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EFFECT OF ADDING ALEURONE FLOUR AT DIFFERENT LEVELS ON PAN BREAD QUALITY

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ABSTRACT: *Aleurone flour (ALF) was prepared and wheat flour (72%) was replaced using ALF at levels of 0, 11, 22, and 33 % as a rich source of folic acid (618.13µg/100 gm). Wheat flour was used to produce rich folic acid (FA) pan bread. FA was added to wheat flour used in pan bread production for the comparison. The bread was prepared by sponge dough method and the FA was dissolved in a portion of the water used. Chemical composition, rheological properties, color attributes, baking test and organoleptic properties were evaluated. The obtained results revealed that ALF is considered a good source for FA, protein, fat, fiber and ash. The concentration of FA in the produced pan bread ranged from 148.18(control) to 342.37 µg/100g (pan bread with 33%ALF), while, adding of FA (220 µg /100g) to wheat flour led to a higher FA content in the produced pan bread (363.21µg/100g). Rheological properties of dough containing ALF were negatively affected. Darkness of pan bread increased as ALF level increased. Loaf volume and specific volume decreased as ALF level increased, while, freshness of pan bread was enhanced by using ALF up to 22%. Finally, it could be concluded that, acceptable pan bread with high contents of FA, protein, fat, fiber and minerals could be produced by using ALF up to 22%, while, adding FA (220 µg /100gm flour) to wheat flour did not cause any significant effects on rheological properties, chemical composition, baking quality and organoleptic evaluation parameters.*

Key words: *wheat flour, aleurone, folic acid, rheological properties, organoleptic and pan pread.*

INTRODUCTION

The fortification of cereals with FA has been implemented in several countries, such as Untied Kingdom, Canada, Australia and the United States. There is a convincing evidence that FA supplementation, when taken from the onset of pregnancy, reduces the prevalence of neural tube defects (Scott *et al.*, 1995). An additional effect of FA fortification may be lowering the risk of cardiovascular diseases (Boushey *et al.*, 1995). FA lowers homocystine level in blood, and the patients with a high homocysteine level in blood have a higher risk of developing coronary heart disease (Wald *et al.*,2002) and Alzheimer 's disease (Aisen *et al.*, 2003). In Italy, a study has revealed that a diet rich in folate was associated with a lower rate of colon cancer (Lavehia *et al.*, 2002).

Carmichel *et al.*, (2006) indicated that 96% of women from several regions of the United States took FA supplements during the pregnancy. Folic acid fortification of cereal grains resulted in a higher folate intake than would have occurred in the absence of fortification in women at a high risk for cervical cancer (James *et al.*, 2004). The FDA allows more folic acid to be added to ready –to- eat cereals. Most cereals provide 25% of Recommended Dietary Allowance (RDA); however, highly fortified cereals provide 400 µg of FA per serving (Albertson and Marquart, 1999).

There is some concerns that eating foods that are naturally rich in folate may not provide reliable intake of folate required to prevent spin bifida (Cuskelly *et al.*, 1996). Therefore, it is important to identify novel, naturally rich sources of folate and to test their dietary strategies based on such foods which may be effective for the optimization of tissue folate in the general population. Wheat aleurone flour (ALF) is a novel food product that has the potential to make an important contribution to the intake of natural folate (Clysedale, 1994). One of the most notable features of the composition of this product is the high level of folate that is present which has a concentration between 400 and 600 µg/100g wet ALF). This natural level of folate is higher than that observed in wheat bran, fruits and vegetables which usually ranged between 20 and 200 µg/100g wet (Bailey, 1995) and is comparable to folate /FA levels in fortified flour and cereal that provide 50% of the RDI per a serve assuming RDI of 400µg and serving size of 40 g wet weight (Crane *et al.*, 1995). This study aims to prepare a pan bread rich in folic acid and to investigate the effect of adding FA rich ALF or FA standard on chemical composition, rheological properties of dough and bread quality.

MATERIALS AND METHODS

I-Materials:

-ALF was purchased from the North Cairo Mills Company, Egypt.

-Wheat flour, shortening, sugar, salt and yeast were obtained from a local market, Cairo.

-FA was obtained from Sigma Company, USA.

II-Methods:

Preparation of ALF

The ALF was dried at 40° C in oven and ground to obtain fine powder (60 mesh).

Preparation of flour mixtures

Wheat flour (72%) was partially replaced by ALF to obtain flour mixtures containing11, 22, and 33% ALF or 220 µg FA /100 gm wheat flour. All prepared mixtures were used to manufacture pan bread.

Chemical evaluation

-Gross chemical composition

ALF, wheat flour, and the prepared pan bread were chemically analyzed for moisture, crude protein (% N×5.71), ether extract, total ash, crude fiber, total sugars, reducing sugars and non reducing sugars according to methods described in A.O.A.C.(1990).Total carbohydrates were calculated by the difference (100-fat, protein, ash, and fibers on dry weight basis).

-Selected minerals content

Magnesium, calcium, potassium, sodium, copper, iron and phosphorus in all samples of pan bread were determined according to method described by Chapman and Pratt (1978).

Rheological properties

Blends of 0, 11, 22 and 33 % ALF substituted flour or 220 µg FA/100 gm were subjected to dough rheology as determined by Farinograph (Model Type No: 81010 (31, 50 and 63 rpm), ©Brabender ® OHG, Duisburg, 1979 Germany) as described in [A.A.C.C., 2000]. Parameters measured were, water absorption, arrival time, development time, stability time, weakening and mixing tolerance index (MTI). The elastic properties of dough with different levels of ALF or 220 µg FA flours were measured by using an Extensograph (Model Type No: 81010 (31, 50 and 63 rpm), ©Brabender ® OHG, Duisburg, 1979 Germany) according to the method of A.A.C.C. 2000. The parameters studied were resistance to extension (R), extensibility (E), proportional number (R/E) and energy (Area). Falling number of dough with different levels of flours were measured as described in A.A.C.C. (2000).

Baking tests:

A-Pan bread preparation

Pan bread was prepared by sponge- dough method as described in A.A.C.C.(2000).

B-Organoleptic evaluation

Pan bread loaves were bean scored for external and internal properties, according to the method of Kulp *et al.*, (1985)

C-Statistical analysis

Data of organoleptic evaluation of pan bread were subjected to analysis of variance and least significant difference (L.S.D) at 0.05 level according to the method described by McClave and Benson (1991).

D- Color

Color of different samples was measured by using a Spectro-Colorimeter (Tristimulus Color Machine) with CIF lab color scale (Hunter, Lab Scan XE, Germany).

E- Loaf volume and weight

Loaf volume was measured by rapeseed displacement. Both loaf weight and loaf volume were determined according to the method described by Kulp *et al.*, (1985).

Specific volume =loaf volume/loaf weight

F-Freshness of ban bread

Freshness of each formula was tested by alkaline water retention capacity (AWRC) determination according to the method of Yamazaki (1953), with some modifications stated by Kitterman and Rubenthaler (1971).

Determination of folic acid by HPLC

FA was determined by HPLC method in ALF and all pan bread samples during mixing, after fermentation and after baking as described by the Analytical Methods Committee (2000). Amount of 5 g sample was weighed into polypropylene bottle and 25 ml of 0.1 *M* hydrochloric acid was added. The suspension was homogenized by using an ultrasonic processor for 30s and then heated in water bath at 90°C for 30 min. When the suspension was cold, pH was adjusted to 4 by using 1*M* sodium acetate and 0.1 g of taka-diastase was added. The sample was maintained in a water bath with magnetic stirring at 50 °C for 2h., then, 1 ml of 50% (w/v) trichloroacetic acid was added and the bottle was again introduced into the water bath at 90 °C for 10 min. When the sample was cold, pH was adjusted to 6 with 10 M potassium hydroxide and it was quantitavely transferred to a 50- ml volumetric flask using the mobile phase buffer (10 mM potassium dihydrogenphosphate (pH 6). Aliquots were centrifuged at 6000 rpm for 10 min, filtered through a 0.45-μm nylon millipore chromatographic filter and injected into the chromatograph. Certified reference samples were analyzed in the same way.

RESULTS AND DISCUSSION

1-Chemical composition of raw materials, pan bread and pan bread fortified with ALF or FA

A-Gross chemical composition

Data presented in Table (1) show gross chemical compositions of wheat flour 72%, ALF and the prepared pan bread. It is clear that ALF is a good potential source for crude protein, ether extract, crude fibers, and total ash. Moisture, crude protein, ether extract, crude fiber, and ash contents increased with increasing the ALF level in wheat flour, whereas total sugars and total carbohydrates contents decreased in pan bread fortified with ALF compared to the control. The increase in protein, fat, ash and fibers of ALF supplemented pan bread can be attributed to the high content of those ingredients in ALF. Such findings were also obtained by Shouk (1996) and Michael *et al* (1999).

Table (1): Gross chemical compositions of pan bread and pan bread with FA or ALF (calculated on dry weight basis).

Constituents (%)	Wheat Flour 72%	ALF	Control from flour 72%	Pan bread with FA	Pan bread with 11% ALF	Pan bread with 22 % ALF	Pan bread with 33 % ALF
Moisture	11.12	7.12	35.74	34.33	35.86	36.39	38.11
Protein	12.00	24.05	11.91	11.87	13.31	15.49	17.80
Ether extract	1.56	7.18	2.56	2.55	3.00	3.21	3.36
Crude fiber	1.02	16.23	0.94	0.98	4.88	8.56	12.22
Total Ash	0.86	5.33	1.79	1.87	2.26	3.18	4.00
Total carbohydrates	84.56	47.21	82.80	82.73	76.55	69.56	62.62
Total sugars	2.22	6.20	3.15	3.12	2.77	2.38	2.86
Reducing	0.79	1.56	0.94	0.75	0.75	0.65	1.01
Non reducing	1.43	4.64	2.21	2.37	2.02	1.73	1.85

b- Minerals content of pan bread

Data presented in Table (2) show minerals content of both pan bread (control) and pan bread fortified with either ALF (11, 22 and 33%) or FA. It is noticed that pan bread (control) and pan bread fortified with FA had a lower content of tested minerals than pan bread supplemented with ALF. Minerals content is increased with increasing the ratio of substitution of ALF in wheat flour. This might be attributed to a higher minerals content in ALF compared to that in wheat flour (72%). This could be, due to the fact that aleurone cells, together with the germ and testa, contain the essential nutrients required for the growth and development of the embryo (Clysedale, 1994 and Saxalpy &Venn-Brown, 1980). Vitamins and minerals in aleurone cells may be lost when wheat grain is refined. Bran contains a higher content of minerals than flour (Dikeman *et al.*, 1982 and Shouk, 1996).

Table (2): Minerals content of pan bread (calculated on dry weight basis)

Minerals (mg/100g)	Control (from flour 72%)	Pan bread with FA	Pan bread with 11% ALF	Pan bread with 22% ALF	Pan bread with 33 % ALF
Magnesium	104.61	105.70	123.70	146.07	169.31
Calcium	23.00	23.0	36.56	40.70	42.56
Potassium	102.0	105.0	180.37	238.67	283.49
Iron	1.71	2.11	3.31	4.43	5.57
Copper	0.30	0.34	0.42	0.48	0.57
Sodium	630.18	640.75	736.73	824.74	971.97
Phosphorus	190.12	188.22	280.16	320.17	378.33

2- Color attributes of pan bread as affected by adding ALF

Data presented in Table (3) represent color attributes of pan bread, pan bread with FA and pan bread with ALF. As shown, pan bread supplemented with ALF was darker than control or pan bread with FA where L-values (lightness) and b-values (redness) decreased as ALF level increased in pan bread. The same trend was observed regarding a-values (yellowness) and ΔE. Both a and ΔE values increased as ALF level increased. ALF is darker than wheat flour so, darkness increased as a result of presence of ALF in pan bread. Such findings were also observed by Kim *et al.*(1997a), Kordonowy &Young (1985) and Ramy *et al.* (2002).

Table (3): Mean Hunter color values of pan bread .

Samples	L	a	b	ΔE
Control	66.13	9.59	31.23	42. 92
Pan bread with FA	60.71	12.00	37.43	50.86
Pan bread with 11% ALF	52.56	13.77	29.96	52.10
Pan bread with 22 % ALF	46.03	15.60	28.09	56.81
Pan bread with 33 % ALF	44.19	15.07	27.19	57.74

3- Baking quality of pan bread

The physical characteristics of the produced pan bread are presented in Table (4). Loaf volume decreased as ALF level increased while loaf weight increased as ALF level increased. This effect may be due to high fiber and protein contents in ALF. Fibers and proteins are characterized by its higher water holding capacity. Sekhan *et al.* (1997) reported that bread volume decreased but muffin volume increased with the addition of different types of bran to wheat flour. Also, Tangkanakul *et al* (1995) stated that fibers enrichment of wheat flour breads led to decrease in loaf volume and increased the density of bread where fiber enrichment increased hardness of bread. The highest reduction in loaf volume was in bread made from wheat flour blended with ALF at 33 % level. The drop in loaf volume could be due to the dilution effect on gluten due to addition of ALF flour to wheat flour and less retention of CO_2 gas can be contributing (Sharma & Chauhan, 2000).

From the same table, specific loaf volume of pan bread containing 22 or 33% ALF had lower values compared with that of control sample. On the other hand, the addition of FA to wheat flour increased loaf weight, loaf volume and specific loaf volume compared to those of the control. Reductions in loaf volume were probably related primarily to the overall decrease in gluten content (Kawka *et al.*, 1999). Similar results were reported by Cavallero *et al*, (2002).

Table (4): Effect of adding FA and ALF on the baking quality and freshness of pan bread stored at room temperature.

Samples	Baking quality			Water retention capacity (Freshness)		
	Weight (g)	Volume (cc)	Specific volume	Zero time	After24hrs	After 48hrs
Control	130	275	2.12	380	320	270
Pan bread with FA	131	310	2.37	375	310	264
Pan bread with 11% ALF	132.5	280	2.11	390	325	273
Pan bread with 22% ALF	133.5	240	1.80	400	350	280
Pan bread with 33% ALF	135	180	1.33	360	280	230

4- Freshness of bread

Data in Table (4) also show the effect of addition of ALF on the freshness of pan bread which was stored at room temperature for 48 hrs. It is clear that pan bread with ALF (11 and 22%) was fresher than control or pan bread with FA under the same conditions due to its higher water retention capacity and consequently improvement of its staling rate. This might be due to higher contents of fats and proteins in ALF fortified pan bread than control or pan bread with FA and the presence of fat caused such effect. The pan bread with 11 or 22% ALF had a higher water retention capacity compared to control or pan bread with FA. Such increase can be related to a higher hydrophilic nature of proteins (Barron and Espinoza, 1993). It was noticed that fortification level of 11or 22% ALF produce pan bread with a better consistency or high texture characteristics.

5-Rheological properties of wheat flour dough and the effect of FA and ALF addition on these properties

a-Farinograph properties of dough

From the results in Table (5), it could be that water absorption, arrival time and stability time of dough increased with increasing level of added ALF compared with those of control sample or dough with FA. This increase may be due to high protein and fibers contents of ALF compared to wheat flour. Protein and fibers tend to bind more water as fibers are characterized by its high water holding capacity. Proteins and fibers in ALF may be interacting with wheat flour ingredients and added water, consequently stability of dough increased. In this respect, Kim *et al.* (1997b) reported that water absorption and stability of dough increased as rice grain dietary fibers increased. Urooj *et al.* (1998) and Hussein *et al* (2006) reported that increasing proportion of barley flour in the blend with white flour resulted in a progressive increase in water absorption, arrival time and dough stability.

On the other hand, dough development time, dough weakening and mixing tolerance index were reduced by adding ALF to wheat flour at all levels replacement while, addition of FA to wheat flour has no effect on wheat flour dough characteristics.

Similar results were reported by Kawka *et al* (1999), Cavallero *et al* (2002) and Hussein *et al.* (2006). Who indicated that water absorption and arrival time were increased while dough development and mixing tolerance index were decreased as a result of adding whole barley flour to white wheat flour.

b- Extensograph properties of dough:

As shown in Table (5) resistance to extension, dough energy and proportional number increased by adding different levels of ALF to wheat flour while, the extensibility of the dough decreased by ALF addition. This effect may be related to the presence of fibers in ALF that dilute the gluten content of dough. A viscoelastic property of wheat dough depends on gluten quality and quantity. So, as gluten content increases, viscoelastic properties are improved.

c- Falling of the used:

From data presented in Table (5), it could be noticed that the addition of ALF at any level, to wheat flour dough resulted in a lower falling number compared to that of control or dough with FA. This could be related to a higher amylase activity of ALF compared to that of wheat flour 72% (Rao *et al.*, 1985).

Table (5): Effect of added FA and ALF on rheological properties of doughs.

Parameters	Control	Dough with FA	Dough with 11% ALF	Dough with 22% ALF	Dough with 33% ALF
Farinograph test					
Water absorption(%	66	66	68.5	72	74
Arrival time(min)	1.25	1.50	1.5	2.00	3.00
Development time(min)	10	10	9	7	6
Stability time (min)	6.5	6.5	10	12	14
Weakening (BU)	120	120	90	70	60
Mixing tolerance index (BU)	90	90	40	30	25
Extensograph test					
Extensibility(E)	145	145	110	120	110
Resistance to extension(R)	165	165	305	330	380
Proportional number (R/E)	1.14	1.14	2.77	2.75	3.45
Energy (cm2)	24	24	37	57	35
Falling number					
Falling No.	338	321	254	281	305

6-Changes in folic acid content during of pan bread making

Data presented in Table (6) show the changes in FA content during processing of pan bread, where FA content in ALF was 618.13µg/100g calculated on dry weight basis. From the same table, it is evident that FA content of doughs made from wheat flour (72%) and wheat flour fortified with FA or with ALF at different levels (11,22 and 33%) was much higher. This trend could be attributed to the presence of yeasts and ALF. FA content of wheat flour (130.87µg/100g) was lower than that of such fortified with FA (350.88µg/100g) or that fortified with ALF (190.76, 251.55 and 320.89 µg/100g) at level of 11, 22 and 33%, respectively and this was due to the high content of FA in ALF.

FA content was increased in all doughs after the fermentation and this increase might be due to the synthesis of FA by yeast during the fermentation (Abd El- Lateef, 2002). All baked breads had lower level of FA as compared to the corresponding doughs.These results are in agreement with those obtained by Christine *et al.* (1997) who prepared fortified white wheat bread with FA using the sponge- dough method of bread baking as used in the commercial practice, where The bread was fortified with FA at a concentration of 1.4 µg/g flour. They found a loss of about 33%in folate content in their study. Osseyi *et al.* (2001) and Kariluoto *et al.* (2004) found a significant increase in total folate during sourdough fermentation in both wheat and rye flours. They stated that this increase was associated, mainly with the growth of yeasts which can synthesize folates and depended on type and amount of added yeasts. On the other hand, Gujska and Majewska, (2005) found that there was a decrease in FA content during the processing flour rye and wheat to bread.

Table (6): Effect of fermentation and baking on folic acid contents in pan bread pan bread fortified with FA and pan bread with ALF (µg/100gm).

Samples	Sponge dough method		
	After mixing	After fermentation	After baking
ALF	618.13		
Control	130.87	156.22	148.18
Pan bread with FA	350.88	391.2 0	363.2 1
Pan bread with 11% ALF	190.76	222.29	212.33
Pan bread with22% ALF	251.55	274.95	265.06
Pan bread with 33% ALF	320.89	359.98	342.37

7-Organoleptic characteristics of pan bread :

The effects of folic acid and ALF supplementations on the organoleptic properties of pan breads are presented in Table (7). With increasing of ALF level, sensory scores for taste, aroma, mouth feel, crumb texture, crumb color, break& shred, crust color and symmetry of shape of pan bread sharply decreased. There was no significant differences between pan bread sampls containing FA and control sample with respect to all tested parameters.

With regard to taste and aroma, there was no a significant difference between control and that containing 11%ALF. A significant difference was detected when ALF level was increased over than 11%. The adverse effect of ALF level (33%) was proportional to lower scores in taste and aroma. Also, the results showed significant differences in crumb texture and crumb color between samples supplemented with 11, 22 and 33% and those of pan bread (control or fortified with folic acid). The adverse effect of ALF flour was clearer in crumb color and crumb texture. These effects may be due to a high fiber content of ALF which affect color and texture of crumb. As the level of ALF in blends was increased, crust color of breads changed from white creamy to dull brown. A significant difference in crust color was observed in all blended breads. The data suggested that the darkest color was observed in bread prepared from ALF (22and 33 % levels) blended flours. The darker crust color may be due to greater amounts of millard reactions between reducing sugars and proteins (Raidi&Klein, 1983). Also, it could be detected that, as ALF level increased adverse effect increased regarding all tested characteristics.

Table: (7): Organoleptic properties of pan bread as affected by FA and ALF additions.

Samples	Taste (20)	Aroma (20)	Mouth feel (10)	Crumb texture (15)	Crumb color (10)	Break & Shred (10)	Crust color (10)	Symmetry shape (5)
Control	18.00^{a}	18.30^{a}	9.4^{a}	13.70^{a}	8.20^{a}	8.95^{a}	8.90^{a}	4.15^{a}
Pan bread with FA	18.00^{a}	18.01^{a}	9.00^{a}	13.50^{a}	8.00^{a}	8.80^{a}	8.80^{a}	4.02^{a}
Pan bread with 11% ALF	15.00^{ab}	15.40^{ab}	7.10^{bcd}	11.90^{bc}	6.75^{b}	7.35^{b}	6.15^{bc}	3.18^{b}
Pan bread with22% ALF	12.5^{bcd}	15.00^{ab}	6.95^{bcd}	11.00^{c}	5.50^{b}	6.20^{b}	5.30^{c}	2.75^{bc}
Pan bread with 33% ALF	11.10^{bc}	14.50^{b}	3.70^{d}	9.10^{c}	3.40^{c}	5.80^{b}	5.25^{c}	$2..75^{bc}$
L.S.D at 0.05	3.233	3.616	1.413	1.775	1.417	1.269	1.113	0.772

In this respect, Zumbado *et al.* (1997) reported that sensory scores decreased with increasing level of rice bran. The deterioration in the crumb texture and crumb color of wheat bread due to such supplementations was observed by other workers (Rathna&Neelakantan, 1995; Sharma *et al.*, 1999; Carson *et al.*, 2000).

CONCLUSION

A high FA with better nutritional value bread could be produced by replacing wheat flour with ALF till 22% without affecting technological and sensory properties. Meanwhile, it was noticed that deteriorative effects have occurred a more than 22% ALF was added. There were no significant differences between pan bread containing FA and control sample with respect to all tested parameters.

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تأثير إضافة دقيق الاليرون كمصدر لحمض الفوليك على جودة الخبز الافرنجى

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الملخص العربي

تم إعداد دقيق الاليرون واستخدامه في تدعيم دقيق القمح استخلاص ٧٢% بنسب ٠-١١-٢٢و٣٣% كمصدر لحمض الفوليك(٦١٨,١٣ميكروجرام/١٠٠جرام) لإنتاج خبز افرنجي غنى فى حمض الفوليك٠وذلك بالطريقة الأسفنجية ٠إضافة حمض الفوليك تمت بذوبانه في جزء من الماء المستعمل في عملية العجن وتم تقيم التركيب الكيميائى والخواص الريولوجية واللون وجودة الخبز والصفات الحسية ونسبة الفوليك في الخبز الناتج وأوضحت النتائج المتحصل عليها الآتى:

إن الاليرون مصدر غنى فى حمض الفوليك وانه تم رفع نسبته فى الخبز الافرنجى من ١٤٨,١٨ ميكروجرام/١٠٠جرام فى العينة الكنترول الى٣٤٢,٣٧ ميكروجرام/١٠٠جرام فى الخبز المحتوى على ٣٣%اليرون، فى حين أن إضافة حمض الفوليك إلى الدقيق رفعت نسبته فى الخبز الناتج إلي ٣٦٣,٢١ ميكروجرام/١٠٠جرام٠ ويعتبر الاليرون مصدر جيد للبروتين والدهن والالياف والأملاح المعدنية مما أدى إلى رفع القيمة الغذائية للخبز الناتج .ولقد تاثرت الصفات الريولوجية للعجائن المحتوية على الاليرون سلبيا ٠كما ان اللون ازداد دكانه في الخبز الناتج بزيادة نسبة الإضافة من الاليرون ولقد قل كل من حجم الرغيف والوزن النوعي بزيادة نسبة الإضافة فى حين ان الطزاجة زادت بزيادة نسبة الاليرون حتى ٢٢%٠واخيرا يمكن التوصية بإنتاج خبز مقبول حسيا وذو قيمة غذائية عالية باستعمال دقيق الاليرون حتى نسبة ٢٢%فى حين ان إضافة حمض الفوليك إلى الدقيق لم يؤثر سلبيا على جودة الخبز الناتج٠