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Treatment of Anaemia and Malnutrition by Shamy Bread Fortified with Spirulina, Quinoa and Chickpea flour

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Abstract

The present investigation was conducted to promote the nutritional value of Shamy bread by supplementation of wheat flour (WF) with 20% of quinoa flour (QF) or 20% chickpea flour (CF), 0.7% spirulina powder (SP) and other mixture samples (15% CF + 5% QF + 0.70% SP). According to the obtained results, SP recorded the highest value of protein, fat, ash, magnesium, zinc, and iron, which reflected on the produced Shamy bread as final product. Also, QF recorded a higher value of protein, ash, crude fiber, zinc, and iron compared to WF. The results indicated that according to statistical analysis there are evident discrepancies for all treatments concerning protein, ash, and total carbohydrates compared to the control bread sample (100% WF). The replacement of WF by 20% QF led to a decrease in the dough stability while replacement of WF by 20% CF led to highly increase the stability of dough and recorded the highest values of alkaline water retention capacity (AWRC) during all storage periods consequently the same sample recorded the highest value of overall acceptability. Thus, the mixture sample recorded the highest value of protein content with the lowest value total carbohydrate for the produced beard. Keywords: wheat flour, quinoa flour, spirulina, chickpea flour, anemia, malnutrition.

Keywords: Wheat flour; Quinoa flour; Spirulina; Chickpea flour; Anemia; Malnutrition.

1. Introduction

Wheat is considered as the most important crop in Egypt. Wheat represents almost 10 % of the total value of agricultural production and about 20% of all agricultural imports. In spite of a decrease in the share of cereals as a source of food energy, mostly to the benefit of fat and protein-rich foods, wheat remains the main energy source in terms of daily calorie intake in Egypt and bread remains the main staple food in the typical Egyptian diet [1]. Meanwhile, the Egyptian Shamy bread is the staple food consumed by a majority of low-income groups, therefore the iron deficiency and in generally malnutrition was occurred [2].

Accordingly, iron deficiency anemia (IDA) is the most common nutritional disorder in the world and a major problem in developing countries, especially in Egypt. Anemia affects the population and is concentrated in preschool-aged children and women, making it a global health problem [3]. and the prevalence of anemia in adolescents especially females is one of the major public health concerns worldwide especially in adolescent girls is alarmingly high accordingly, food fortification and food supplementation are important alternatives that may help to satisfy the iron needs [4] such as soaking and cooking of legumes with ascorbic acid led to improve the bioavailability of iron and iron deficiency anemia [5] also, Thompson [6] reported the addition of legumes can slightly improve the iron content of cereal- and tuber-based diets. On the other side, worldwide, 165 million children were affected with undernutrition, of which 26% were stunted. This percent reduced by 35% from 253 million in 1990. The prevalence of stunting was 27% in Asia and 36% in Africa. These remain a public health problem, one that

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often goes unrecognized. More than 90% of stunted children in the world have been living in Africa and Asia. An estimated 80% of the world's stunted children lived in just fourteen countries (India, Nigeria, China, Pakistan, Indonesia, Bangladesh, Ethiopia, Democratic Republic of Congo, Philippines, United Republic of Tanzania, Egypt, Kenya, Uganda, and Sudan). Sub-Saharan Africa and South Asia were the home to three-fourths of the world's stunted children, 40% and 39%, respectively [7, 8]. Malnutrition is a major public health problem in the world. Developing countries are highly affected. Asian and Sub-Saharan African countries, including Ethiopia, contribute the highest of all [9].

Anemia is defined as a decrease in the total amount of hemoglobin or the number of red blood cells. Iron deficiency anemia is a form of a normal red blood cell. Iron deficiency anemia is typically caused by inadequate intake of iron, chronic blood loss, or a combination of both [10]. However, Africa has the highest prevalence rates of anemia in preschool children (65.40 %), non-pregnant women (44.70%), and pregnant women (55.0 %). Asia has the highest number of cases of anemia with about half of the world's anemic women living in the Indian Subcontinent, the majority of whom develop anemia during pregnancies. In India, the National Family Health Survey (1998-1999) [11]. Therefore, it is not possible to meet the recommended levels of iron from staple-based diets unless some meat, poultry, or fish is included. However, many potential dietary sources need to be urgently promoted including many leafy vegetables and legumes that contain important quantities of iron, with special emphasis on increasing the consumption of animal products that are high in bioavailable iron and iron absorption enhancers. In Kenya, a study showed that meat intake in children less than 3 years of age was positively related to hemoglobin, suggesting low meat intakes are an important cause of anemia in this age group [12].

Legumes including beans and chickpea (*Cicer arietinum* L.) are important crops because of their nutritional quality. They are rich sources of complex carbohydrates, vitamins, and minerals [13]. Also, legumes have been considered a rich source of protein throughout the world and contain approximately three times more proteins than cereals. Chickpea is one of the top five important legumes on the basis on whole grain production. The world annual of chickpea production is about 14.24 million tones and Egypt produce annually about 4.70 thousand tones [14]. Also, chickpea flour is a good source of proteins, fibers, minerals and other bioactive compounds and it could be an ideal ingredient for improving the nutritional value of bread and bakery products [15].

Thus, quinoa (*Chenopodium quinoa* Willd.) is a plant species of the Chenopodiaceae family, which originated in the Andean region and can adapt to different types of soil and climatic conditions. It is a pseudo grain with high nutritional value as it is rich in proteins, lipids, fiber, vitamins, and minerals [16]. Quinoa also considered as a gluten-free pseudo-cereal that contains a high amount of fiber, high biological-value proteins, essential fatty acids (ω-3 and ω-6), vitamins, and minerals [17], which plays a very important role in improving rheological, technological and sensory properties of baking products, could be used for produce bakery products [18]. On the other hand, spirulina are important for bread supplementation which is one of the blue-green algae rich in protein 61.57% and contains a high proportion of essential amino acids (38.81% of the protein) and a source of naturally rich in vitamins especially vitamin B complex such as vitamin B12 (193 mg / 10 g) and folic acid (9.66 mg / 100 g), which helps the growth and nutrition of the child brain, also rich in calcium and iron it containing (1043.62 and 338.76 mg / 100 g, respectively) to protect against osteoporosis and blood diseases as well as a high percentage of natural fibers [19]. So, the present study is conducted to produce some of the functional foods such as Shamy bread highly in iron content to reduce malnutrition in children, by fortification wheat with different ratios of some natural food sources such as spirulina, chickpeas and quinoa, also, this study aims to evaluate the final product concerning chemical, physical, and biological characteristics.

2. Materials and Methods

2.1. Materials

Wheat flour (72% extraction) was obtained from the North Cairo Flour Mills Company, Egypt. Commercial quinoa seed sample (grown in Egypt in 2017 season) was purchased from the ministry of agriculture in season 2017–2018 and kept at 3-4°C until used in technological studies. Spirulina was purchased from Nor El-Hoda Co., Cairo, Egypt. Chickpea was obtained from the Agriculture Research Center, Giza, Egypt.

2.2. Methods

2.2.1. Preparation of raw materials

Dry cereals and legumes were cleaned from impurities and then washed thoroughly with tap water, the washed cereals and legumes were separately soaked in tap water overnight, except rice was soaked for 30 minutes, according to Soliman et al. [20]. The peeled chickpea was cooked separately in a pressure cooker for 5 to 10 minutes. After cooking, the remaining water was eliminated. After that, the cooked materials were dried in solar dryer at 45- 60ºC. Dried cereals and legumes were milled in the electrical mill and then sieved through a silk sieve (60 mesh) [20]. Quinoa seeds were washed with hot water to remove saponins. The seeds were washed many times with cold or hot water to remove saponins until there was no more foam in the washing water, and they were then dried at 50°C for 6 h. then, quinoa seeds were ground to a fine powder in an electric grinder stainless steel using a Laboratorial disc mill (Quadrumat Junior flour mill, Model Type No: 279002, ©Brabender ® OHG, Duisburg 1979, Germany) and sifted through a 60 mesh, and finally stored at 4OC until used [21].

2.2.2. Chemical composition

Standard Association of Official Analytical Chemistry methods, [22] were adopted for estimating moisture, ash, crude fiber, protein, and fat contents. Total carbohydrates were calculated as: 100 - (protein + fat + moisture + ash).

2.2.3. Minerals determination

Calcium (Ca), zinc (Zn) and iron (Fe) contents were determined using a Pye Unicom SP 19000 atomic absorption spectroscopy in Food Technology Research Institute, Agriculture Research Center, Giza, Egypt as described by AOAC [22].

2.2.4. Rheological tests

The viscoamylograph, Farinograph, and extensograph tests were carried out in laboratories of the Egyptian Baking Technology Center (EBTS), Elahram St., Giza according to the procedure mentioned by the AACC [23].

2.2.5. Pasting properties

The viscoelastic properties of the prepared flours were examined using a Rapid Visco Analyser-4 (Newport Scientific, Australia) in chemitic international laboratory, 6 October City, Giza, Egypt according to the method described in AACC [23].

2.2.6. Falling number test

The falling number of wheat flour samples as an indicator of α-amylase enzyme activity was conducted laboratories of the Egyptian Bread Technology Center (EBTC), Elahram St., Giza according to the standard method of AACC [23].

Falling number was calculated on a 14% moisture basis, by using the following equation:
Liquefaction No. (14% mb) = Falling No. x 100-14/100-Moisture% of sample.

2.2.7. Amino acid analysis

Amino acids content was determined in National Research Center, Doki, Giza, Egypt, where the protein quantification was done with micro-Kjeldahl method. Amino acid analysis procedure involves acid/alkaline hydrolysis, separation by cation exchange column, post-column derivatization with Ninhydrin and detection using UV/Vis detector at 570 nm as described in the Korean Food Code [24]. These procedures in the Korean Food Code were established based on AOAC [22] Official Method 960.52 for micro-chemical determination of nitrogen (micro-Kjeldahl).

2.2.8. Processing of Shamy bread

Shamy bread was prepared as El Guindi et al. [25] with some modification. 100 g of flour was well blended with 1.5g salt and 0.5g dry yeast. Flour samples and all ingredients were mixed in a kneader. Dough was kneaded until reaching adequate consistency (3-5 min). The dough was left to ferment for 30 min at 30°C and 85% relative humidity and then divided to 125 g pieces. The pieces were arranged on a wooden board that had been sprinkled with a thin layer of fine bran and were left to re-ferment for about 30 min at the same temperature and relative humidity. The pieces of fermented dough were flattened to about 20 cm in diameter. The flattened loaves were proofed for 10-15 min at 30-35°C and 85% relative humidity and then baked at 400-450°C for 1-2 min in Egyptian Baking Technology Center, Elahram St., Giza. The loaves of bread were allowed to cool on racks for about 1 h before evaluation.

2.2.9. Organoleptic characteristics of Shamy bread

Shamy bread samples were organoleptically evaluated by ten panelists of Egyptian Baking Technology Center staff for its sensory characteristics: General appearance (10), crust color (20), odor (20), separation of layer (10), taste (20), roundness (10), distribution of crumb (10) and overall acceptability (100) as the method described by Yaseen et al. [26] with some modification.

2.2.10. Color determination

Objective evaluation of surface Shamy bread color was measured [27]. Hunter a*, b* and L* parameters were measured with a color difference meter using a spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with the white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26.

Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16).

2.2.11. Bread staling measurement

Shamy bread loaves freshness was tested after wrapping in Egyptian Baking Technology Center, Elahram St., Giza using polyethylene bags and stored at room temperature (1, 2, and 3 days) using the Alkaline Water Retention Capacity test (AWRC) [28]. AWRC (%) =[(Weight of tube with sample after centrifuge–weight of empty tube)/Weight of sample]x100.

2.2.12. Statistical analysis

The obtained results were statistically analyzed using SPSS statistical package (Version 9.05) according to Rattanathanalerk et al. [29], analysis of variance (ANOVA). Duncan's multiple range test and least significant difference (LSD) was chosen to determine any significant difference among various treatments at probability 5%.

3. Results and discussion

3.1. Approximate composition of raw materials

The chemical composition of raw materials was estimated and the obtained results were presented in Table 1. From the results, it could be noticed that the lowest value of moisture content was observed by chickpea flour (7.60%) followed by 9.10% which recorded by spirulina powder with significant. While no significantly difference between wheat flour and quinoa flour in terms of moisture contents. Concerning the protein content, the lowest value was recorded by wheat flour (11.20%) with significant while, the quinoa flour and chickpea flour recorded the higher values of protein content (20.60 and 24.73%, respectively) compared to wheat flour with significant. In terms of fat contents, the wheat flour recorded the lowest value (1.4%) with significant, on contrary to the fat content of spirulina which significantly recorded the highest value (7.5%), meanwhile, the quinoa and chickpea flour recorded higher values of fat content (6.10% and 5.36% respectively) with no significant between them.

These results are in agreement with those obtained by Gadallah et al. **[30]** they reported that the chickpea flour contained 23.26% crude protein, 6.20% fat, 1.91% ash, 2.45% crude fiber. The obtained results nearly in agreement with those of Sharoba et al. **[31].** They determined the approximate composition of wheat flour 72% extraction. and found that the contents of moisture, protein, fat, ash, crude fiber, and available carbohydrates were 11.99%, 11.85%, 1.06%, 0.52%, 0.54% and 86.04% respectively. Also, these results were in agreement with those of Abou-Zaid et al. **[18]** they determined the chemical composition of crude quinoa and reported that it contained 11.65% moisture, 14.10% protein, 5.90% fat, 3.79% ash, 4.35% crude fiber and 71.86% total carbohydrates. Wheat flour also recorded the lowest value of fiber contents (0.65%) in contrary to the quinoa which recorded the highest value of fiber content (5.70%) followed by 3.91% which recorded by chickpea, while spirulina recorded the lowest value of fiber content (1.70%) with significant between all samples.

However, spirulina powder recorded the highest value of protein and ash contents (59.80% and 8.40% respectively) with significant. Consequently, the spirulina powder recorded the lowest value of carbohydrate content (22.60%) with significant compared to the other samples of raw materials where the wheat flour sample recorded the highest value of carbohydrate content (86.22%) followed by 65.17% which observed by quinoa flour with significant these results were in agreement with those of Dewi et al. [32] they studied the approximate composition of spirulina and reported that it contained 59.16% crude protein and 24.50% carbohydrate content. Also, these results were in agreement with those of Tömösközi et al. [**33**] they studied the chemical composition of wheat flour and reported that it contained 11.54% protein, 0.98% fat and 0.55% ash content.

TABLE 1 Proximate composition of raw materials.

Composition (%)	Wheat flour100%	Quinoa	Chickpea	Spirulina
Moisture	13.3±0.4^a	13.2±0.6^a	7.6±0.7^c	9.1±0.2^b
Ash	0.53±0.01^c	2.43±0.1^b	1.96±0.1^b	8.40±0.1^a
Protein	11.20±0.03^d	20.6±0.4^c	24.73±0.3^b	59.8±0.6^a
Crude Fat	1.4±0.1^c	6.1±0.1^b	5.6±0.1^b	7.5±0.2^a
Crude fiber	0.65±0.04^d	5.7±0.1^a	3.91±0. 1^b	1.70±0.2^c
Carbohydrates	86.22±0.04^a	65.17 ±0.13^b	63.81±0.54^b	22.6±0.7^c

Values with the different letters in the same column were significantly different at $p \leq 0.05$.

3.2. Mineral contents of raw materials

The mineral content of used materials considered as an important factor in reducing malnutrition, and according to the obtained results which presented in Table 2. It could be noticed that the wheat flour appeared as a poor material in its mineral content (Ca, Mg, Mn, Cu, Fe, and Zn) compared to the other used materials. Also, the spirulina recorded the lowest value of potassium content (43.51mg/100g). On the other hand, the spirulina recorded the highest values of magnesium, manganese, iron, and zinc contents. Whilst, the heist values of calcium and potassium contents were observed by chickpea flour (37.46mg/100g and 118.20mg/100g respectively), whereas, the highest value of copper content was recorded by quinoa flour (0.83mg/100g). These results were in agreement with Sharoba [34] according their results obtained during the study which conducted on the nutritional value of spirulina and its use in the preparation of some complimentary baby food formulas. Also, these results have confirmed the results of Hadeer [35] during their study on mineral contents of wheat and quinoa flour.

3.3. Proximate composition of prepared mixtures.

The mixtures were prepared with the replacement of wheat flour of quinoa, chickpeas, and spirulina, and the results were shown in Table 3 and the obtained results indicated that the sample contained 100% wheat flour (WF) recorded the lowest values of ash and crude protein contents with significant (0.52% and 11.20% respectively) and the highest value of carbohydrate content significantly was observed by the same aforementioned sample (86.23%). Consequently, the replacement of wheat flour by different levels of the other used materials led to increasing important nutrients like protein and minerals (ash) where the replacement of wheat flour by 20% of quinoa flour led to a relative increase in ash content about 78.84% and the protein content was a relatively increased about 16.96% and about 138.46% for fiber content. Also, the replacement of WF by 20% of chickpea led to relative increase in ash, protein, fat and crude fiber contents about 59.61%, 24.19%, 60%, and 86.15% respectively. While in the case of replacement of WF by 0.7% spirulina, the protein and ash are considered to be the most important factors for which relative increase occurred (13.46% and 3.21% respectively). Also, the mixture sample which contained 79.30% WF + 0.7% spirulina + 15% chickpea flour + 5% quinoa flour recorded a higher value of ash content and also recorded the highest value of protein content with relative increment rate about 71.15% for ash content and 26.51% for protein content. These results are in agreement with those obtained by Gadallah et al. [30] they studied the chemical composition of composite wheat flour with 20% chickpea and found that it contained 14.87% protein, 2.05% crude fat, 1.25% crude fiber and 80.85% nitrogen-free extract.

TABLE 2. Mineral contents of raw materials (mg/100g)

Minerals (mg/100g)	WF100%	Quinoa	Chickpea	Spirulina	*M2
Ca	4.66	18.95	37.46	15.86	14.11
Mg	7.37	74.26	37. 35	89.75	16.59
K	52.21	88.95	118.2	43.51	83.35
Mn	0.27	0.56	1.4	4.83	0.47
Cu	0.26	0.83	0.45	0.51	0.28
Fe	0.44	4.13	2.14	34.92	1.23
Zn	0.45	2.19	0.83	12.16	0.88

WF =Wheat flour, *M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%)

TABLE 3. Proximate composition of prepared mixtures.

Composition (%)	WF 100%	WF-Q (80/20)	WF-C (80/20)	WF-S (99.3/0.7)	M
Moisture	13.34±0.04^{a}	13.34±0.01^{a}	12.205±0.01^{c}	13.42±0.01^{a}	13.04±0.00^{b}
Ash	0.52±0.01^{e}	0.93±0.01^{a}	0.83±0.01^{c}	0.59±0.00^{d}	0.89±0.00^{b}
Protein	11.20±0.03^{d}	13.10±0.05^{b}	13.91± 0.01^{a}	11.56±0.02^{c}	14.17±0.14^{a}
Crude Fat	1.40±0.01^{c}	2.34±0.01^{a}	2.24±0.04^{b}	1.41±0.01^{c}	2.32±0.01a^{b}
Crude fiber	0.65±0.01^{d}	1.55±0.02^{a}	1.21±0.03^{c}	0.66±0.02^{d}	1.40±0.03^{b}
Carbohydrate	86.23±0.10^{a}	82.08±0.03^{c}	81.81±0.05^{d}	85.86±0.06^{b}	81.22±0.02^{e}

Values with the different letters in the same column were significantly different at $p \leq 0.05$.
* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina
*M= Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%)

3.4. Rheological properties of wheat flour and used mixture

Table 4 presented farinograph and extensograph parameters of wheat flour and their mixture with other components of quinoa flour, chickpea flour, and spirulina powder. The results presented in the same Table indicated that, as for farinograph parameters, wheat flour recorded 64% water absorption and the replacement of wheat flour by 20% quinoa flour led to increasing the percentage of WA to 67.0%, and the highest value of WA was recorded by the sample contained 20% chickpea flour (75.0%). Flour sample containing chickpea observed more water absorption due to an increase in protein content of composite. Enhanced protein content results, also may be due to an increase in pentosans, especially ribose and deoxyribose which has a higher water holding capacity **[36]**. And concerning to the arrival time all sample recorded 1min with except the sample contained 79.30% WF + 0.7% spirulina + 15% chickpea flour + 5% quinoa flour (mixture sample) recorded the highest value of arrival time (1.5min). Meanwhile, the stability of tested samples was recorded highly differences in dough stability values where the control sample (100% WF) recorded 5min of stability which decreased to 1.5min after the replacement of WF by 20% quinoa flour, however, the replacement of wheat flour by 0.7% spirulina led to increasing the stability to 6.0min, while the stability was decreased to 4.0min with the replacement of wheat flour by 20% chickpea. These results are in agreement with those by Salim et al. [37]; El-Sherief [38] and Abdelghafor et al. [39]. They reported the low dough stability of the flour blends might be due to the higher fiber content which destroyed the gluten matrix [40]. The same trend also was noticed by the addition of whole chickpea flour. However, these results were not agreed with Paraskevopoulou et al. [41] they studied the effect of lupine protein isolates as a high protein material and reported that the stability of dough was an increase by supplementation of wheat flour with lupine, and the results obtained are discussed in terms of a possible action of LPI particles as a filler of the gluten network and partly in terms of possible interactions that take place between the gluten protein constituents and those of lupine.

TABLE 4. Farinograph and Extensograph parameters

Parameters	WF 100%	WF-Q 80/20%	WF- C 80/20%	WF-S 99.3/0.7%	M
WA (%)	64.0	67.0	75.0	64.8	73.5
AT (min)	1.0	1.0	1.0	1.0	1.5
DDT (min)	1.5	2.0	1.0	1.5	5.0
DS (min)	5.0	1.5	4.0	6.0	3.0
MTI(BU)	50.0	75.0	70.0	60	40.0
DW(BU)	60.0	85.0	80.0	75	85
Ex. (mm)	160.0	140	130.0	165.0	140.0
R(BU)	770.0	465	380.0	720.0	355.0
(R/E)	3.43	2.28	2.46	2.70	2.10
DE (cm^2)	126.0	81.0	65.0	145.0	63.0

* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina, *M = Mixture (wheat flour 79.30%-Quinoa 5%-Chickpea 15% –Spirulina 0.7%). WA= Water absorption, AT= Arrival time, DW= Dough weakening, E= Extensibility, R= Resistance to extension, DE= Dough energy, DDT= Dough development time, DS= Dough stability.

Concerning the mixing tolerance index and dough weakening the highest values were investigated by the sample contained 20% quinoa flour followed by the sample contained 20% chickpea (75 BU and 70 BU respectively) with contrast to the sample contained the control sample (60 BU). In the same pattern dough weakening (DW) the sample contained 20% quinoa flour recorded the highest value of DW (85 BU) in contrast to 60 BU which observed by the sample contained 100% wheat flour. The same Table showed extensograph parameters and the results concluded that the highest value of extensibility was recorded by the sample contained 20% chickpea flour (165mm) followed by 160mm which observed by the control sample. While the highest value of resistance to extension (R) was observed by the control sample (770BU) followed by 720BU which recorded by the sample contained 20%chickpea flour, while the other samples recorded more decrement ratios compared to the two aforementioned samples ranged from 465BU to 355 BU. Also, the control sample recorded the highest value (3.43) of proportional number (R/E) while the mixture sample recorded the lowest value for the same parameter (2.10). These results are in agreement with those found by Aly *et al.* [42] & Hegazy and Faheid [43] they reported that the addition of chickpea flour at different ratios to the wheat flour caused a decrease in the resistance to extension of the dough. In contrast, by increasing the different levels of the chickpea flour, the resistance to extension of the dough decreased as the result of increasing their fiber content that destroyed the gluten matrix in the dough, regardless of their higher content of protein as compared to the control sample **[40]**.

However, the sample contained 20% chickpea flour recorded the highest value of dough energy (DE) in contrast to the mixture sample which recorded the lowest value of the same parameter ($63cm^2$). These results could be explained by that the less formation of gluten. Similar results were observed by Hamaker [44] and Sulieman et al. [45], they reported that the fixed gluten amounts with increasing chickpea flour substitution level, dough strength, and extensibility decreased significantly. Also, Gadallah et al. [30] reported results from this study concluded that the substitution of wheat flour with chickpea flour caused significant increases in crude protein, lipids, fiber, and ash contents. Water absorption of wheat flour was gradually increased with the increased level of chickpea flour. The substitution level which improved the extensibility and extensibility at maximum elasticity compared with the control sample was 15% of chickpea flour. Replacement of wheat flour with chickpea flour gave the highest amounts of gas rather than those of sorghum flour. However, these results did not agree with those of Harra et al. [46] mentioned that, in addition to augmenting the nutritional value, the addition of quinoa flour has shown positive effects on the rheological and sensory characteristics of bakery products, such bread, and cookies.

3.5. Pasting properties of wheat flour and used mixtures

Pasting properties of wheat flour and the replacement samples presented in Table 5 and from the results it could be observed that, the control sample recorded the lowest values concerning peak viscosity

Table 5. Pasting properties, falling number, and damaged starch of wheat flour and their mixtures

Parameters	WF 100%	WF- Q 80/20%	WF- C80/20%	WF- S 99.3/0.7%	M
Peak1	1795±8.08^{b}	2170±2.60^{a}	1907±3.28^{d}	2632±2.08^{c}	2251±3.18^{e}
Trough	1020±1.15^{b}	1184±1.76^{a}	1115±2.91^{d}	1551±1.45^{c}	1283±3.53^{e}
Breakdown	781±2.19^{c}	986±0.58^{a}	791±2.19^{d}	1074±2.65^{b}	966±2.03^{e}
Final Visc	1918±1.20^{b}	2274±2.33^{a}	2035±2.08^{d}	2816±2.08^{c}	2365±2.33^{e}
Setback	898±0.88^{c}	1092±1.53^{a}	921±0.88^{d}	1258±1.53^{b}	1077±1.45^{e}
Peak Time	5.93±0.02^{a}	6.09±0.01^{a}	5.87±0.01^{c}	6.20±0.06^{ab}	6.15±0.08^{bc}
Pasting Temp	69.46±0.05^{ab}	67.75±0.03^{ab}	68.43±0.27^{bc}	68.77±0.15^{c}	68.95±0.22^{a}
F.N. (sec)	347.80±2.13 ^{d}	465±2.64 ^{a}	428±7.22 ^{b}	374.40±7.22 ^{c}	383±1.63 ^{c}
Falling time	405±2.64^{a}	368±7.22^{b}	314±7.22^{c}	323±1.63^{c}	287±2.13^{d}
L.N	14.44±0.09^{a}	15.88±0.32^{b}	18.53±0.44^{b}	18±0.09^{c}	20.15±0.14^{d}
S.D (AACC)	5.28±0.01^{d}	5.73±0.01^{c}	6.40±0.01^{a}	6.14±0.01^{b}	5.70±0.05^{c}

Values with the different letter in the same column were significantly different at $p \leq 0.05$.
* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina
*M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%)
*L.N. = liquefaction number
S.D = starch damage

(1795cp), trough (1020cp), breakdown (781cp), final viscosity (1918cp) and setback (898cp) with significant differences with the other samples concerning all the aforementioned parameters, meanwhile the replacement of wheat flour by 20% of all quinoa and chickpea flour led to increasing all the previous parameters where the peak viscosity recorded (2170cp and 1907cp, respectively) and (1092cp and 921cp, respectively) for setback. On other hand, the sample contained 0.7% spirulina led to more increase peak viscosity (2632cp), trough (1551cp), breakdown (1074cp), final viscosity (2816cp) and setback (1258cp) with significant compared to the other samples. However, no significant differences between the control sample and the samples contained 20% quinoa flour and 20% of the sample contained chickpea flour in terms of pasting temperatures. Also, the same Table showed falling number for all samples where the sample contained 20% quinoa flour recorded the highest value (465sec.) followed by 428sec. for the sample contained 20% chickpea flour with significant between them. While, the lowest value 347sec. was recorded by control sample.

However, no significant differences between the samples contained 20% chickpea flour and the sample contained 0.7% spirulina in terms of falling time, while the mixture sample recorded the lowest value of falling time (287) with significant. As for the damaged starch the highest percentage was observed by the sample contained 20% chickpea flour (6.40%) followed by 6.14% which recorded by the sample contained 0.7% spirulina with significant. However, no significant differences between the control sample and the samples contained 20% quinoa flour and the mixture sample in terms of the percent of damaged starch (5.73% and 5.70% respectively). Changes in viscosity during the breakdown indicate the paste stability and changes accurately during setback, it might show the consistency of gel and viscosity of both type of starch increases during cooling time that may have occurred due to aggregation of amylose molecules [47]. Also, Ahmed et al. [48] explained pasting properties have a significant influence on the composition of starch and amylose content; they found lower pasting parameters due to lower amylose content in starch.

3.6. Color measurement of Shamy bread

Color considered as an important factor of bread quality, Table 6 presented the color parameters (L*, a*, and b*) and the results indicated that the control bread sample was more lightness and significantly recorded the highest value of L* parameter (65.27) followed by 63.92 which recorded by the bread sample contained 0.7% spirulina, whilst, the mixture bread sample significantly recorded the lowest value of L* (more darkness). Also, the results indicated that the bread sample contained 20% quinoa was darker than the bread sample contained 20% chickpea flour with significant. These results

TABLE 6. Hunter color of Shamy bread

Parameters	WF 100%	WF- Q 80/20%	WF- C 80/20%	WF-S 99.3/0.7%	M
L*	65.27±0.01[a]	60.14±0.02[d]	62.08±0.01[c]	63.92±0.02[b]	58.43±0.02[e]
a*	11.19±0.02[a]	8.17±0.01[b]	9.15±0.01[c]	2.05±0.02[d]	1.56±0.01[e]
b*	24.15±0.04[d]	27.03±0.01[c]	35.25±0.04[a]	19.42±0.02[e]	28.16±0.04[b]

Values with the different letters in the same column were significantly different at $p \leq 0.05$.
* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina
*M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%)

confirmed the results of Thejasri et al. [49] they reported that the addition of quinoa flour to wheat flour led to a decrease in the lightness of biscuit samples and they approved that to the higher value of protein in quinoa flour compared to wheat flour. According to the obtained results presented in the same Table a* values (redness) of bread, samples showed a significant difference between all bread samples where, the control bread sample was more redness than the other samples recorded the highest value (11.19) followed by 9.15 which recorded by the bread sample contained 20% chickpea flour, and 8.17 for the bread sample contained 20% quinoa flour, while the mixture bread sample was darker compared to the other bread sample (1.56). In terms of b* values (yellowness) the bread sample contained 20% chickpea flour recorded. These results were agreed with Codină et al. [50] they reported that the addition of quinoa flour to wheat flour led to decrease L* value (more darkness) and decrease the redness while the b* value was increased. Also, Hemeda et al. [51] studied the effect of chickpea addition to wheat flour and they reported that, the addition of chickpea led to increase the darkness of bread and decrease a* and b* values compared to the control bread which consists from 100% wheat flour 72% extraction.

3.7. Approximate composition of Shamy bread

The chemical composition of Shamy bread was estimated and the results were presented in Table 7. From the results in the same Table it could be observed that, in terms of moisture content the bread sample contained 20% quinoa recorded the highest value (34.18%) with significant followed by 33.28% which investigated by the bread sample contained mixture of flour, while the lowest two values (31.63% and 31.77%) were recorded by the control bread sample and the sample contained 0.7% spirulina respectively with no significant between them and with significant with the other samples. As for ash content, the lowest value was obtained by the control bread sample, consequently, all replacement samples recorded higher values of ash content compared to the control sample with significant where, the highest value of ash content (2.01%) significantly was recorded by the bread sample contained 20% quinoa flour. Concerning protein content, also the lowest value (12.92%) was investigated by the control bread sample with significant, while the bread samples contained 20% chickpea and the bread sample contained mixture of flour recorded the highest two values of protein content (15.71% and 15.77% respectively) with no significant between them and with significant differences compared to the other bread samples. On the other hand, the control bread sample recorded the lowest values in terms of crude fat and crude fiber contents (1.41% and 0.71% respectively) with no significant only compared with the bread sample contained 0.7% spirulina for the same aforementioned parameters (1.45% and 0.74% respectively). While, the control bread sample significantly recorded the highest value of carbohydrate content (83.38%) whilst, the lowest two values of carbohydrate contents were investigated by the bread sample contained 20% quinoa flour and the bread sample contained 20% chickpea flour (78.72% and 78.71% respectively) with no significant between them and with significant difference compared to the other samples.

Table 7. Proximate composition of Shamy bread

3.8. Mineral contents of Shamy bread

Mineral content (Ca, Mg, K, Mn, Cu, Fe, and Zn) of Shamy bread samples were summarized in Table 8 and also illustrated in Fig. 1 as a relative increase in mineral contents after replacement of wheat flour by used materials. From the results it could be noticed that the mineral contents of wheat flour were the lowest content for all study items elements, consequently any replacement level of any used materials led to increasing the mineral content, where

Samples	WF 100%	WF-Q (80/20)	WF-C (80/20)	WF-S (99.3/0.7)	M
Moisture	31.63±0.02[d]	32.19±0.12[c]	34.18±0.05[a]	31.77±0.05[d]	33.28±0.01[b]
Ash	1.58±0.01[e]	2.01±0.00[a]	1.88±0.01[c]	1.64±0.01[d]	1.97±0.01[b]
Protein	12.92±0.01[d]	15.05±0.01[b]	15.71±0.01[a]	13.30±0.01[c]	15.77±0.01[a]
Crude Fat	1.41±0.00[c]	2.42±0.03[a]	2.30±0.02[b]	1.45±0.01[c]	2.40±0.01[a]
Crude fiber	0.71±0.01[d]	1.80±0.02[a]	1.43±0.02[c]	0.74±0.01[d]	1.51±0.01[b]
Carbohydrates	83.38±0.04[a]	78.72±0.03[c]	78.71±0.04[c]	82.87±0.01[b]	78.35±0.05[d]

Values with the different letters in the same column were significantly different at $p \leq 0.05$.
* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina
*M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%).

Table 8. Mineral contents of Shamy bread (mg/100g)

*Sample	WF 100%	WF- Q 80/20%	WF- C 80/20%	WF-S 99.3/0.7%	M
Ca	7.41	8.82	12.52	9.87	10.35
Mg	8.56	20.18	15.18	9.15	17.42
K	33.53	75.1	74	66.52	71.93
Mn	0.27	0.35	0.49	0.31	0.39
Cu	0.32	0.34	0.45	0.36	0.39
Fe	1.07	1.71	1.41	1.31	1.68
Zn	0.74	1.02	1.1	0.76	1.75

* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina
*M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%)

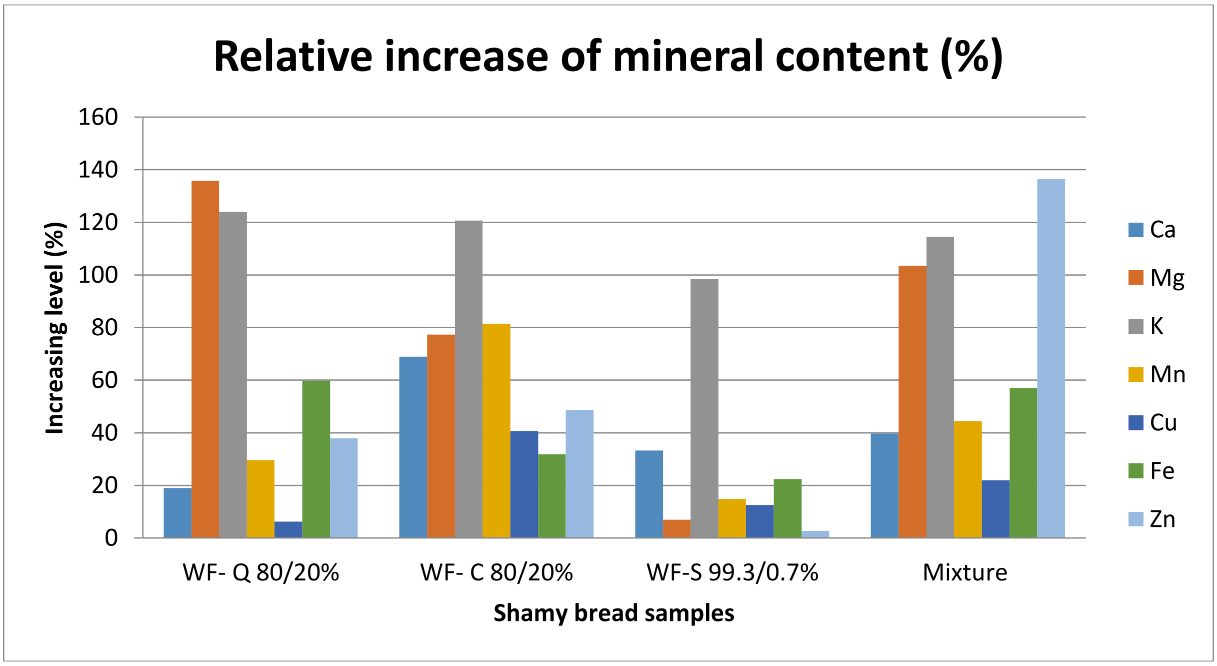

Fig. 1 Relative increase of mineral content for supplemented Shamy bread samples compared to control Shamy bread sample (100% wheat flour)

the replacement of wheat flour by 20% quinoa flour led to increase the calcium content from 7.41mg/100g to 8.82mg/100g, while the same sample recorded the highest values of magnesium, potassium and iron (20.18, 75.10 and 1.71mg/100g respectively). However, the highest value of calcium, manganese, and copper contents was observed by the sample contained 20% chickpea flour (12.52, 0.49, and 0.45mg/100g respectively). Whereas, the mixture sample recorded the highest value of zinc content (1.75mg/100g) compared to 0.74mg/100g which investigated by the control sample.

3.9. Alkaline water retention capacity and decreasing rate of Shamy bread

The bread staling test was estimated and the results were presented in Tables 9 and 10 according to the results showed in Table 9 it could be concluded that the alkaline water retention capacity of Shamy bread sample containing 20% of chickpea significantly recorded the highest values (376, 333, 312, and 300) during zero time, 24h, 48h and 72h respectively. In contrast to the sample containing 20% quinoa flour which recorded the lowest value of AWRC during all storage periods with significant. The results also indicated that no significant differences were observed between the control sample and the sample consist of 99.3% wheat flour + 0.7% in terms of AWRC during zero time, 48h and 72h.

Consequently, (Table 10) the lowest values of rate of decrease (RD%) were observed by the sample containing 20% chickpea flour with significant during all storage periods, also no significant differences between the control sample, the sample consists from 0.7% spirulina + 99.70% wheat flour and the mixture sample (WF 79.3%+QF 5%+CF 15 %+ SP 0.7%) after 48h and 72h of storage. And these results were in a harmony with Külen et al. [52] they reported that high protein contents led to an increase in the alkaline water retention capacity of the supplemented bead.

3.10. Amino acid composition of Shamy bread samples

Nutritional quality of protein depends on its essential amino acids, and of course, the quality of plant protein is lower compared to the animal protein. However, some cereals protein was used in human nutrition and it is possible to improve their protein efficiency ratio (PER) by amino acid supplementation **[53]**. Composite flour in bread making took place during recent years to improve the nutritional value of diets such as improving the balance of essential amino acid and carbohydrate contents. Supplementation of wheat flour with legume flours has great potential in developing countries for improving the nutritional value of different baked products **[45]**.

Since legumes are important sources of carbohydrate, protein, high quality of dietary fiber, several B. complex vitamins, and minerals, they can be used for fortification of various products. Legume proteins alone are poor quality, sulfur-containing amino acids are the first limiting amino acid in legumes and lysine is limited in cereals. As legume and cereal are consumed together, a complementary the effect is obtained **[54]**. Table 11 presented the amino acids composition (mg/100g) of Shamy bread samples and the results demonstrated that concerning the essential amino acids the highest values of valine (6.29g/100g) was recorded by control bread sample (100% wheat flour) followed by 6.26g/100g which recorded by the sample contained 0.70% spirulina, while the bread sample which contained 20% quinoa recorded the highest value of threonine (10.15g/100g) followed by 8.99g/100g which observed by the mixture bread sample. Meanwhile, the sample contained 20% chickpea flour recorded the highest value of isoleucine (4.02g/100g) in contrast to the sample contained 20% quinoa flour which recorded the lowest value of isoleucine, whereas the highest value of leucine was investigated by the control sample, also the control sample recorded the highest value of phenylalanine (6.33mg/100g), methionine 5.12g/100g and cysteine 1.82g/100g.

However, the sample contained 20% quinoa flour recorded the highest value of histidine (3.62g/100g, whilst the highest values of tyrosine and lysine were recorded by the sample contained 20% chickpea flour (3.05 and 6.39g/100g respectively). As for the non-essential amino acids, the highest values of aspartic acid, serine, glutamic acid, and arginine were recorded by the sample contained 20% chickpea flour (7.38, 4.32, 13.57 and 8.73g/100g respectively). In contrast to the sample contained 20% quinoa flour which recorded the lowest values of aspartic acid and serine. However, the control sample recorded the highest values of proline, glycine, and tryptophan. While the sample contained 0.70% spirulina recorded the highest value of alanine (4.94g/100g). These results have confirmed the results of Sharoba [30]

TABLE 9. Alkaline water retention capacity of Shamy bread

Samples	Alkaline water retention capacity			
	Zero time	After 24 h.	After 48 h.	After 72 h.
WF 100%	350c±3.15	300d±2.25	273c±7.11	266c±8.24
WF-Q 80/20%	300d±3.21	244e±2.63	221d±11.24	207d±10.26
WF-C (80/20%)	376a+4.85	333a±4.50	312a±9.25	300a±7.36
WF-S (99.3/0.7%)	355c±2.95	306c±3.41	277bc±8.74	273bc±6.25
M	366b±3.11	316b±2.28	286b±11.08	278b±6.75

Values with the different letters in the same column were significantly different at $p \leq 0.05$.

TABLE 10. Decreasing rate of Shamy bread

Samples	Rate of decrease (%)		
	After 24 h.	After 48 h.	After 72 h.
WF 100%	14.14b±0.725	22.00b±2.01	24.00b±2.22
WF-Q (80/20%)	18.49a±0.360	26.33a±1.97	31.00a±2.15
WF-C (80/20%)	11.30d±0.524	16.88c±1.85	20.08c±1.55
WF-S (99.3/0.7%)	13.80bc±2.14	21.97b±2.11	22.95b±1.88
M	13.66c±0.314	21.72b±2.36	24.04b±1.36

Values with the different letters in the same column were significantly different at $p \leq 0.05$.

TABLE 11. Amino acid composition of Shamy bread (mg/100g)

Amino acid	Samples				
	WF (100%)	WF- Q (80/20%)	WF- C (80/20%)	WF- S (99.3/0.7%)	*M
			Essential amino acids (EAA)		
Valine	6.29	5.62	5.65	6.26	5.63
Threonine	5.94	10.15	5.54	5.93	8.99
Isoleucine	3.94	3.42	4.02	3.96	3.58
Leucine	7.57	6.75	7.56	7.58	6.97
Tyrosine	2.81	2.95	3.05	2.82	2.99
Phenylalanine	6.32	5.86	6.29	6.33	5.96
Histidine	3.52	3.62	3.44	3.52	3.56
Lysine	6.13	5.73	6.39	6.12	5.89
Methionine	5.12	4.99	4.34	5.11	4.81
Cysteine	1.82	1.65	1.7	1.81	1.66
			Non- essential amino acids (NEAA)		
Aspartic acid	6.49	6.51	7.38	6.52	6.75
Serine	4.12	3.92	4.32	4.12	4.03
Glutamic acid	12.95	13.03	13.57	12.95	13.18
Proline	6.03	5.62	5.58	6.02	5.6
Glycine	5.92	5.63	5.82	5.92	5.68
Alanine	4.92	4.93	4.79	4.94	4.92
Arginine	8.11	7.58	8.73	8.11	7.87
Tryptophan	2.01	2.01	1.85	2.01	1.97

* WF = wheat flour -Q = Quinoa- C = Chickpea – S = Spirulina

*M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%).

concerning the amino acid content of wheat flour and spirulina.

3.11. Organoleptic characteristics of Shamy bread samples

Shamy bread was tested for sensory characteristics and results are shown in Table 12 and from the results it could be concluded that, no significant differences between the control bread sample, bread sample contained 20% chickpea flour and mixture bread sample in terms of the odor and general appearance characteristics, also the bread samples contained 20% quinoa and the samples contained 0.70% spirulina recorded lower values of odor test with significant compared to the other values and with no significant between them. As for the test and separation of layer characteristics, the sample contained 20% quinoa flour significantly recorded the lowest value of taste and separation of layer (15.60and8.20 respectively) while the other samples did not record any significant difference concerning taste according to the statistical analysis. However, the sample contained 0.70% spirulina recorded the lowest value of crust color (14.0) followed by 15.80 which recorded by the sample contained 20% quinoa with no significant, meanwhile, all samples did not record any significant differences concerning the roundness and distribution of crumb of bread. Generally, the sample contained 20% chickpea flour significantly recorded the highest value of overall acceptability (91.60).

TABLE 12. Organoleptic evaluation of Shamy bread

Sensory characteristics	Samples				
	WF 100%	WF- Q 80/20%	WF- C 80/20%	WF-S 99.3/0.7%	M
Odor (20)	17.20±0.73^{ab}	15.80±0.86^{b}	19.20±0.37^{a}	15.20±0.37^{b}	18.60±0.51^{a}
Taste (20)	18.20±0.66^{ab}	15.60±0.81^{b}	19.20±0.37^{a}	17.20±0.58^{ab}	19.20±0.58^{a}
Crust color (20)	19±0.63^{a}	15.80±0.58^{bc}	19.60±0.24^{a}	14±1.22^{c}	15.40±0.68^{ab}
Separation layers (10)	9.80±0.37^{a}	8.20±0.73^{b}	9.60±0.40^{a}	9.20±0.37^{a}	9.20±0.49^{a}
Roundness (10)	9.20±0.20^{a}	8.60±0.24^{a}	9.40±0.24^{a}	9.60±0.24^{a}	9.40±0.24^{a}
Distribution (10)	8.40±0.24^{a}	7.60±0.24^{a}	8.20±0.37^{a}	8.20±0.37^{a}	8.40±0.40^{a}
General appearance (10)	9.80±1.98^{a}	7.60±2.38^{c}	9.20±0.80^{a}	8.40±1.81^{b}	9.20±1.50^{a}
Overall acceptability (100)	91.6±2.00^{b}	82.2±2.50^{c}	94.4±1.00^{a}	81.8±2.00^{c}	89.4±1.5^{b}

* WF = wheat -Q = Quinoa- C = Chickpea – S = Spirulina.
*M = Mixture (wheat flour 79.30%-Quinoa 5%- Chickpea 15% –Spirulina 0.7%) .

3. Conclusion

Anemia and malnutrition, in general are among the most important problems that need to be addressed, especially by using natural materials, and since chickpeas, quinoa and spirulina are high in the percentages of mineral elements, especially iron and zinc, so each of them was used to reduce such diseases, and the results of this study indicated through chemical analysis the nutritional value of the products supplemented with these aforementioned substances were higher in terms of protein, iron, zinc, and potassium contents compared to the control sample, which contributes to reducing anemia.

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