	2.00
ω1,2 gliadins	0	3	2.00	1.00	0.58	1.00	3.00
	2	3	1.67	1.15	0.67	1.00	3.00
	4	3	4.33	1.15	0.67	3.00	5.00
$\alpha + \beta$ gliadins	0	3	4.00	1.00	0.58	3.00	5.00
	2	3	3.00	1.00	0.58	2.00	4.00
	4	3	3.00	1.00	0.58	2.00	4.00
γ gliadins	0	3	5.67	0.58	0.33	5.00	6.00
	2	3	6.67	1.52	0.88	5.00	8.00
	4	3	7.00	1.00	0.58	6.00	8.00
Total proteins	0	3	14.33	0.58	0.33	14.00	15.00
	2	3	12.33	1.53	0.88	11.00	14.00
	4	3	16.33	0.58	0.33	16.00	17.00
ANOVA (ω5)	F(2.6) = 4.75, Sig. = 0.06 > 0.05, eta square = 4.22/6.89 = 0.61						
ANOVA (ω1,2)	F(2.6) = 5.18, Sig. = 0.05, eta square = 12.67/20.00 = 0.63						
ANOVA ($\alpha + \beta$)	F(2.6) = 1.00, Sig. = 0.42 > 0.05, eta square = 2.00/8.00 = 0.25						
ANOVA (γ)	F(2.6) = 1.18, Sig. = 0.37 > 0.05, eta square = 2.89/10.22 = 0.28						
ANOVA (TAP)	F(2.6) = 12.00, Sig. = 0.008 < 0.05, eta square = 24.00/30.00 = 0.80						

Table 5. Total gliadin and gliadins by fractions in muffins made from wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) stored for 0 weeks, with absorbance measurement at 210 nm

Таблица 5. Общий глиадин и глиадины по фракциям в кексах, изготовленных из пшеничной муки (100, 75, 50 и 25 %) и муки киноа (0, 25, 50 и 75 %), после 0 недель хранения (измерение оптической плотности проводилось при 210 нм)

Proteins	Wheat vs. quinoa, %	N	Average protein (Xav)	Standard deviation	Standard error	Minimal value	Maximal value
ω5 gliadins	100:0	3	4.00	1.00	0.58	3.00	5.00
	75:25	3	2.67	0.58	0.33	2.00	3.00
	50:50	3	3.00	0	0	3.00	3.00
	25:75	3	2.67	1.15	0.67	2.00	4.00
ω1,2 gliadins	100:0	3	2.67	0.58	0.33	2.00	3.00
	75:25	3	3.00	1.00	0.58	2.00	4.00
	50:50	3	1.67	1.15	0.67	1.00	3.00
	25:75	3	2.00	1.00	0.58	1.00	3.00
$\alpha + \beta$ gliadins	100:0	3	4.33	1.15	0.67	3.00	5.00
	75:25	3	5.00	1.00	0.58	4.00	6.00
	50:50	3	3.67	0.58	0.33	3.00	4.00
	25:75	3	4.00	1.00	0.58	3.00	5.00
γ gliadins	100:0	3	15.00	1.73	1.00	14.00	17.00
	75:25	3	8.33	1.15	0.67	7.00	9.00
	50:50	3	8.67	0.58	0.33	8.00	9.00
	25:75	3	5.67	0.58	0.33	5.00	6.00
Total proteins	100:0	3	26.00	1.73	1.00	25.00	28.00
	75:25	3	19.67	1.53	0.88	18.00	21.00
	50:50	3	17.00	2.65	1.53	14.00	19.00
	25:75	3	14.33	0.58	0.33	14.00	15.00
ANOVA (ω5)	F(3.8) = 1.79, Sig. = 0.23 > 0.05, eta square = 3.58/8.92 = 0.40						
ANOVA (ω1,2)	F(3.8) = 1.21, Sig. = 0.37 > 0.05, eta square = 3.33/10.67 = 0.31						
ANOVA ($\alpha + \beta$)	F(3.8) = 1.06, Sig. = 0.42 > 0.05, eta square = 2.92/10.25 = 0.28						
ANOVA (γ)	F(3.8) = 37.58, Sig. = 0.000 < 0.05, eta square = 140.92/150.92 = 0.93						
ANOVA (TAP)	F(3.8) = 23.67, Sig. = 0.000 < 0.05, eta square = 224.92/250.25 = 0.90						

Table 6 shows the total amount of gliadin proteins and the amount of protein per fraction in the muffin samples with wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) after two weeks of storage.

In this experiment, gliadin protein was extracted from the muffin samples with wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) after two weeks of storage. The highest amount of protein within the ω5 gliadin fraction was isolated from the samples without quinoa (Xav = 2.00) while the lowest came from the samples with the wheat vs. quinoa ratios of 50:50 and 25:75 (Xav = 1.00). As for ω1,2 gliadin, the highest amount of Xav = 3.67 was detected in the sample without quinoa, as well as in the sample with 75% wheat flour and 25% quinoa. The lowest amount within this fraction came from the samples with 25% wheat flour and 75% quinoa, equaling Xav = 1.67. The ANOVA test registered a statistical difference: F(3.8) = 4.71, Sig. = 0.03 < 0.05. According to the Tukey's HSD test, a statistically significant difference existed between the samples with the following wheat vs. quinoa ratios: 100:0 and 25:75; 75:25 and 25:75. The highest amount of protein within the $\alpha + \beta$ gliadin fraction was observed in the samples that contained no quinoa (Xav = 6.00) while the lowest belonged to the samples with equal shares of wheat and quinoa flours and the samples with 25% wheat and 75% quinoa (Xav = 3.00). The ANOVA detected a statistically significant difference: F(3.8) = 8.00, Sig. = 0.008 < 0.05. According to the Tukey's HSD test, this statistically significant difference occurred between the samples with the following wheat vs. quinoa ratios: 100:0 and 50:50; 100:0 and 25:75. Within the γ gliadin fraction, the highest amount of protein was isolated from the samples with no quinoa and reached Xav = 8.67 whereas the lowest belonged to the samples with 25% wheat flour and 75% quinoa (Xav = 6.67). The highest total amount of proteins was extracted from the muffins cooked without quinoa (Xav = 20.33). The lowest total amount of proteins belonged to the samples with 25% wheat flour and 75% quinoa flour, being as low as Xav = 12.33. The ANOVA detected a statistical difference: F(3.8) = 14.22, Sig. = 0.001 < 0.05. A subsequent comparison by Tukey's HSD test showed that this statistically significant difference took place between the samples with the following wheat vs. quinoa ratios: 100:0 and 50:50; 100:0 and 25:75; 75:25 and 25:75.

Table 6. Total gliadin and gliadins by fractions in muffins with wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) stored for 2 weeks, with absorbance measurement at 210 nm

Таблица 6. Общий глиадин и глиадины по фракциям в кексах, изготовленных из пшеничной муки (100, 75, 50 и 25 %) и муки киноа (0, 25, 50 и 75 %), после 2 недель хранения (измерение оптической плотности проводилось при 210 нм)

Proteins	Wheat vs. quinoa, %	N	Average protein (Xav)	Standard deviation	Standard error	Minimal value	Maximal value
ω5 gliadins	100:0	3	2.00	1.00	0.58	1.00	3.00
	75:25	3	1.67	0.58	0.33	1.00	2.00
	50:50	3	1.00	0	0	1.00	1.00
	25:75	3	1.00	0	0	1.00	1.00
ω1,2 gliadins	100:0	3	3.67	0.58	0.33	3.00	4.00
	75:25	3	3.67	0.58	0.33	3.00	4.00
	50:50	3	2.67	0.58	0.33	2.00	3.00
	25:75	3	1.67	1.15	0.67	1.00	3.00
$\alpha + \beta$ gliadins	100:0	3	6.00	1.00	0.58	5.00	7.00
	75:25	3	4.00	1.00	0.58	3.00	5.00
	50:50	3	3.00	0	0	3.00	3.00
	25:75	3	3.00	1.00	0.58	2.00	4.00
γ gliadins	100:0	3	8.67	1.15	0.67	8.00	10.00
	75:25	3	7.67	0.58	0.33	7.00	8.00
	50:50	3	7.33	0.58	0.33	7.00	8.00
	25:75	3	6.67	1.53	0.88	5.00	8.00
Total proteins	100:0	3	20.33	2.08	1.20	18.00	22.00
	75:25	3	17.33	1.53	0.88	16.00	19.00
	50:50	3	14.33	1.15	0.67	13.00	15.00
	25:75	3	12.33	1.53	0.88	11.00	14.00
ANOVA (ω5)	$F(3.8) = 2.25$, Sig. $= 0.16 > 0.05$, eta square $= 2.25/4.92 = 0.46$						
ANOVA (ω1,2)	$F(3.8) = 4.71$, Sig. $= 0.03 < 0.05$, eta square $= 8.25/12.92 = 0.64$						
ANOVA ($\alpha + \beta$)	$F(3.8) = 8.00$, Sig. $= 0.008 < 0.05$, eta square $= 18.00/24.00 = 0.75$						
ANOVA (γ)	$F(3.8) = 1.92$, Sig. $= 0.20 > 0.05$, eta square $= 6.25/14.92 = 0.42$						
ANOVA (TAP)	$F(3.8) = 14.22$, Sig. $= 0.001 < 0.05$, eta square $= 110.25/130.92 = 0.84$						

Table 7 shows the total amount of gliadin proteins and the amount of protein per fraction in the muffin samples with wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) after four weeks of storage.

The highest amount of protein within the ω5 gliadin fraction belonged to the samples that contained no quinoa and reached Xav = 2.33. The lowest amount of protein was extracted from the samples with the following wheat vs. quinoa ratios: 100:0, 50:50, and 25:75 (Xav = 2.00). Within the ω1,2 gliadin fraction, the highest amount of protein was obtained from the samples with 25% wheat flour and 75% quinoa flour (Xav = 4.33) while the lowest came from the samples without quinoa (Xav = 3.33). As for gliadin $\alpha + \beta$, the highest amount of protein was isolated from the samples with 75% wheat flour and 25% quinoa flour (Xav = 3.67) whereas the lowest amount belonged to the samples with equal shares of the flours and the one with 25% wheat and 75% quinoa (Xav = 3.00). The highest amount of protein within the γ gliadin fraction was isolated from the samples that contained no quinoa (Xav = 9.33). The lowest amount of protein came from the samples with equal shares of wheat and quinoa (Xav = 6.67). The ANOVA recorded a statistical difference: $F(3.8) = 4.55$, Sig. $= 0.04 < 0.05$. The Tukey's test showed that the samples without quinoa and the sample with equal shares of the flour were significantly different. The highest total amount of proteins was extracted from the muffins that contained no quinoa flour (Xav = 18.00) whereas the lowest came from the samples with equal shares of both flours and equaled Xav = 15.33.

Bialek *et al.* could replace wheat flour with 33% pumpkin seed flour in muffins meant for children [17].

Bhaduri conducted a comprehensive study on the physical properties of gluten-free flour-fortified muffins [20]. Based on the obtained results, the muffins containing 100% rice flour and 75% rice flour + 25% quinoa flour were the softest and the most acceptable for consumers.

Kurek and Sokolova improved bread quality with quinoa flour of different particle sizes and percentages of wheat flour replacement [21]. The most suitable combination was 219 µm and 5.41%.

To our knowledge, no publications are available on the effect that replacing part of wheat flour with quinoa flour exerts on gliadin proteins in muffins. In this research, the highest amount of gliadin proteins was isolated from the muffin samples made from wheat flour

Table 7. Total gliadin and gliadin by fractions in muffins with wheat flour (100, 75, 50, and 25%) and quinoa flour (0, 25, 50, and 75%) stored for 4 weeks, with absorbance measurement at 210 nm

Таблица 7. Общий глиадин и глиадин по фракциям в кексах, изготовленных из пшеничной муки (100, 75, 50 и 25%) и муки киноа (0, 25, 50 и 75%), после 4 недель хранения (измерение оптической плотности проводилось при 210 нм)

Proteins	Wheat vs. quinoa, %	N	Average protein (Xav)	Standard deviation	Standard error	Minimal value	Maximal value
ω5 gliadins	100:0	3	2.00	1.00	0.58	1.00	3.00
	75:25	3	2.33	0.58	0.33	2.00	3.00
	50:50	3	2.00	0	0	2.00	2.00
	25:75	3	2.00	0	0	2.00	2.00
ω1,2 gliadins	100:0	3	3.33	0.58	0.33	3.00	4.00
	75:25	3	3.67	0.58	0.33	3.00	4.00
	50:50	3	3.67	0.58	0.33	3.00	4.00
	25:75	3	4.33	1.15	0.67	3.00	5.00
$\alpha + \beta$ gliadins	100:0	3	3.33	1.15	0.67	2.00	4.00
	75:25	3	3.67	1.15	0.67	3.00	5.00
	50:50	3	3.00	1.00	0.58	2.00	4.00
	25:75	3	3.00	1.00	0.58	2.00	4.00
γ gliadins	100:0	3	9.33	1.53	0.88	8.00	11.00
	75:25	3	8.33	0.58	0.33	8.00	9.00
	50:50	3	6.67	0.58	0.33	6.00	7.00
	25:75	3	7.00	1.00	0.58	6.00	8.00
Total proteins	100:0	3	18.00	2.00	1.15	16.00	20.00
	75:25	3	17.67	1.15	0.67	17.00	19.00
	50:50	3	15.33	0.58	0.33	15.00	16.00
	25:75	3	16.33	0.58	0.33	16.00	17.00
ANOVA (ω5)	F(3.8) = 0.25, Sig. = 0.86 > 0.05, eta square = 0.25/2.92 = 0.08						
ANOVA (ω1,2)	F(3.8) = 0.90, Sig. = 0.48 > 0.05, eta square = 1.58/6.25 = 0.25						
ANOVA ($\alpha + \beta$)	F(3.8) = 0.26, Sig. = 0.85 > 0.05, eta square = 0.92/10.25 = 0.09						
ANOVA (γ)	F(3.8) = 4.55, Sig. = 0.04 < 0.05, eta square = 13.67/21.67 = 0.63						
ANOVA (TAP)	F(3.8) = 3.04, Sig. = 0.09 > 0.05, eta square = 13.67/25.67 = 0.53						

only. We deted the lowest amount of gliadin proteins in the muffin samples made from 25% wheat flour and 75% quinoa.

Quinoa is rich in protein, but it is also gluten-free, so gliadin content was at its lowest in the samples with 25% wheat flour and 75% quinoa flour. In the samples with the wheat vs. quinoa ratios of 100:0, 75:25, and 50:50, the content of the gliadin protein decreased with the increase in the storage time (0, 2, and 4 weeks). However, the content of gliadin protein extracted from the samples with 25% wheat and 75% quinoa increased with time. This phenomenon can be explained by protein denaturation, modification of the amino acid chain, formation of protein polymers, reduction of protein solubility, aggregation, and carbonyl concentration increase [22]. Oxidized lysine, arginine, proline, and threonine can produce carbonyl derivatives, which, in their turn, can change the conformation of proteins, thus affecting their functional properties [23]. In the muffin samples with the wheat vs. quinoa ratios of 100:0, 75:25, 50:50, and 25:75, the total amount of protein decreased after 0 and 2 weeks of storage. However, the total amount of protein went up in the samples stored for 4 weeks.

Conclusion

In this study, we replaced part of wheat flour in muffins with quinoa flour and stored the obtained products for 0, 2, and 4 weeks. After extracting gliadin proteins, we obtained the following results. The highest amount of gliadin was isolated from the muffins that contained wheat flour only (Xav = 26.00). The lowest amount of protein was isolated from the muffin samples with 25% wheat flour and 75% quinoa flour (Xav = 14.33). The gliadin protein content decreased because quinoa is gluten-free, even though it is rich in protein. In the muffin samples with the wheat vs. quinoa ratios of 100:0, 75:25, and 50:50, the content of gliadin went down as the storage time reached four weeks. However, the samples with 25% wheat flour and 75% quinoa flour demonstrated an increase in the content of gliadin protein. The protein content decreased to the wheat vs. quinoa ratio of 50:50 during storage because of protein denaturation, modification of amino acid chains, formation of protein polymers, and low protein solubility.

Quinoa can be recommended to celiac disease patients because it is rich in proteins but contains no gluten. When we replaced part of wheat flour with quinoa flour in muffins, the amount of gliadin protein kept decreasing until the ratio of wheat flour and quinoa flour reached 50:50. The results obtained in this study could be a good starting point for the development of high-fiber, gluten-free, and more nutritionally valuable muffins.

Contribution

V.S. Gojković Cvjetković and Ž.R. Marjanović-Balaban developed the recearch concept, worked with the software. D.P. Vujadinović designed the methodology. D.M. Škuletić and D.Z. Rajić performed the formal analysis. V.S. Gojković Cvjetković is responsible for the data curation; the original draft belonged to V.S. Gojković Cvjetković and V.M. Tomović. V.S. Gojković Cvjetković, D.P. Vujadinović, and V.M. Tomović reviewed and edited the final version. All the authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Критерии авторства

В. С. Гойкович Цветкович и Ж. Р. Марьянович-Балабан – концепция исследования, программное обеспечение. Д. П. Вуядинович – методология исследования. Д. М. Шкулетич и Д. З. Раич – формальный анализ. В. С. Гойкович Цветкович – обработка данных. В. С. Гойкович Цветкович и В. М. Томович – первоначальный проект исследования. В. С. Гойкович Цветкович, Д. П. Вуядинович и В. М. Томович – редактирование окончательной версии статьи. Все авторы ознакомлены и согласны с итоговой версией рукописи.

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

References

1. Rahaie S, Gharibzahedi SMT, Razavi SH, Jafari SM. Recent developments on new formulations based on nutrient-dense ingredients for the production of healthy-functional bread: A review. Journal of Food Science and Technology. 2014;51(11): 2896–2906. https://doi.org/10.1007/s13197-012-0833-6

2. Valková V, Ďúranová H, Havrlentová M, Ivanišová E, Mezey J, Tóthová Z, *et al.* Selected physico-chemical, nutritional, antioxidant and sensory properties of wheat bread supplemented with apple pomace powder as a by-product from juice production. Plants. 2022;11(9). https://doi.org/10.3390/plants11091256

3. Rajiv J, Soumya C, Indrani D, Rao Venkateswara G. Effect of replacement of wheat flour with finger millet flour (*Eleusine corcana*) on the batter microscopy, rheology and quality characteristics of muffins. Journal of Texture Studies. 2011;42(6):478–489. https://doi.org/10.1111/j.1745-4603.2011.00309.x

4. Kaur M, Singh B, Kaur A. Influence of different oilseed cake incorporation on batter rheology, proximate composition, texture, antioxidant and sensory properties of wheat flour muffins. International Journal of Food Science and Technology. 2022;57(11):7107–7115. https://doi.org/10.1111/ijfs.16050

5. Ndinchout AS, Chattopadhyay D, Ascension NM, Kaur V, Singh N, Paul MF. Muffins fortified with *Dacryodes macrophylla* L. fruit: quality and sensory evaluation. Foods and Raw Materials. 2022;10(1):40–50. https://doi.org/10.21603/2308-4057-2022-1-40-50.

6. Shewry P. What is gluten – Why is it special? Frontiers in Nutrition. 2019;6. https://doi.org/10.3389/fnut.2019.00101

7. Gojković V, Marjanović-Balaban Ž, Grujić R, Vujadinović D, Vukić M. The influence of extraction and chromatographic separation on the ability to identify gliadins from the wheat flour. Journal of Hygienic Engineering and Design. 2019; 26:118–126.

8. Schopf M, Wehrli MC, Becker T, Jekle M, Scherf KA. Fundamental characterization of wheat gluten. European Food Research and Technology. 2021;247:985–997.

9. Marjanović-Balaban Ž, Gojković Cvjetković V, Grujić R. Gliadin proteins from wheat flour: the optimal determination conditions by ELISA. Foods and Raw Materials. 2021;9(2):364–370. https://doi.org/10.21603/2308-4057-2021-2-364-370

10. de Bock P, Daelemans L, Selis L, Raes K, Vermeir P, Eeckhout M, *et al.* Comparison of the chemical and technological characteristics of wholemeal flours obtained from amaranth (*Amaranthus* sp.), quinoa *(Chenopodium quinoa*), and buckwheat (*Fagopyrum* sp.) seeds. Foods. 2021;10(3). https://doi.org/10.3390/foods10030651

11. Elsohaimy SA, Refaay TM, Zaytoun MAM. Physicochemical and functional properties of quinoa protein isolate. Annals of Agricultural Sciences. 2015;60(2):297–305. https://doi.org/10.1016/j.aoas.2015.10.007

12. Agrawal RS. Quinoa – supergrain of the future: A review. The Pharma Innovation Journal. 2018;7(11):249–251.

13. Bhathal S, Grover K, Gill N. Quinoa – a treasure trove of nutrients. Journal of Nutrition Research. 2015;3(1):45–49. https://doi.org/10.55289/jnutres/v3i1.2

14. Agarwal N, Kolba N, Khan N, Even C, Turjeman S, Koren O, *et al.* Quinoa soluble fiber and quercetin alter the composition of the gut microbiome and improve brush border membrane morphology in vivo (*Gallus gallus*). Nutrients. 2022; 14(3). https://doi.org/10.3390/nu14030448

15. Bazile D, Pulvento C, Verniau A, Al-Nusairi MS, Ba D, Breidy J, *et al.* Worldwide evaluations of quinoa: Preliminary results from post international year of quinoa FAO projects in nine countries. Frontiers in Plant Science. 2016;7. https://doi.org/10.3389/fpls.2016.00850

16. The future of food and agriculture. Trends and challenges. Rome: FAO; 2017. 180 p.

17. Bialek M, Rutkowska J, Adamska A, Bajdalow E. Partial replacement of wheat flour with pumpkin seed flour in muffins offered to children. CyTA – Journal of Food. 2015;14(3):391–398. https://doi.org/10.1080/19476337.2015.1114529

18. Wieser H, Antes S, Seilmeier W. Quantitative determination of gluten protein types in wheat flour by reversed-phase high-performance liquid chromatography. Cereal Chemistry. 1998;75(5):644–650. https://doi.org/10.1094/CCHEM.1998.75.5.644

19. Gojković Cvjetković V, Grujić R, Marjanović-Balaban Ž, Stanojević L, Stanojević JS, Cakić MD. Gliadin analysis by reversed-phase high performance liquid chromatography. Advanced Technologies. 2019;8(2):30–36. https://doi.org/10.5937/savteh1902030G

20. Bhaduri S. A comprehensive study on physical properties of two gluten-free flour fortified muffins. Journal of Food Processing and Technology. 2013;4(7). https://doi.org/10.4172/2157-7110.1000251

21. Kurek MA, Sokolova N. Optimization of bread quality with quinoa flour of different particle size and degree of wheat flour replacement. Food Science and Technology. 2020;40(2):307–314. https://doi.org/10.1590/fst.38318

22. Savanović D, Grujić R. Protein changes during freezing and storage meat and products from meat. Veterinary Journal of the Republic of Srpska. 2017;17(1):105–118.

23. Stevanović J, Borozan S, Božić T, Jovičić S, Đekić T, Dimitrijević B. Oxidative stress. Veterinarski Glasnik. 2012; 66(3–4):273–283.