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Effect of Wheat Flour Replacement and Enrichment by Eggshells on the Quality Properties of a Laboratory Produced Biscuit

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Abstract: ***The aim of the study was studying effect of wheat flour replacement by eggshell on the quality properties of the produced biscuit. The ratios of the added eggshells were 5%, 10%, 15%, 20% and 25%. The sensory, microbial and chemical properties of the produced biscuit samples were studied, and it was found that 10% - replacement ratio (A3) treatment was superior in the treatments sensory properties followed by A2, A4, A1 and lastly A6 . while at the microbial content of the produced biscuit samples, it was noticed that the bacterial numbers values were higher in A3 treatment and at low values in A4, A5 and A1 treatments the yeasts numbers were at their highest values in A4 treatment and at lowest values in A5 treatment and there was no yeasts growth in A1 and A2 treatments. From the result of the study it was also noticed absence of Escherichia coli and Salmonella bacteria growth in biscuit samples. The chemical content results of the produced biscuit samples showed that using eggshells gave a clear increase in calcium ratio with increasing of replacement ratio and calcium / iron ratio and phosphorus in the enriched biscuits. It may be recommended to add eggshell powder as a good calcium source in baking (biscuit and cake) production especially to the kids who don't eat milk and its products.***

Keywords: eggshell, enrichment, biscuit

1. Introduction

The eggshell is considered as a secondary products resulted from the activity of foods manufacturing companies , hatchers , houses and restaurants. The united states of America produced alone 600.000 tons yearly of eggshell as a secondary products , while in Taiwan , the estimated waste of eggshells yearly was 104-109 tons and most of the wastages were ignored and not treated by pushing them to the garbage and this may cause an dangerous environmental problem . As a result of that , the USA forced the food manufacturing companies to find alternatives to limit this problem and give a clear advantage (26). Lastly ,techniques were put to isolate the membranes from the eggshell commercially and this makes a good possibility in developing products having additional value from all the materials (4). Eggshell have a crude materials which are considered as good source of the activation compounds such as hyaluronic acid and sulfur rich proteins (15) and (25). Generally , eggshell consists of 94% calcium, 1% $MgCO_3$, 1% $CaHPO_4$ and 4% organic materials , besides $CaCO_3$ characterized as not having residues of Pb , Al , Cd and Hg such as $CaCO_3$ of oysters shells (13 and 11). It was mentioned by (3) that eggshells have $CaCO_3$,SO_4,some protein and also Fe, Cu and zinc (12) . The egg and its shell and components were recognized by the American Agency of Foods Control (AAFCO) as a sale materials when they are added to human and animals (cattle) foods since 1982 (6) . Many studies mentioned that the using of eggshell in treating bones fragility in the elderly people by reduction of pains and increasing bones density (BMB, bone mineral density), (12 and 20). It was emphasized by (22) that giving eggshell powder with vitamin .D and magnesium to woman at 50-70 year age for 12 months increased their bone density. It was also mentioned by (24) that using eggshell in the pharmaceutical sector such as medicine discs to release the medicine inside human body . In study carried by (11) about evaluation of eggshell membrane safety as a food supplement and it was found that it contained glycosaminogly can and the necessary proteins that occur in natural way to maintain joints and connective tissues health and it was cleared that this was safe in human consumption at levels reach more than 500mg day $^{-1}$. The shortage of studies that related with eggshell in foods nutrition manufacturingespecially in Iraq makes the aims of this study to investigate effect of eggshell powder addition to the laboratory produced biscuit on its nutrient content and study the consumption acceptance of biscuit by using the sensory evaluation.

2. Materials and Methods

1) Eggshell powder preparation

The red and white egg shells were collected and many treatments were done on them beforetheir grinding and drying. They were :-

A: un boiled , white egg shells (washed)
B: un boiled , white egg shells (un washed)
C: boiled , white egg shells (washed)
D: : boiled , white egg shells (un washed)
E: : un boiled , red egg shells (washed)
F: : un boiled , red egg shells (un washed)
K: : boiled , red egg shells (washed)
G: : boiled , red egg shells (un washed)

Then , the shells were grinded to fine powder by using electrical mill, and the resulted powder was dried at an electrical oven at 105 C^{o}for 3 hours and put in polyethylene bags. The microbial tests of the samples was carried out at Labs of Market and Consumer Protection Research Center / University of Baghdad ,and chemical analyses were done on samples to know their mineral elements content (5).

2) The laboratory biscuit preparation
The materials that were used in preparation of the laboratory biscuit are shown in table (1).

Table 1: The used materials

Material	Quantity
Wheat flower	100g
Fat	22.7g
Milk	73.6ml
Baking powder	5g
Salt	2.7g

This kind of biscuit was considered as control treatment with the othertreatments, then the wheat flour was replaced by eggshells powder (5) as follows:

1) Treatment (A1): Without replacement (control treatment), 100% wheat flour.
2) Treatment (A2): Replacement of 5%- eggshells powder by the used wheat flour (5g. eggshells powder + 95 g. wheat flour).
3) Treatment (A3): Replacement 10%- eggshells powder by the used wheat flour (10g. eggshells powder + 90g. wheat flour)
4) Treatment (A4): Replacement 15%- eggshells powder by the used wheat flour (15g. egg shells powder + 85g. wheat flour).
5) Treatment(A5): Replacement of 20%- eggshells powder by the used wheat flour (20g. eggshells powder+ 80g. wheat flour)
6) Treatment (A6): Replacement 25%- eggshells powder by the used wheat flour (25g. egg shells + 75 g. wheat flour).

The laboratory biscuit was prepared according to the method that was mentioned by (7) and it included the following steps:

1) Sieve of wheat flour, baking powder and salt together in mixing dish.
2) Fat was added to the dried components, then, hand rubbing was done till getting a homogenous mixture.
3) Almushbik and the wooden board were sprayed by wheat flour and the paste was brushed in 1-1.5 cm thickness.
4) Biscuit was put in non- fatted tray using special knife and(1-1.5 cm) space between biscuit pieces was selected. Then , the tray was put in an oven.
5) The roasting process was done at 218 C^o for 12-15 min, till getting golden color biscuit .

3) The sensory evaluation
It was done by 20 specialized evaluators who work in home economics Department ,College of education for woman , University of Baghdad, by using an evaluation questionnaire that authorized by food and nutrition Department, Texas University , USA, (1975) (8). The sensory degrees of each property are shown in table(2).

Property	Degree
Color	7
Appearance	7
Texture	7
Freshness	7
Flavor	7
General acceptance	7
Total	42

4) Determination of the mineral elements
Calcium, phosphorus and iron in biscuit were determined by using the method which is put by (5).

5) Microbial tests
25 gram sample in 225 ml of peptone water was taken by using the mixer (Stomacher), and the following tests were carried out after the sample preparation end.

A) Total bacteria counts: It was done by following plate count method and using nutrient agar medium. After preparation of this medium one ml of sample was put inside a plate and then the nutrient agar was poured and left to get solid state and put in an incubator at 27 C^o for 24 hours.

B) Colon bacteria (coliform) numbers determination: It was done by using violet red bile agar . This medium was poured in the plates and left to be solid and then 0.1 ml of the soluble material sample was grown on the plates surfaces by using spreading method , and then the plates were put in an incubator at 37 C^o for 24 hours.

C) Salmonella bacteria numbers determination: Selenite F .broth media was prepared and , ml of it was put in sterilized tubes then (1) ml of the soluble material was put on the media and left in an incubator at 37 C^o for 24 hours. S.S.Agar medium was prepared and left in plates to be solid, then 0.1 ml of selenite F. broth media was taken and spread on the plates by using the spreading method. The plates were put in an incubator at 37 C^o for 24 hour and reading was taken after 3 days.

D) Molds and yeasts number determination: It was done by using plate count method and preparing nutrient agar medium. One ml of the soluble material sample was taken and put in the plate and then some of the medium was poured over it and left to be solid and put in an incubator at (25 C^o- 28 C^o) for 3 days.

6) Biscuit storage: Biscuit was stored in polyethylene bags and they were put in a refrigerator at 4 C^o for one week, then the last microbial tests were done .

7) Statistical analysis:(SAS) program was used in the data statistical analysis for study effect of the studied treatments on the sensory properties. The significant differences between the mediums were compared by testing the least significant difference (LSD)(21).

3. Results and Discussion

1- The chemical analysis of eggshells: The results shown in table (3) and they show that the eggshells powder of the red eggs had the highest calcium content (32.7%) compared with the eggshells powder of the white eggs which had 30.8% calcium. Results of this study was near the results of (1) and (19) who analyzed calcium content in eggshells and they got 35.6% and 34.8% calcium respectively.

Table 3: Eggshells content of mineral elements

sample	Ca	Zn	Fe	P
Eggshells of the red eggs	30.75%	0.009%	0.0074%	0.003%
Eggshells of the white eggs	30.80%	0.008%	0.0072%	0.001%

2- Microbial content of eggshells

Table (4) shows the microbial content eggshells samples, the sample (D) had the highest total number of bacteria (15*10ml colony^{-1}) and the lowest value was 1.6*10 ml colony^{-1} in the sample (F). All the studied samples were free of colon bacteria except sample (F) which contained 5.4*10 ml colony^{-1}.

Results of this study showed no Salmonella bacteria growth in all the samples. It was found in (18) study that the unwashed eggs samples contained Enterobacteriaceae bacteria group which includes E.coli and Salmonella and klebsiella bacteria, while very small ratio of the washed eggs samples contained this bacteria. In another study, it was appeared that the washed eggs samples contained (1-6) bacterial colony (14), while (9) mentioned that washing eggs samples and facing them to 40-48 C^{o}resulted a decrease in theirs Salmonella bacteria content to less than 5 colonies. It was explained by (17) that washing eggs may decrease the bacterial number on the eggshell.

Table 4: Total bacterial , E.coli bacteria and Salmonella bacteria numbers

Treatments	Total bacterial number (ml.colony^{-1})	E.coli number (ml.colony^{-1})	Salmonella number (ml.colony^{-1})
A	2.8*10	ND	ND
B	2.8*10	ND	ND
C	2.2*10	ND	ND
D	15*10	ND	ND
E	2.2*10	ND	ND
F	1.6*10	5.4*10	ND
K	2.8*10	ND	ND
G	2.2*10	ND	ND

3) The sensory evaluation of the laboratory biscuit

It may be noticed in table (5) that 5%- replacement ratio (A2) treatment got the highest degrees of the studied sensory properties except of the chips property in which the 10%-replacement ratio (A3) treatment got the highest value (4.9), while in the rest treatments (A2, A4, A1, A5 and A6) got (4.8), (4.8), (4.7), (4..4), and (4.3) degrees respectively.

With the appearance property evaluation , the 5% -replacement ratio (A2) treatment and 10% replacement ratio (A3) treatment got a medium value (5.6) while, A1, A4, A5 and A6 treatments got the following mediums (5.3), (4.2), (4.7), and (4.0) degrees respectively in the appearance property.

With the texture property, the 5% -replacement ratio (A2) treatment got the highest medium degree (4.8), while the A3, A1, A4, A5 and A6 treatments got (4.7), (4.6), (4.6),(4.5), and (4.5) degree respectively in the same property.

In the freshness property, the 5% replacement ratio (A2) got the highest medium degree (5.4), while the rest treatments A6, A5, A4, A3, and A1 got the mediums (4.2), (4.4), (4.6), (4.8) and (5.1) degrees respectively in freshness property.

The flavor property degree in the 5% - replacement ratio (A2) treatment was in its highest medium value (5.4), while the rest treatments A1, A3 , A4, A5 and A6 got the mediums (5.2), (4.7), (4.7), (4.5), and (4.2) respectively in this property.

The color property of the 5% replacement ratio (A2) treatment got the highest medium degree (5.9), while the rest treatments A3,A1, A4, A5 and A6 got the mediums (5.4), (5.3), (4.6), (4.5) and (4.2) degrees respectively in this property.

The general acceptance property of the 5%- replacement ratio (A2) treatment got the highest medium degree (5.6), while the rest treatments A1, A3, A4, A5 & A6 got the mediums (5.4), (5.2), (5.2) ,(5.0), and (4.1) degrees respectively in this property. From table(4), it may be noticed that the significant differences between the treatments were statistically significant in all the studied properties except texture property. Appearance property got the highest (LSD) value (0.894) while the chips property got the lowest (LSD) value (0.748) compared with the others properties, these results were near the results that got by (10).

Table 5: Effects of the studied treatments on the sensory properties of the laboratory cake

Treatment (replacement ratio)	Medium ± standard error						
	Appearance	Texture	Freshness	Flavor	Chips	Color	General acceptance
A1(control)	5.3±0.48	4.6±0.52	5.1±0.67	5.2±0.72	4.7±0.41	5.3±0.39	5.4±0.56
A2 (5%)	5.6±0.61	4.8±0.58	5.4±0.52	5.4±0.52	4.8±0.46	5.9±0.62	5.6±0.61
A3 (10%)	5.6±0.61	4.7±0.49	4.8±0.49	4.7±0.43	4.9±0.52	5.4±0.42	5.2±0.49
A4 (15%)	4.2±0.34	4.6±0.51	4.6±0.61	4.7±0.43	4.8±0.44	4.6±0.51	5.2±0.62
A5 (20%)	4.1±0.48	4.5±0.38	4.4±0.33	4.5±0.52	4.4±0.35	4.5±0.48	5.0±0.39
A6 (25%)	4.0±0.29	4.5±0.38	4.2±0.28	4.2±0.28	4.3±0.38	4.2±0.37	4.1±0.44
LSD	*0.894	0.759 N.S	*0.816	*0.791	*0.748	*0842	*0.863

4- The microbial tests of the laboratory biscuit

Table (6) shows the results of the microbial test of the laboratorybiscuit before and after storage for one week . All the sample were free of bacteria, yeasts and molds after production directly , while the treatment (A3) contained 54*10 ml colony^{-1} of total bacteria and (A2) contained 15*10 ml colony^{-1} followed by the (A6) treatment (12*10 ml colony^{-1}) after a week of refrigerator storage , while the lowest bacterial number was in the treatments A1, A5 and A4 which contained (3*10),(4*10) ml colony^{-1} of total bacteria, this pollution may be due to the storage condition which was convenient to bacteria growth . In yeast case , it may be noticed in table (6) that the highest yeasts number was in (A4) treatment (8*10 ml colony^{-1}) followed by (A3) treatment (15*10 ml colony^{-1}) and the lowest value was in (A5) treatment (1*10 ml colony^{-1}) there were no yeast and mold growth after a week of storage (2) . allthe samples were free of E. coli bacteria growth as well as Salmonella bacteria growth , this may be due to the high temperature that destroyed this bacteria. (23)

Table 6: Numbers of bacteria and yeasts in the laboratory biscuit

Treatment	Bacteria and yeasts numbers		E. coli before and after storage	Salmonella before and after storage
	Before storage	After week		
A1	ND	4*10 bacterial colony ND-yeasts	ND	ND
A2	ND	5*10 bacterial colony ND-yeasts	ND	ND
A3	ND	54*10 bacterial growth 15*10- yeast colony	ND	ND
A4	ND	3*10-bacterial colony 20*10-yeast colony	ND	ND
A5	ND	3*10- bacterial colony 1*10-yeast colony	ND	ND
A6	ND	12*10- bacterial colony 8*10-yeast colony	ND	ND

ND= No growth

5- Concentration of the mineral elements

Table (7) shows the mineral content variations of biscuit samples. Biscuit content of calcium , Feand P in the control treatments were 152, 0.035 and 8 mg $100g^{-1}$ respectively , while , there was a clear increase of Ca and Fe contents in the laboratory biscuit samples compared with the control treatment , this may be due biscuit enrichment with different ratios of eggshells (10 and 16).

Table 7: The mineral content of 100 gram of the laboratory Biscuit

sample	Replacement ratio	Ca%	Fe%	P%
A1	Control	152%	0.035%	8.0%
A2	5%	181%	0.038%	5.0%
A3	10%	204%	0.035%	4.0%
A4	15%	188%	0.035%	3.9%
A5	20%	193%	0.044%	3.5%
A6	25%	192%	0.034%	3.0%

From the obtained results , it may be concluded that the best replacement ratio was at 5% replacement ratio (A2) treatment and adding the eggshells powder caused a big increase of Ca and Fe contents in the enriched biscuit at different ratios , so it is recommended to enrich the food products by eggshells especially in kids and elderly people foods production.

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