

## EFFECT OF BAKING CONDITIONS AND ANTIOXIDANTS ON ACRYLAMIDE CONTENT OF BISCUIT .

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### Abstract:

The risks of acrylamide (AA) to health and its toxic properties (neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity) were demonstrated by the Scientific Committee on Toxicity, Ecotoxicity and the Environment in 2001. Bakery products account for around 20% of human exposure to AA. Since then, due to the great consumption of dietary sources of AA among people of different ages and in different countries, worldwide efforts have been carried out to reduce the formation of the toxic molecule in foods.

The present work introduces a trial to decrease the carcinogenic compound – AA – in biscuit by using three thermal degrees as 100 °C, 150 °C, 200 °C/20 min., combined with antioxidant plant sources as citrus and cinnamon. The determination of tested samples extended to survey AA content in the local bakery products. The results of AA of collected samples showed that high AA content in local Rusk (Boksomat) (980 µg day<sup>-1</sup>) and dry bread (balady) (81 µg day<sup>-1</sup>). Whereas, the other samples less than 31 (µg day<sup>-1</sup>).

The obtained results of AA content in biscuits, showed that high significant linear relationship between AA content and thermal temperature with high significant of correlation coefficient ( $R^2 = 0.95$ ). The AA determination of tested biscuit showed that lowest values (12.9 µg day<sup>-1</sup>) were recorded at 100 °C. Also, AA content in tested biscuit either with citrus or cinnamon (13.9, 13.3 µg day<sup>-1</sup>) respectively. Whereas, the higher temperature recorded more AA values in the range of 15 to 21 µg day<sup>-1</sup> at 150 °C and 200 °C respectively. Also, no significant effects were observed for using of antioxidants. According to the obtained results, using the temperature at 100 °C are the critical degree for baking biscuit with low AA content. In the future the plan will suppose for testing another antioxidants to minimize AA.

**Keywords :** Acrylamide , Baking, Biscuit, .Thermal Treatments, Acrylamide, Antioxidants.

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### Review

Most biscuits and cookies are chemically leavened baked products. They are stable foods and have advantages such as long shelf life and good eating quality. In Egypt, like most Arabic and Islamic countries, these peoples consume bakery products from time to time, but they consume daily biscuits, sweet cakes especially during Religious events such as Aid al-Fitr after fasting month of Ramadan.

Unfortunately, most of food products under thermal treatments –as biscuit– can contain carcinogenic compounds. Acrylamide (AA) are one of them (Fig.1), which classified as “reasonably anticipated to be a human carcinogen” by the U.S. Department of Health and Human Services and as “possibly carcinogenic to humans” by FAO/WHO(2002,2007). AA have been found in a number of foods that are thermally processed.(Lineback *et al*,2012,Becalski,*et al*,2003, Mottram *et al*,2002). Its forms in food due to a reaction between the amino acid asparagines and reducing sugars such as glucose and fructose. AA formation produce during Maillard reaction, which leads to browning and flavor changes in cooked foods. AA formation occurs primarily at elevated cooking temperatures used when frying or baking (above 120 °C) and in low moisture conditions.(Tereke, *et al*,2003,Zyzak,*et al*,2003).AA has been found primarily in plant-based foods, notably potato products such as French fries and potato chips; cereal foods such as cookies, crackers, breakfast cereals, and toasted bread; and coffee.(Stadler *et al*,2002).

AA can cause cancer in laboratory animals at high doses, although it is not clear whether it causes cancer at the much lower levels found in food. But the meta-analysis studied 25 relevant studies chosen from a much larger database. Relative risks were calculated for an increase of 10( µg day<sup>-1</sup>) of AA intake and were close to 1.0 µg for all the cancers considered. None of the associations was statistically significantly increased. (Pelucchi *et al*. 2011).

Mitigation of AA levels in foods has been based on voluntary and collaborative actions involving both national authorities and companies producing AA containing foods (Lineback *et al*., 2012).The mitigation of AA in bakery products and cereal have been investigation by workers (Claus *et al*,2008 and Sadd *et al*.2008).Other minor ingredients ,such as calcium, glycine ,antioxidants ,phytic acid and organic acids ,have been tested before by different investigators (Capuano *et al*.2009).

The EC (European Commission) 2011 has been actively involved in these extensive efforts since 2002. Table (1) shows summary of reported amounts of AA in different products and product groups as adapted from Mills *et al*. (2009) and Petersen & Tran (2005). The literature is lacking in investigation of the effects of Low temperatures on the formation of AA in bakery products.

Therefore ,the present work introduce trials to prepare biscuit under different thermal treatments besides using antioxidants plant sources with traceability of AA in tested samples ,besides introduce survey of AA in some selected bakery products in Egyptian markets.

## **MATERIALS AND METHODS**

### **1-Survey AA content in local bakery products:**

Six types of bakery products were collected from local markets in Cairo, Egypt. Four replicates were used for determination of AA. These samples were collected from local markets, it involved local Rusk (Boksomat), local biscuit, imported biscuit, corn flakes, dry bread (balady) and corn flakes. Samples were grinded for preparation of AA determination.

#### **2.1. Raw Materials**

Refined wheat flour was procured from local market in Cairo, Egypt. soft flour (750 g), Virgin butter (500g), sugar (200g), milk (100cm<sup>2</sup>), ammonia bicarbonate (20g) and vanilla (5.0g). All ingredients were mixed well to add three eggs, half hanging, for normal biscuit. Whereas, suitable amount of citrus peel powder and cinnamon were used separately as common Egyptian methods.

#### **2.2. Preparation of Biscuits**

Biscuits were prepared according to the American Association of Cereal Chemists (AACC, 1983) method No. 10.52 with a slight modification as Egyptian method. The preliminary step for the preparation of biscuit was creaming. The creaming was carried out by using shortening i.e. hydrogenated fat and sugar in Hobart Mixer for 4 min and subsequently added a mixture of refined wheat flour and baking powder along with measured quantity of water in the Hobart mixer. The mixing was done for five minutes till the soft dough was formed. The prepared dough was subjected to sheeting of 4 mm thickness manually. Finally sheets were cut to 4.5 cm diameter by using the die and were subjected to baking at 100°C, 150°C and 200°C for 20 min. Then, all biscuits samples were cooled at room temperature separately.

#### **2.3. Sampling and treatment:**

The nine main treatments of prepared biscuits can be mentioned as follows:

- 1-The first one as thermal treatment at 100 only, then 100°C + citrus or cinnamon.
- 2-The second one thermal treatment at 150 °C only, then 150 °C + citrus or cinnamon.
- 3-The third one thermal treatment at 200 °C only, then 200 °C + citrus or cinnamon.

### **2. Acryl amide analysis:**

Samples were allowed to swell adding water in an amount normally corresponding to 3 times the weight of the sample (more for exceptionally dry samples). Preparation, 10 g of the homogenate was weighed into a 100ml centrifuge glass with a screw cap and thoroughly mixed with 40 ml of 1-propanol. All analysis steps were done according to Biedermann *et al* (2002a) using GC-MS.

### 3-Statistical analysis

A linear regression analysis was applied using Excel program, a Microsoft computer to get value of  $R^2$ . All values were average of three replicates with standard deviation.

#### Results:

#### AA content of selected bakery samples

As shown in Table (2) the high value of AA were observed with Local-Rusk (Boksomat) near  $980 (\mu\text{g.kg}^{-1})$ , then balady bread ( $81 \mu\text{g.kg}^{-1}$ ). The first one usually produce with sesame seed (*Sesamum indicum*) and dry even brown color. Also, dry bread- usually use for diabetic with brown color. Unfortunately, soft balady bread contained different black or brown spots besides are considered the backbone in breakfast for most of Egyptian peoples daily. The bakery products with brown colour usually contain high content of AA. its results due to activate Maillard reaction products consequently produce AA as showed by different workers. Carcinogenicity of brown spots recorded high values (Haase *et al.*, 2012). Also, high content of AA in crust brown layer of bread was observed by workers (Springer, *et al.*, 2003, Surdyk, *et al.*, 2004; Mustafa, *et al.*, 2005, Bråthen & Knutsen, 2005).

Several factors related to food composition and processing conditions have been shown to influence the formation levels of AA and also other quality characteristics such as browning in thermally processed foods (Friedman, 2003). Significant correlations have been reported between AA and color formations in foods during heating (Gökmen *et al.* 2006a).

Whereas, the rest bakery products contain less content around  $30 (\mu\text{g.kg}^{-1})$  or less. The results of AA content in the rest bakery products were observed as like in Table (1). Mitigation of AA can be done by sodium hydrogen carbonate, addition of asparagine to ferment the dough with yeast, addition of glycine reduces the content of acrylamide in cereal and potato products (Bråthen, *et al.*, 2005).

#### Effect of thermal combined antioxidants on AA content of biscuits:

As shown in Table(3), Fig.(2), the results proved increasing thermal treatments was in positive relationship ( $y=0.074x+4.8$ ) with AA content. The correlation coefficient values were high significant ( $R^2=0.95$ ). According the resulted linear equation the rate of producing AA ( $0.074 \mu\text{g.kg}^{-1}$ ) per each one degree of temperature ( $^{\circ}\text{C}$ ).

In the same time no reduction significantly was observed due to using citrus or cinnamon as natural sources for antioxidants (fig.3, Table 3). The explanation may be due degradation of antioxidants by thermal treatments or during dough. But the best result of lower values of AA ( $12.7-13.3 \mu\text{g.kg}^{-1}$ ) at  $100^{\circ}\text{C}$  for baking biscuit. This degree may be a critical temperature/time zone (20 min.) where AA is formed at low rate than it is destroyed.

## Discussion:

The survey of local markets bakery products showed that high content of AA in local Rusk ( $980 \mu\text{g.kg}^{-1}$ ), dry bread ( $81 \mu\text{g.kg}^{-1}$ ) due to crust surface. Same trends are recorded in Table (1), in bakery product. Rusk surface usually contain high AA content. Many workers showed that AA is found in all baked goods and the most important products are bread, crisp bread, gingerbread, crackers, cookies, and biscuits. AA content was determined in bakery products ( $70 - 430 \mu\text{g.kg}^{-1}$ ), crisp bread ( $800-1200 \mu\text{g.kg}^{-1}$ ), biscuits and crackers ( $30-3200 \mu\text{g.kg}^{-1}$ ) (friedman,2003).

Nevertheless, bread was calculated to contribute more than 10% to the total dietary exposure of AA in Egypt because of its frequent and large consumption (Shehata and Mohamed ,2015). In total, about 30% of the dietary exposure originates from bakery products (Boon et al., 2005). Springer et al. (2003) reported that the AA content of crisp bread. Temperature has an important role in the formation and elimination of AA. It is well-known that AA forms in bakery products as biscuits at high temperatures ( $>120^\circ\text{C}$ ) (Grob et al 2003; Rydberg et al.2003), unfortunately AA can form less than  $100^\circ\text{C}$  (Eriksson S,2005).

Factors of greatest importance in this regard are temperature and air humidity profile during baking. Compared with conventional baking conditions, optimized conditions may result in a 50% reduction in AA formation. It is a common observation that the formation of crust starts when the surface temperature of the bread is over  $100^\circ\text{C}$ . A dehydration process accompanies crust formation.

In the present work ,temperature degrees were  $100^\circ\text{C}$ ,  $150^\circ\text{C}$  ,  $200^\circ\text{C}$  /20min. for biscuit baking. The lower content of AA was recorded at low temperature. Its worth to mention that biscuit samples were grinded to prepare for determination AA ,therefore no crust resulted in tested biscuit samples. Thereby, the obtained results proved that  $100^\circ\text{C}$  are the critical temperature to get biscuit with low content of AA. In the same time the resulted biscuit was light color free from browning area or crust.

However, such crust colors in biscuits would be considered as unacceptable by the consumer.

Using antioxidants in the present work ,were used as citrus or cinnamon powder ,The obtained results showed no high significant effect on reducing AA in biscuits .These ,may be resulted from thermal effects for destroying effect of antioxidants or low used concentration of antioxidants. Some workers succeed to decrease AA content by using of bamboo leaves (AOB) and extract of green tea greatly reduced the AA (Zhang and Zhang,2007), rosemary but failed by using spice dittany ( Hedegaard et al.,2008).

The most recent comprehensive review and meta-analysis of dietary AA role in human cancer was published by a team of European researchers (Pelucchi *et al.* 2011). The meta-analysis studied 25 relevant studies chosen from a much larger database. Relative risks were calculated for an increase of  $10 \mu\text{g day}^{-1}$  of AA intake and were close to 1.0  $\mu\text{g}$  for all the cancers considered. None of the associations was statistically significantly increased.

Therefore, these studies need more research for decrease AA or the other toxic Maillard products by using new techniques during dough by addition of natural antioxidants to reduce the carcinogenic effects.

### **Conclusion :**

There is a strong positive correlation between baking temperature, time, AA formation, many workers used some replacements of reducing sugars with sucrose and the use of flours with a lower asparagine content, others chemical may decrease the AA content of baked foods. The present results high light alarm on presence of carcinogenic asAA in local Egyptian bakery products. Our work succeed to use low temperature to minimize AA in biscuits. The results must need more efforts under big authority responsible consumer health to reduce the levels of carcinogenic compounds as AA in food.

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**Table 1** Summary of reported amounts of acrylamide in different products and product groups. Adapted from Mills et al. (2009) and Petersen & Tran (2005)

Product/product group	Acrylamide range ( $\mu\text{g kg}^{-1}$ )
Potatoes (raw)	<10–<50
Potato chips/crisps	117–4,215
French fries/chips	59–5,200
Bakery products and biscuits	18–3,324
Breads	<10–3,200
Bread (toast)	25–1,430
Breakfast cereals	<10–1,649
Other fruit and vegetable products	<10–70
Chocolate products	<2–826
Roasted coffee	45–935
Coffee substitute	80–5,399
Coffee extract/powder	87–1,188
Meats	<10–116
Dairy products	<10–100
Baby food and infant formula	<10–130

**Table(2)AA content of selected Egyptian bakery products**

Selected samples	AA $\mu\text{g.kg}^{-1}$
Local-Rusk(Boksomat)	980±0.2
Imported biscuits	29±0.4
Local-Biscuits	31 ±0.5
Corn Flakes	19±0.4
Local- loaf(Fino)	21±0.1
Dry bread(Balady)	81±0.3

**Table(3)Thermal treatments combined antioxidants effects on AA content of biscuits.**

Thermal treatment	AA ( $\mu\text{g.kg}^{-1}$ )
-First treatment-	12.7±0.2
-100°C	13.9±0.1
-100 °C+citrus	13.3±0.2
-100 °C+Cinnamon	
Second treatment	14.90±0.2

-150°C	14.98±0.3
-150 °C+citrus	14.70±0.5
-150 °C+Cinnamon	
Third treatment	
-200°C	20.1±0.1
-200 °C +citrus	20.7±0.3
-200 °C+ Cinnamon	21.2±0.4

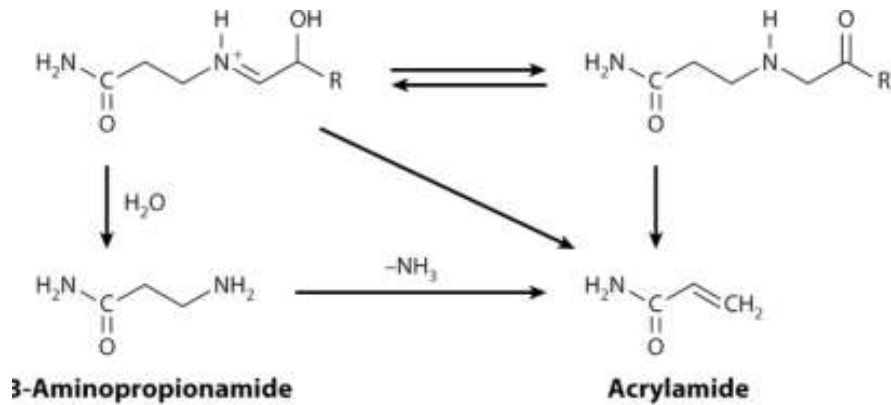
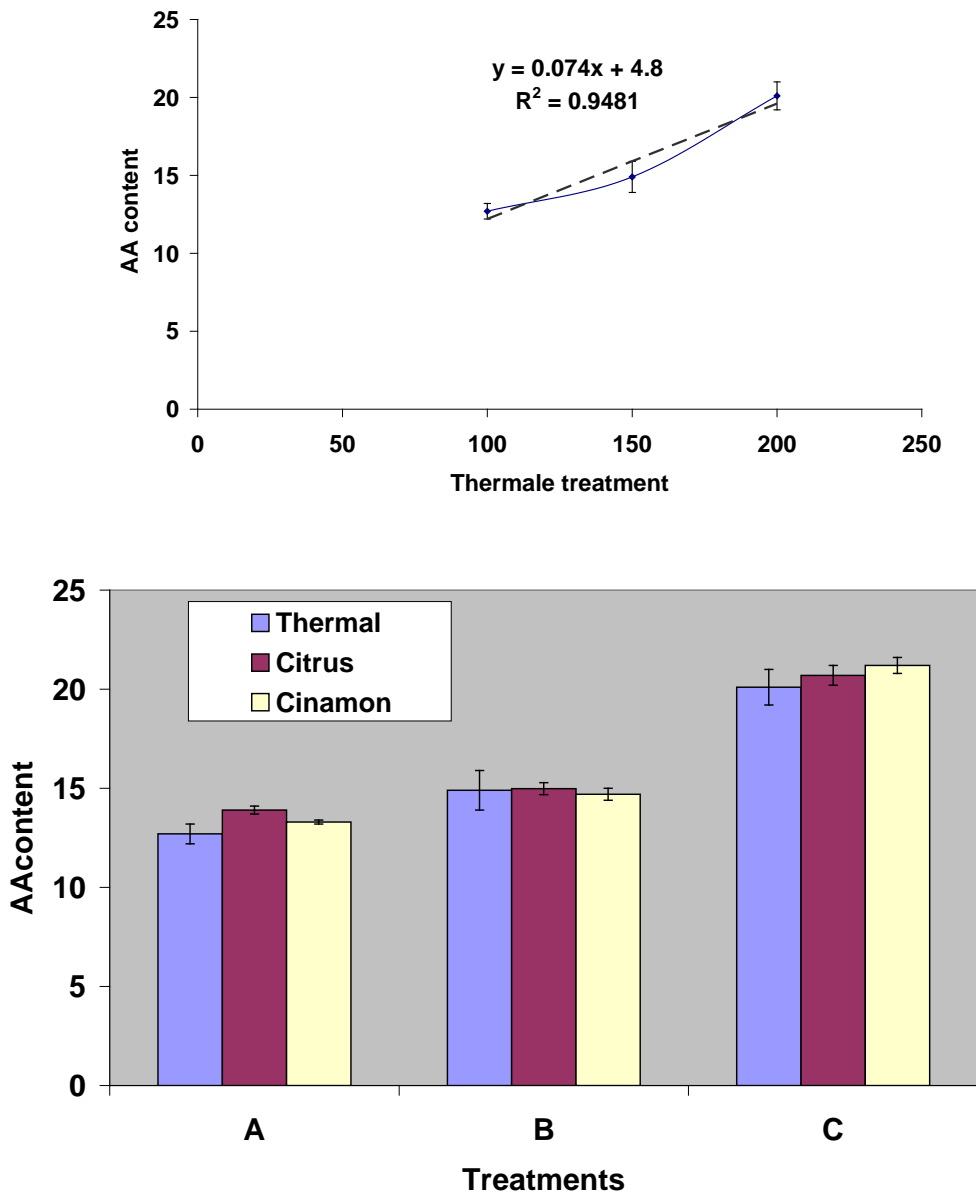


Fig. 1: Formation of acrylamide.

Fig.( 2 ) Change in AA content ( $\mu\text{g.kg}^{-1}$ ) of biscuit during baking process



**Fig.(3 ) Effect of thermal treatments combined with antioxidants on AA content ( $\mu\text{g.kg}^{-1}$ ) of biscuit.**

**A=100°C +citrus or cinnamon ,B=150°C + citrus or cinnamon ,C=200 °C + citrus or cinnamon**