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Chemical, Rheological, Sensorial and Microbial Evaluation of Supplemented Wheat Flour Biscuit with Guava Seeds Powder

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ABSTRACT

In this present research, biscuit was prepared with the addition of guava seeds powder at the ratio of 5, 10 and 15 %. Chemical, rheological, sensorial and microbial properties were evaluated for different biscuit samples. Addition of guava seeds improved the functional of biscuits which prepared from it. Incorporation of guava seeds powder in biscuit dough revealed an increase in dough stability, Elasticity, P.N and energy (cm^2) values and a decrease in water absorption and degree of softening values. Result of sensory evaluation of biscuits samples which prepared from guava seeds powder showed non-significant differences at ($P \leq 0.05$) between all samples of biscuits formulas. Addition guava seeds powder to biscuits samples caused an increase in total essential amino acids from 50.1 to 57.0, 64.7 and73.9 mg/g while total amino acids represented 65.3, 68.4, 73.4 and 80.5 mg/g in biscuits samples supplemented with 0, 5, 10, 15 % guava seeds powder, respectively. Supplementation of guava seeds powder with the ratio of 5, 10 and 15 % to biscuits formulas reduced microbial counts. It concluded that the additions of guava seeds powder to wheat flour based biscuit improve its nutritional and rheological functions properties.

Keywords: wheat flour, rheological properties, guava seeds, biscuit processing, amino acids, microbial load.

INTRODUCTION

Bakery products namely bread, cakes and biscuits are played role in human nutrition which was consumed daily. Biscuits (Crackers and cookies) are one of the most bakery products and the major ingredient is wheat flour which provides structure and bulk (Lai and Lin, 2006). In recent years biscuits prepared with many different nutritional level, flavors, texture, odor and taste (Lara, *et al.,* 2011; Patrignani, *et al.,* 2016; Huang *et al.,* 2018). Many efforts have been studied to substitute many essential ingredients of biscuits with nutrients and healthy substances (Jisha, *et al.,* 2009; Koffi *et al.,* 2013; Patel and Pradhan, 2015). Seed, peel, and pulp leftovers of guava represented up to 30% of their fruit as healthier processed food by-products due to the presence of bioactive compounds and their sensory characteristics (Lima *et al.,* 2019). Moreover Bernardino-Nicanor *et al.,* (2006) discarded guava seeds represent 12 g/100 g of their fruit weight and use a source of protein and oil for animal and human feeding (Bernardino-Nicanor *et al.,* (2001). Guava seeds considered as a waste in food industries and the seeds contained high amount of lignocellulosic compounds, globulins, gluterlins, hemicellulose, cellulose, lignin and hemicelluloses (Elizalde-González and Hernández-Montoya 2009 ; Anisuzzaman *et al.,* 2016). Therefore Bernardino-Nicanor *et al.,* (2006) mentioned that the proximate analysis of the guava seeds on a dry weight basis was72.1±0.1 g/100 g, 12.5±0.5 g/ 100 g, 7.2±0.1 g/100 g, 6.8±0.1 g/100 g and 1.50±0.05 g/100 g for raw fiber, lipids, proteins, carbohydrates and ashes, respectively. Guava seed proteins consists of peptide which contains the glycine-rich protein group and it can used as antimicrobial of gastrointestinal infections in human due to their action to inhibit the growth of gram-negative bacteria and this peptide used to delaying the bacterial resistance process as new technic to treatment of infections caused by *Klebsiella sp.* and *Proteus sp.* (Pelegrini and Franco 2011). Guava seeds contain the glycine-rich proteins which have activity against human pathogenic such as *Proteus sp., Pseudomonas aeruginosa, Escherichia coli and Klebsiella sp.* (Gram-negative bacteria) and Gram-positive bacteria such as *Staphylococcus aureus and Staphylococcus epidermides*), these peptides use as a biotechnological tool and antimicrobial peptides for infectious disease treatment, The action mechanism of these peptides involve permeability alterations, ion imbalance and lipid bilayer depolarization that may induce electrostatic interactions which case membrane disruption , additional these peptides casing modifying enzyme activities, alteration of several gene expressions and improving protein synthesis (Sachetto-Martins, Franco and de Oliveira 2000 ; Tanaka 2001; Pelegrini *et al.,* 2008 ; Lu and Chen 2010 ; Tavares *et al.,* 2012)

This study aims to evaluate the rheological properties namely farinograph and extinsograph) of wheat flour supplemented with guava seeds powder as well as effect additional of guava seeds powder on dough and final biscuit properties. Moreover examine the total bacterial count, spore-forming bacteria and total molds and yeasts counts for biscuit during storage at room temperature (25 ± 3).

MATERIALS AND METHODS

Materials

Wheat flour (*Triticum sativum*) and guava seeds which separated from guava fruits were purchased from a market in Benha city in the Kalyobiya governorate, Egypt. Chemicals were purchased from Sigma-Aldrich Company.

Methods

1. Guava seeds powder preparation

Guava seeds were separated from the pulp, cleaned with tape water and then dried at 45 ℃ using Binder oven. Grinding was performed using a knife mill (Moulinex 85g stainless steel, made in France), and the powder obtained passed through a sieve and stored in polyethylene bags at 4°C until use for further formulation of biscuit.

2. Biscuit preparation

The biscuit were prepared according to the method described by Uchoa *et al.,* (2009) and Sharoba *et al.* (2014) with some modifications. The biscuit were prepared as mentioned in table (1) by the replacement of wheat flour with 5, 10, and 15% guava seeds powder. Wheat biscuit sample were prepared without any addition of guava seeds powder and used as control one. Prepared biscuit formulas were baked on an electric oven at 170°C for 15 min. the biscuits samples were allowed to cool for 30 min and stored in polypropylene bags at room temperature (25±3°C).

3. Rheological properties of flour

Farinograph were obtained by brabender farinograph as showed by AOAC (2000) farinograph method for flour (Absorption, dough development time (DDT) and stability) were calculated while the extensibility, (P.N) and energy values were determined using extinsograph, at Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt.

Table 1. Ingredients used for biscuit preparation

Ingredients (g)	Substitutions level			
	Control	5%	10%	15%
Wheat flour	112.5	106.875	101.25	95.625
Butter	30	30	30	30
Sugar	60	60	60	60
Distilled water	20	20	20	20
Salt	1.05	1.05	1.05	1.05
Guava seeds powder	-	5.625	11.25	16.875

4. Gross chemical composition

Moisture, lipid, protein, crude fibers and ash contents were determined according to official method (AOAC 2000). Total carbohydrates were calculated by the differences as mentioned by Egan *et al.,* (1981) using the following equation:

Total carbohydrates % = 100 – (Moisture % + Crude protein % + Total lipids % + Ash %+ Crude fibers %)

5. Determination of the amino acids

Amino acids contents were determined according to the method described by Pellet and Young, (1980) at National Center for Radiation research and Technology (N.C.R.R.T), Atomic Energy Authority, Nasr City, Cairo Egypt.

6. Sensory evaluation of prepared biscuits

Sensory evaluation of prepared biscuits samples were examined and panel tests consisted of ten members from our laboratory, including appearance, odor, texture, color and taste and scores were obtained as described by Sharoba *et al.* (2014) and Patel et al. (2019)

7. Microbial determination

Total bacterial count were counted by plating on plate count agar medium and incubated at 30°C for 3-5 days as mentioned by APHA, (1992) while total molds and yeasts were counted according to Oxoid, (1998). Spore-forming bacteria count determined according to FDA, (2002).

8. Statistical analysis

The statistical analysis was calculated as described by Bezerra *et al.,* (2008), using Excel to analyze of variance (ANOVA) by test at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Gross chemical composition of guava seeds powder

The percent composition of the guava seeds on a dry weight basis was determined and the major component was crude fiber (69.42 %), followed by total lipids (14.32%), crude protein (9.15 %), total carbohydrates (4.10 %) and ash (3.01 %). Nearly the same results were reported by Bernardino-Nicanor *et al.,* (2006) who mentioned that the proximate analysis of the guava seeds on a dry weight basis was72.1±0.1 g/100 g, 12.5±0.5 g/ 100 g, 7.2±0.1 g/100 g, 6.8±0.1 g/100 g and 1.50±0.05 g/100 g for crude fiber, lipids, proteins, carbohydrates and ashes, respectively.

Table 2. Chemical properties of guava seeds powder used in biscuit preparing

Properties	%
Moisture	4.76 ± 0.3
Total lipids*	14.32 ± 0.1
Crude protein*	9.15 ± 0.2
Ash*	3.01 ± 0.4
Crude Fiber*	69.42 ± 0.3
Total Carbohydrates*	4.10

Values in table indicate the mean and standard deviation of three replicates
***% on dry weight basis**

Effect of guava seed flour on rheological properties of wheat flour

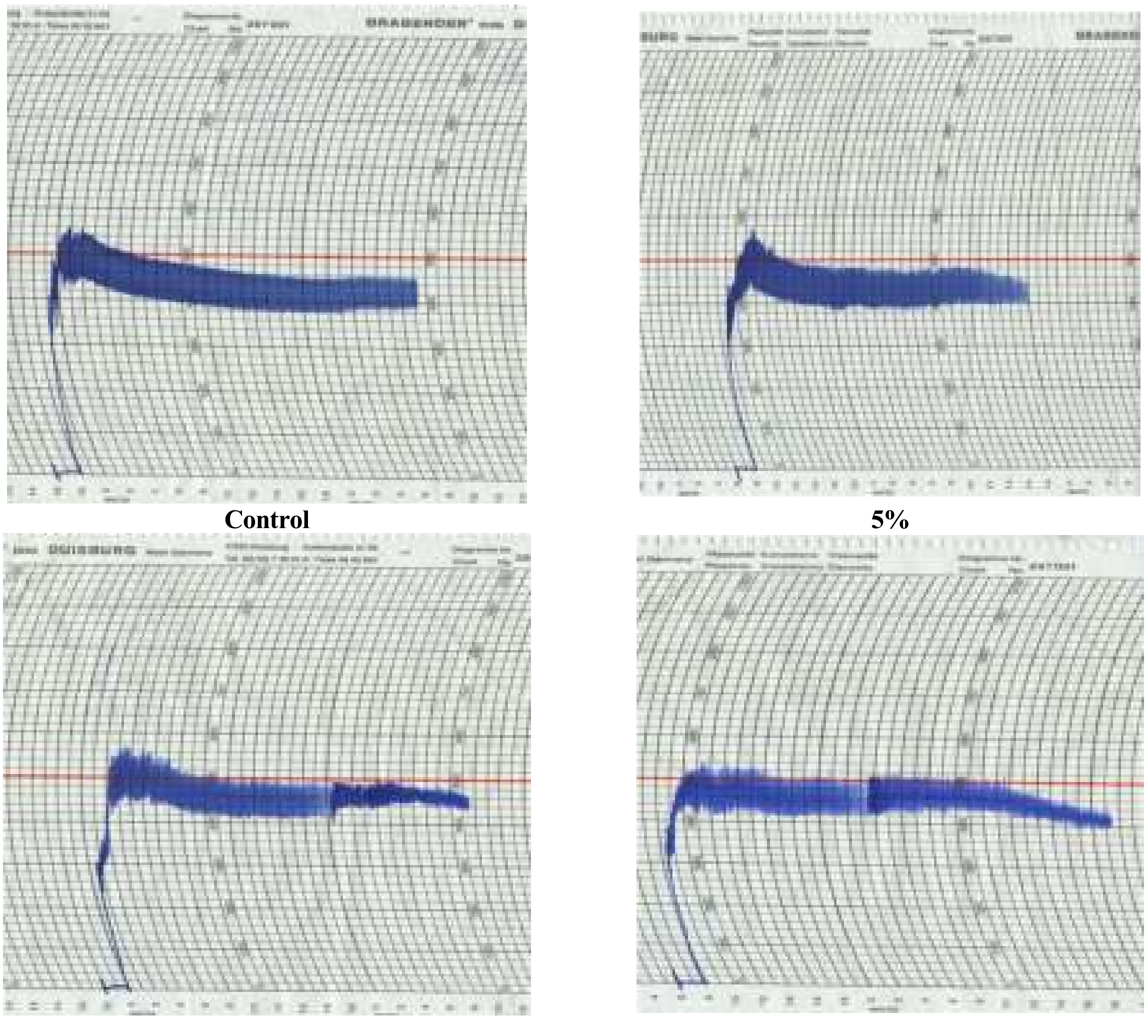
All measured values of rheological evaluation (Farinograph and extinsograph) of wheat flour samples supplemented with guava seeds are shown in Table (3) and figures 1 and 2 . And as can be clearly seen, the values for water absorption decreased from 57.4% for control wheat flour sample to 56.1%, 55.1% and 53 %for wheat flour samples supplemented with5, 10 and 15 % guava seeds powder, respectively. The same phenomenon was also observed for degree of softening (B.U.) value, which reached (60, 40 and 30 B.U) for wheat flour samples supplemented with 5, 10 and 15 % guava seeds powder, respectively as compared to control wheat flour sample (80 B.U.). On the other hand, dough stability (min), Elasticity (B.U), P.N and energy (cm2) values of wheat flour sample supplemented with guava seeds powder increased compare with control sample. These increasing may be due to the proteins pattern of guava seeds powder which has positive effects on some pasting and rheological properties of their flour (Sulieman *et al.,* 2019)

Table 3. Rheological properties of wheat flour supplemented with different concentrations of guava seeds powder

Rheological properties		Wheat flour supplemented with different ratio of guava seeds powder			
		Control	5%	10%	15%
Farinograph test	Water absorption (%)	57.4	56.1	55.1	53
	Arrival time (min.)	1	1	1	1
	Dough development(min.)	1.5	2	2	1.5
	Dough stability time (min.)	3	2.5	4.5	11
	Degree of softening(B.U)	80	60	40	30
Extensograph test	Elasticity (B.U)	190	200	280	320
	Extensibility(mm)	105	100	110	95
	P.N	1.9	1.9	2.5	3.3
	Energy(cm^2)	20	25	35	45

Gross chemical composition of biscuits

Chemical compositions of control biscuits samples and supplemented with different percentages of guava seeds powder (on dry weight basis) are tabulated in Table (4). Moisture, total lipids, crude protein, ash, and total carbohydrates content of the samples were ranged between 1.01 to 1.3 %, 16.5 to 18.9%, 10.3 to 14.8%, 1.2 to 1.5% and 65.1 to71.7 %, respectively. Similar observations were reported by Adebiyi, *et al.,* 2017; Sulieman, *et al.,* 2019. Moreover the addition of guava seeds powder to biscuit samples increased total lipids and crude protein and decreased the ash and total carbohydrates as compare with control biscuit sample, these changes may be due to ingredients of guava seeds powder.



Control   5%

10%   15%

Fig. 1. Farinograph test of wheat flour supplemented with different concentrations of guava seeds powder

Table 4. Chemical composition of biscuits supplemented with different percentages of guava seeds powder

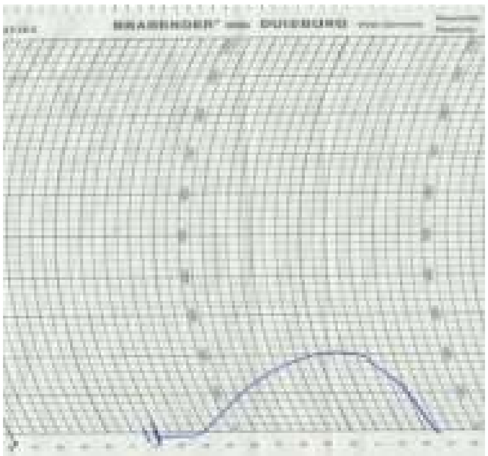
Chemical composition	Treatments: Control	Biscuit supplemented with 5%guava seeds powder	Biscuit supplemented with10%guava seeds powder	Biscuit supplemented with 15% guava seeds powder
Moisture	1.02±0.21	1.3±0.01	1.01±0.04	1.02±0.06
Total lipids*	16.5±0.1	17.2±0.03	18.1±0.07	18.9±0.03
Crude protein*	10.3±0.20	11.6±0.02	12.9±0.04	14.8±0.09
Ash*	1.5±0.10	1.4±0.01	1.3±0.08	1.2±0.12
Total carbohydrates*	71.7	69.8	67.7	65.1

Values in table indicate the mean and standard deviation of three replicates
***% on dry weight basis**

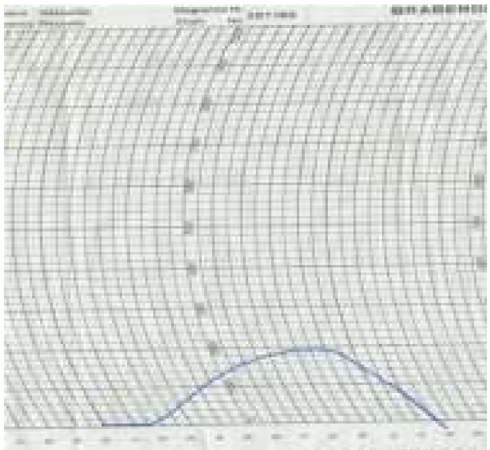
Amino acids profile of biscuits

There are two groups of amino acids: non-essential amino acids that can synthesize via the body and essential amino acids which can't synthesize by body and should be obtained via foods. Furthermore amino acids are important metabolic activities in biological required for biosynthesis of human body (Adebiyi *et al.,* 2017; Sulieman *et al.,* 2019). Table 5 showed that the total amino acids were determined in biscuits samples supplemented with different ratio of guava seeds powder. Leucine is the major essential amino acid and it ranged between 16.4 mg/g (control sample) and 18.8 mg/g (Biscuit sample supplemented with 15%guava seeds powder), followed by valine which ranged between 6.5mg/g and 9.8mg/g, respectively. While arginine acid was the highest non-essential amino acids, it recorded 3.1 mg/g for control sample and1.3 mg/g for biscuit sample supplemented with 15%guava seeds powder followed by aspartic and glutamic.

It is clearly seen that the total amino acids represented 65.3, 68.4, 73.4 and 80.5 mg/g in biscuits supplemented with 0, 5, 10, 15 % guava seeds powder, respectively. From the same table, it could be observed that addition guava seeds powder during preparation of biscuits increase the essential amino acids of biscuits due to their amino acids profiles. Similar observations were reported by Patel, *et al.,* (2019); Sulieman *et al.,* (2019) who mentioned that the guava seeds protein profile very similar focusing patterns of glutelin fractions.



Control



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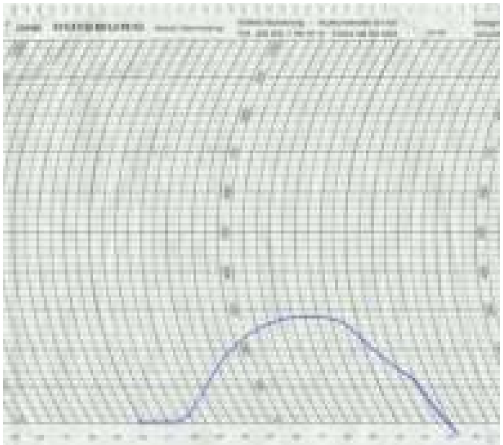

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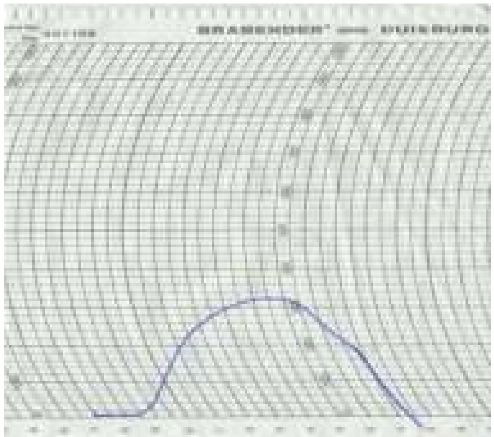

15%

Fig. 2. Extensograph test of wheat flour supplemented with different concentrations of guava seeds powder

Table 5. Amino acids profile of biscuits supplemented with different percentages of guava seeds powder

Amino acids (mg/g)		Treatments: Control	Biscuit supplemented with 5%guava seeds powder	Biscuit supplemented with10%guava seeds powder	Biscuit supplemented with 15%guava seeds powder
Essential amino acids	Therionine	1.5	2.1	2.9	3.6
	Valine	6.5	7.2	8.5	9.8
	Methionine	2.2	3.1	3.9	4.5
	Isoleucine	5.7	6.3	7.1	8.7
	Leucine	16.4	17.2	17.9	18.8
	Tyrosine	7.5	7.9	8.5	9.1
	Phenyalanine	6.3	7.3	8.2	8.9
	Lysine	2.1	3.4	4.3	5.7
	Histidine	1.9	2.5	3.4	4.8
Total essential amino acids		50.1	57	64.7	73.9
Non-essential amino acids	Aspartic	2.5	2.1	1.5	1.1
	Serine	1.3	1.0	0.7	0.6
	Glutamic	2.1	1.5	1.1	0.8
	Prolin	1.2	1.1	0.9	0.6
	Glycine	1.9	1.4	1.1	0.7
	Alanine	1.4	1.3	1.0	0.8
	Cystine	1.7	1.1	0.9	0.7
	Arginine	3.1	1.9	1.5	1.3
Total non-essential amino acids		15.2	11.4	8.7	6.6
Total amino acid		65.3	68.4	73.4	80.5

Sensory evaluation score of biscuits

Measurement of different attributes (Appearance, taste, texture, color and odor) were examined for sensory evaluation score of biscuits prepared from different ratio of guava seed powder (5,10 and 15%) are reported in Tables 6 and 7. Data in these tables showed that there were not significant differences at ($P \leq 0.05$) for biscuits prepared from different percentages of guava seeds powder applied in compare those of control sample.

Table 6. Mean attributes comparison for sensory evaluation score of biscuits supplemented with different percentages of guava seeds powder

Attributes	Treatments: Control	Biscuits supplemented with 5% guava seeds powder	Biscuits supplemented with 10% guava seeds powder	Biscuits supplemented with 15% guava seeds powder
Appearance	9.01±0.61	8.99±0.28	8.84±0.28	8.94±0.19
Taste	9.20±0.32	9.00±0.29	9.05±0.16	9.02±0.06
Texture	8.86±0.76	8.98±0.19	8.47±0.52	8.49±0.54
Color	9.03±0.20	8.99±0.12	9.04±0.42	8.9.8±0.24
Oder	8.99±0.74	8.89±0.25	8.81±0.23	8.90±0.32

Means ± SD with the same latter in the same row are not significantly different ($P \leq 0.05$)

Microbial Examination

Results showed that in table (8). Total bacterial count, spore-forming bacteria and molds and yeasts counts of biscuits supplemented with guava seeds powder during storage at room temperature (25±3°C) and the initial counts were 1.8×10^2, 0.5×10^2 and 8.3×10^2 cfu/g, respectively. It could be noticed that the addition of guava seeds powder (5, 10 and 15 %) to biscuits formulas reduced the counts of microbial load , this reduction could be attribute that guava seeds powder effect positional against some fungi and bacteria .These results are in agreement with those reported by Pelegrini *et al.,* (2008) ; Lu and Chen (2010) ; Pelegrini and Franco (2011); Tavares *et al.,* (2012) whom reported that guava seeds contain the glycine-rich proteins which have activity against human pathogenic such as *Proteus sp., Pseudomonas aeruginosa, Escherichia coli and Klebsiella sp.* (Gram-negative bacteria) and Gram-positive bacteria such as *Staphylococcus aureus and Staphylococcus epidermides*), these peptides use as a biotechnological tool and antimicrobial peptides for infectious disease treatment, The action mechanism of these peptides involve permeability alterations, ion imbalance and lipid bilayer depolarization that may induce electrostatic interactions which case membrane disruption , additional these peptides casing modifying enzyme activities, alteration of several gene expressions and improving protein synthesis.

Table 7. Analysis of variance for sensory evaluation score of biscuits supplemented with different percentages of guava seeds powder

Analysis of variance between treatments	Attributes				
	Appearance	Taste	Texture	Color	Oder
Main square values	0.06 ^{NS}	0.08 ^{NS}	0.67 ^{NS}	0.07 ^{NS}	0.05 ^{NS}
F value	0.41	1.49	2.28	0.64	0.29
F crit.	2.87	2.87	2.87	2.87	2.87
Degree of freedom	3	3	3	3	3

^{NS}: Non-significant.
Values are means of ten replicates. Data were analyzed by ANOVA (Single factor), F test means ($P \leq 0.05$).

Table 8. Microbial load of biscuits supplemented with different percentages of guava seeds powder during storage at room temperature (25±3°C)

Microbiological parameters (Cfu/g)	Storage (Months)	Microbial counts of biscuits supplemented with guava seeds powder			
		Control	biscuits supplemented with 5% guava seeds powder	biscuits supplemented with 10% guava seeds powder	biscuits supplemented with 15% guava seeds powder
Total bacterial counts	Zero time	1.8×10^2	1.7×10^2	1.4×10^2	1.2×10^2
	1	4.6×10^3	3.9×10^3	3.2×10^3	2.8×10^3
	2	8.2×10^3	8.7×10^3	7.6×10^3	6.2×10^3
	3	9.9×10^3	9.0×10^3	8.5×10^3	7.1×10^3
Spore-forming bacteria	Zero time	0.5×10^2	0.3×10^2	0.2×10^2	0.1×10^2
	1	1.9×10^2	1.5×10^2	1.0×10^2	0.8×10^2
	2	3.9×10^2	2.8×10^2	2.0×10^2	1.2×10^2
	3	7.8×10^2	6.0×10^2	4.1×10^2	2.5×10^2
Total molds& yeasts	Zero time	8.3×10^2	7.9×10^2	7.8×10^2	7.6×10^2
	1	8.9×10^2	8.1×10^2	7.8×10^2	6.7×10^2
	2	9.7×10^2	8.5×10^2	8.0×10^2	7.2×10^2
	3	9.9×10^2	8.8×10^2	8.3×10^2	7.9×10^2

CONCLUSION

The biscuit formulas were prepared by replacing wheat flour with 5, 10, and 15% guava seeds powder. Farinograph and extinsograph tests estimated for wheat flour samples supplemented with guava seeds (5, 10 and 15 %). The results showed that a decrease in water absorption and degree of softening (B.U.) values and an increase in dough stability (min), Elasticity (B.U), P.N and energy (cm^2) values by increase a addition of guava seeds powder. Biscuits were developed using guava seeds powder via addition it to their formula. The result of sensory attributes of biscuits formulas had non-significant differences ($P\leq0.05$) between all samples. Guava seeds powder had positive effects on amino acids profiles (Increasing an essential amino acids), rheological and microbial properties (Lowering total bacterial molds & yeasts counts). Further studies on the antimicrobial activities of guava seeds powder are needed to understand effectiveness against many fungi and bacteria of biscuit during storage at room temperature (25±3°C). Hence guava seeds powder can be widely applied to developing wheat flour and quality of biscuits.

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التقييم الكيميائي والريولوجي والحسي والميكروبي لدقيق بسكويت القمح المدعم بمسحوق بذور الجوافة

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في هذا البحث تم تحضير البسكويت بإضافة مسحوق بذور الجوافة بنسبة ٥ و ١٠ و ١٥٪. وتم تقييم الخواص الكيميائية والريولوجية والحسية والميكروبية لعينات البسكويت المختلفة. أدت إضافة بذور الجوافة الى تحسين الخصائص الوظيفية للتقييم الميكروبي للبسكويت الذي أعد منها. أظهرت النتائج ان دمج مسحوق بذور الجوافة في عجينة البسكويت عن زيادة في ثبات العجين، قيم المرونة ، والطاقة (Cm2) وانخفاض في درجة امتصاص الماء وقيم درجة المرونة و أظهرت نتيجة التقييم الحسي لعينات البسكويت المحضرة من مسحوق بذور الجوافة اختلافات غير معنوية ($P\leq0.05$) بين جميع عينات البسكويت. بالإضافة إلى ذلك ، إضافة مسحوق بذور الجوافة لعينات البسكويت أدى الى زيادة الأحماض الأمينية الأساسية من ٥٠.١ إلى ٥٧.٠ و ٦٤.٧ و ٧٣.٩ ملجم / جرام ، وبالتالي فإن إجمالي الأحماض الأمينية يمثل ٦٥.٣ و ٦٨.٤ و ٧٣.٤ و ٨٠.٥ ملجم / جرام للبسكويت المدعم بمسحوق بذور الجوافة بنسبة ٠ ، ٥ ، ١٠ ، ١٥ ٪ ، على التوالي. تدعيم عينات البسكويت بمسحوق بذور الجوافة بنسب ٥ ، ١٠ و ١٥ ٪ أدى الى خفض الحمولة الميكروبية. يمكن أن نستنتج أن إضافة مسحوق بذور الجوافة إلى دقيق القمح يحسن الخصائص الوظيفيه الغذائية والريولوجية. هذا العنوان تم اقتراحه من قبل المحكم الخارجى نرجو التوجيه من معاليكم هل يتم الاخذ به ام لا Rheological Characteristics, Sensory Properties, Microbial Evaluation and Amino Acids of Biscuit mead from Wheat Flour Supplemented with Guava Seeds Powder الخصائص الريولوجية والصفات الحسية والتقييم الميكروبيولوجى والاحماض الامينية للبسكويت المصنع من دقيق القمح المدعم بمسحوق بذور الجوافة