3. MATERIALS AND METHODS

3.1. Materials:-

3.1.1. Flaxseeds: were obtained from Food Technology Research Institute (FTRI), Agriculture Research Center (ARC), Giza, Egypt.

3.1.2. Okra: Fresh immature pods of okra were purchased from a local market in Alexandria city.

3.1.3. Butter: butter, (Lurpak) from Oral Foods Amba, Dc-8260 Vebe Geh, Denmark, used as a fat source in the production of cakes and cookies was purchased from local market in Alexandria city.

Wheat flour (72%extraction), Sugar, brown sugar, egg, baking powder, vanillin and bicarbonate: were purchased from the local market in Alexandria city.

3.2. Methods:-

3.2.1. Preparation of flaxseed gum: Flaxseed gum was extracted according to the method described by Bhatty (1993) with some modifications. Flaxseeds were mixed with water (1:20 w/v) and stirred for 30 min. at 100˚C then left for 8hr. at room temperature. The extract was separated by filtration using glass wool. The gum solution was then concentrated on a rotary evaporator at 40˚C, where the final measured viscosity was 46.4 m pas*s (Millipascal) at 100 RPM (Spindle RV 02).

3.2.2. Preparation of okra gum: Okra gum, derived by water extraction from immature pods of the okra plant (*Hibiscus escutentus* L.), was prepared according to the method described by Romanchik *et al.,* (2002) with some modification as follows:

Seeds were removed from okra pods using a knife as they contain small traces of fat. In a covered saucepan 150g okra was boiled in 474g distilled water for 15 minutes. The mixture was strained to remove okra, and then was cooled to room temperature, where the final measured viscosity was 136 m pas*s (Millipascal) at 100 RPM (Spindle RV 02). This was reached after a serial of preliminary studies to reach the atmost viscosity with out affecting the organoleptic properties.

3.2.3. Processing of cakes and cookies:

3.2.3.1. Cake processing:

Cakes were processed according to the method of Shaltout *et al*., (2004). The formulation of cake consisted mainly of: One hundred gram flour (72%extraction), 100g sugar, 100g butter, 100g egg, 2.5g baking powder, 3g vanillin (Table 1). The procedure of processing

was as follows: The dry ingredients (i.e. flour, baking powder, and vanillin) were weighed and sifted together. The butter and sugar were creamed for 1min. on low speed in Goldi mistral (model HM301) and then they were creamed for additional 1 min. at the high speed. Eggs were added gradually while creaming and whole mixture was creamed for additional 2 min. on the high speed. After scraping down the mixing bowl, the batter was mixed again for 1 min. at the high speed. The cake batter was immediately deposited into rectangular pans (size 23.8X8.8cm, bottom diameter 4cm), and baked at 170℃±10℃ for 25min. in preheated oven. The cakes were allowed to cool for 1hr. and were then removed from the pan. The cooled cakes were packed in polypropylene bags at room temperature before physical, chemical and sensory analyses were performed. For low-fat application, the fat replacers (okra gum and flaxseed gum) were added in the creaming phase to replace 25, 50, 75 and 100% of butter used in cake formula as shown in Table (1).

3.2.3.2. Cookies processing:

Cookies were prepared according to the method of (Smith, 1972) which consisted of: 297.9g flour (72%extraction), 131g sugar, 143g brown sugar, 100g butter, 46g egg, 3.3g baking powder, 9.5g bicarbonate, 2g vanillin (table 2). To prepare the control treatment, the butter and sugar were creamed for 2 min. using Goldi mistral (model HM301) till smooth and evenly blended. Eggs with vanillin were added gradually while mixing for additional 2 min. The blended dry ingredients (flour and baking powder) were mixed and sifted, then added to the mixture and beaten for 4min. The dough was then cooled in the refrigerator for 2hr. after that, dough was flattened with hand and shaped to 0.4cm. Thickness, then they were cut using a 3 cm diameter round cutter. Cookies were transferred to baking sheet and baked for 10 min. at 200˚C. After baking, cookies were left from baking sheets with a wide spatula and were left to cool. For low-fat formulations, okra gum and flaxseed gum were used to replace 25, 50, 75 and 100% of butter (by weight) and they were mixed together in the creaming step as shown in Table (2).

3.3. Analytical methods:

3.3.1. Proximate chemical composition:

Chemical constituents of bakery products cakes and cookies were determined as follows:

3.3.1.1. Moisture content:

The moisture content in samples was determined by drying samples in an air oven 105±5℃ till constant weights according to the method **(**AOAC, 2000**).**

Table 1: Formulations of low-fat cake prepared using fat replacers

Ingredient	Control (gm)	25%		50%		75%		100%	
		A	B	A	B	A	B	A	B
Flour	100	100	100	100	100	100	100	100	100
Sugar	100	100	100	100	100	100	100	100	100
Butter	100	75	75	50	50	25	25	-	-
Fat replacer	-	25	25	50	50	75	75	100	100
Egg	100	100	100	100	100	100	100	100	100
Baking powder	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vanillin	3	3	3	3	3	3	3	3	3

(A) low-fat cakes prepared with okra gum, **(B)** low fat cakes prepared with flaxseed gum.

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Table 2: Formulations of low-fat cookies prepared using fat replacers

Ingredient	Control (gm)	Replacement level							
		25%		50%		75%		100%	
		A	B	A	B	A	B	A	B
Flour	297.9	297.9	297.9	297.9	297.9	297.9	297.9	297.9	297.9
Sugar	131	131	131	131	131	131	131	131	131
Brown sugar	143	143	143	143	143	143	143	143	143
Butter	100	75	75	50	50	25	25	-	-
Fat replacer	-	25	25	50	50	75	75	100	100
Egg	46	46	46	46	46	46	46	46	46
Baking powder	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Bicarbonate sodium	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Vanillin	2	2	2	2	2	2	2	2	2

(A) low-fat cookies prepared with okra gum, **(B)** low fat cookies prepared with flaxseed gum.

3.3.1.2. Crude protein

Crude protein content was determined by the micro Kjeldahl´s method as described by (AOAC, 2000). A sample (700mg) was placed in Kjeldahl digestion tube. 5g K_2SO_4 +0.5g $CuSO_4$ and 25ml conc. Sulphuric acid were added to the sample. The sample was digested for one hr. 20ml deionized water was added to the sample after allowing it to cool. After adding 25ml NaOH(40%),the sample was then distilled and the ammonia liberated was collected in boric acid and titrated with 0.1N hydrochloric acid. A blank was prepared and treated in the same manner except that the tube was free of sample.

Protein percentage was calculated according to the formula

Crude protein (%) = (sample titer- blank titer) x14x5.75x100/sample weight. Where, 14is molecular weight of nitrogen and 5.75is the nitrogen factor.

3.3.1.3. Crude fat

Five grams of dried sample remaining after moisture determination were transferred to a thimble and plugged the top of the thimble with a wide of fat- free cotton. Each sample was tested by running through soxhlet apparatus with n-hexane (60-80) for 16hr (AOAC, 2000). The crude fat was calculated using following formula

Crude fat (%) = Extracted fat /sample weight) x100.

3.3.1.4. Total ash

The sample was incinerated in muffle furnace according to the method of (AOAC, 2000). Sample of 1g was subjected to pre-ashing, and then the ashing was completed in an electric muffle at 550C̊ to constant weight.

3.3.1.5. Carbohydrate

Carbohydrate was determined by difference as described in (AOAC, 2000). Carbohydrate was calculated as following
Carbohydrate (%) =100-(Crude protein+ Crude fat+ Total ash).

3.3.1.6. Caloric value:

Caloric value of products was calculated according to the following equation:

E=4(total carbohydrate%+ protein %) + 9(fat %)

Where E=Calories per 100g product.

3.3.2. Physical measurements of cakes:

The pH of cake was measured according to the method of AOAC (2000) using Micro Processor pH Meter (HANNA instruments pH 2011). Cake was weighed after removal from the pan. Cake volume was measured by rapeseed displacement after cooling for 1hr. at room temperature (~25ºC) (AACC, 1983). The height (cm) was measured in the center of the cake. The specific volume of each cake was determined as the ratio of the volume to its weight (Pong *et al*., 1991).

3.3.3. Physical properties of cookies:

After cooling, cookies were subjected to physical measurements (i.e. width and height) according to the method described by **(**A.A.C.C., 1983**)**. As follows: cookies were laid edge to edge and width was measured. They were rotated 90° and remeasured to obtain average diameter (width, W). Cookies were stacked on top of one another and thickness (height) was measured, restacked in different order and remeasured to get average thickness (T). Average weight of 5 cookies was measured in grams. Volume of cookies was measured in centimeter cub (cm3) by rapeseed displacement. The specific volume of each cookie was determined as the ratio of the volume to its weight.

3.3.4. Viscosity measurement:

The viscosity of okra gum and flaxseed gum were measured using viscometer model (Brookfield DV-II+Pro). Measurements were in m pas*s (Millipascal) at 100 RPM (spindle RV 02).

3.3.5. Texture profile analysis:

Crumb texture was determined by a universal testing machine (Cometech, B type, Taiwan), provided with software. An Aluminum 40 mm diameter cylindrical probe was used in a "Texture Profile Analysis" (TPA) double compression test to penetrate to 50% depth, at 1mm/s speed test. Firmness (N), gumminess (N), chewiness (N), adhesiveness (N.s), cohesiveness, springiness and resilience were calculated from the TPA graphic. Both, springiness and resilience, give information about the after stress recovery capacity. While, the former refers to retarded recovery, the latter concerns instantaneous recovery (immediately after the first compression, while the probe goes up). Texture determinations were carried out, after removing the crust, in (40*40*30) mm-sized samples.

Crumb texture in cookies was determined by penetration test which was performed with a universal testing machine (Cometech, B type, Taiwan) operated at a crosshead speed of 100mm min^{-1}. The force needed to penetrate the sample with a flat ended probe (1mm thickness) to 3mm of the sample height was registered. All measurements were performed at ambient temperature 20°c **(**Bourne, 2003**).**

3.3.5.1. Calculations of texture profile analysis:-

3.3.5.1.1. Hardness: hardness or firmness, is the force (N) required to compress the pre-set distance and is determined directly from the experimental data (FD) (Fig 1).

3.3.5.1.2. Cohesiveness: cohesiveness is determined from the strength of the internal bonds in the food structure and is affected by moisture content. It can be determined by Eq (2) (Figure 1).
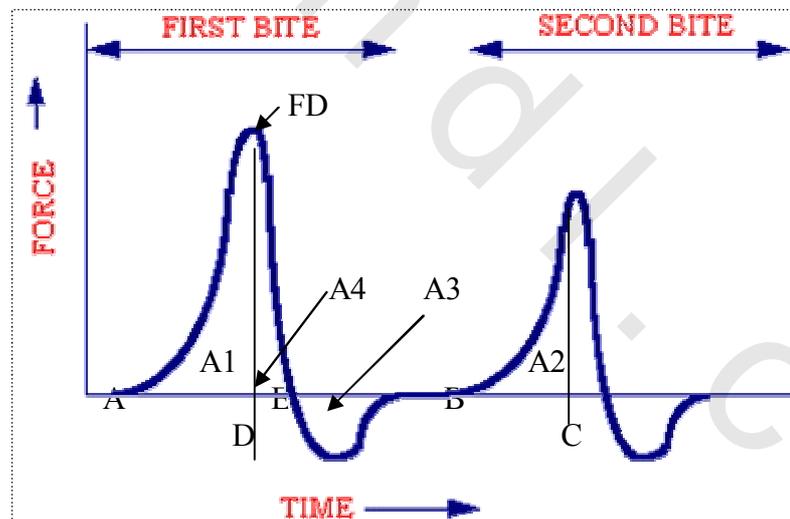
Cohesiveness = A_2/A_1 (2)
Where: A1 and A2 are areas under TPA curve (refer to Figure 1).

3.3.5.1.3. Gumminess: gumminess is the force required to disintegrate a semi-solid food before it is ready for swallowing (Rosenthal, 1999). It can be determined by Eq. (3) to experimental data (Figure 1).

Gumminess = FD (A_2/A_1) = (hardness x cohesiveness)   (3)
Where: FD is the force (N).

Figure 1: typical experimental data from texture profile analysis on cake sample.

3.3.5.1.4. Chewiness: chewiness is the force required to chew a solid food until it is ready for swallowing. It can be determined by Eq. (4) to the experimental data (Figure1).
Chewiness = FD (A_2/A_1) $(t_{B:C} / t_{A:D})$ = (gumminess x springiness)   (4)
Where: $t_{B:C} = t_B - t_C$ = time to reach B- time to reach C (refer to Figure 1)
      $t_{A:D} = t_A - t_D$ = time to rich A – time to reach D (refer to Figure 1).

3.3.5.1.5. Springiness: springiness describes how the crumb reacts following removal of a compressing force. It is, therefore, related to the strength of the crumb cell network and is considered to be a reliable indicator of the onset of staling (Cauvain &Young, 2000). Springiness can be determined by Eq. (5) (Figure 1).

Springiness = $(t_{B:C}/t_{A:D})$

3.3.5.1.6. Resilience: resilience is how well a product " fights to regain its original position". The calculation is the area during the withdrawal of the first compression, divided by the area of the first compression. Eq.(6) to the experimental data (Figure 1).
Resilience = A_4/A_1 (6)

3.3.6. Sensory evaluation of cakes& cookies:

  Eight trained panelists of staff member of Food Science department evaluated cakes for colour, flavour, texture, over all acceptability (Mansour *et al.,* 2003**)**. A 5-point hedonic scale was used to rate the sensory properties. The 5 choices on the hedonic scale were: like extremely, like very much, like moderately, dislike moderately, dislike very much. Every panelist was asked to test and check how much did he/she like the characteristic under test and also to show their attitude by checking the point that best described their feeling about the characteristic. After that, their ratings were given numerical values ranging from 5(for like extremely) to 1(for dislike extremely).

3.3.7. Statistical analysis:
  Data of physical measurements, chemical analysis and sensory evaluation of bakery products were analyzed by analysis of variance using General Linear Model (GLM) procedure within a package program of Statistical Analysis System (SAS 2001). Means were separated using Duncan's test (Duncan 1955) at a degree of significance ($P \leq 0.05$). The factorial analysis was used to evaluate the effects of fat replacer type and level on the physical properties, proximate chemical composition and sensory characteristics