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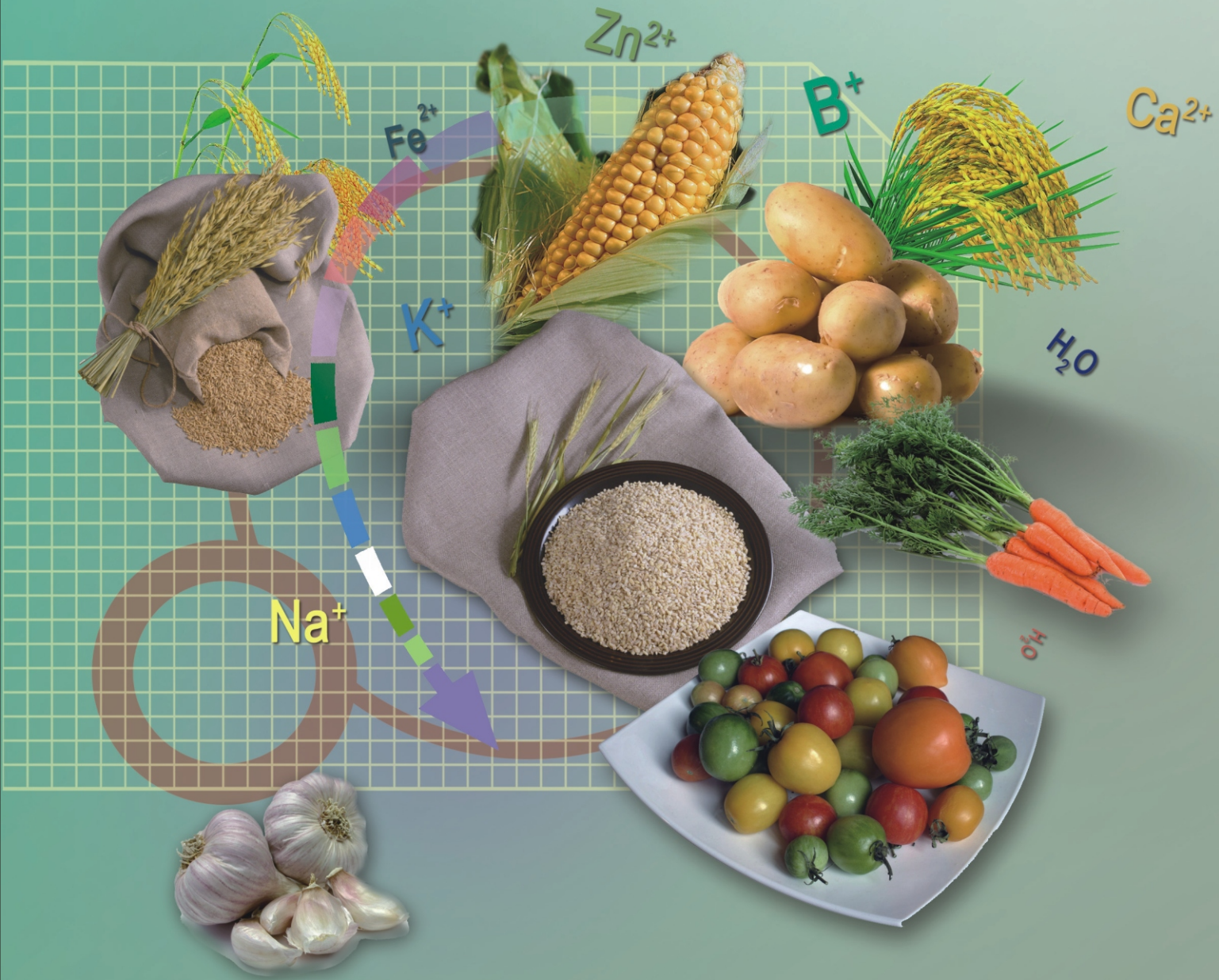

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Sodium Bicarbonate—A Potent Ergogenic Aid?

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ABSTRACT

This report aims to look at the role of Sodium bicarbonate as a potent Ergogenic aid and its role in improving the performance of athletes. It includes the mechanism of action of sodium bicarbonate during high-intensity exercise. The report also shows the various types of athletes who can be benefited from sodium bicarbonate loading, evidences for improvement in performance, conflicting evidences, recommended dosages and side-effects for bicarbonate loading.

Keywords: *Sodium Bicarbonate*, *Ergogenic Aid*, *High-Intensity Exercise*, *Anaerobic Glycolytic System*

1. Introduction

An ergogenic aid is defined as any substance, food, chemical, or training method that helps the body work harder and perform better. Ergogenic aids are any external influences that can positively affect physical or mental performance [1]. These include mechanical aids, pharmacological aids, physiological aids, nutritional aids, and psychological aids. Athletes at all levels of competitions are constantly striving for a means to obtain a leading edge over their opponents and many of them use ergogenic aids to improve their energy and performance [2]. Ergogenic aids may directly influence the physiological capacity of a particular body system thereby improving performance, remove psychological constraints which impact performance, and increase the speed of recovery from training and competition [3].

The term Ergogenic means "to produce work". Nutritional ergogenic aids are dietary manipulations that can increase physical power or energy production, enhance mental strength, or provide a mechanical edge and thereby improve sport performance [4]. Dietary manipulations encompass 3 major efforts: alteration of food choices, addition of macronutrients for specific uses in sports and exercise, addition of micronutrients for specific uses in sports and exercise [3].

2. Energy System for High-Intensity Exercise

Of the 3 primary energy pathways, the anaerobic glycolytic system provides fuel sources, primarily carbohydrates, for high-intensity exercises (exercises lasting between 20 seconds up to about 90 seconds). Anaerobic glycolysis provides the primary fuel source for exercise of near-maximal intensity lasting longer than about 30 seconds. High-intensity exercises (anaerobic work) involve the breakdown of carbohydrates (muscle glycogen). As the glycogen stores are rapidly depleted, there is a resulting increase in hydrogen ion (H^+) concentrations and lactic acid in both the muscle and blood. It is this increase in H^+ concentration (drop in pH) that causes a progressive increase in the acidity of the muscle cells (intracellular environment). The increase in H^+ produces, among other reactions, an inhibition of calcium release from the sarcoplasmic reticulum and an inhibition of the interaction between actin and myosin [5]. The drop in pH as a result of lactic acid accumulation is thought to inhibit the resynthesis of adenosine triphosphate (ATP) as well as inhibit muscle contraction. This in turn results in muscular fatigue defined as a decrease in force production in the presence of increased perception of effort and an inability to maintain high exercise intensities [6,7].

3. Sodium Bicarbonate

Sodium bicarbonate or sodium hydrogen carbonate is the chemical compound with the formula $NaHCO_3$. Sodium bicarbonate is a white solid that is crystalline but often appears as a fine powder. It has a slight alkaline taste resembling that of washing soda (sodium carbonate). It is a component of the mineral natron and is found dissolved in many mineral springs. The natural mineral form is known as nahcolite. It is also produced artificially. Since it has long been known and is widely used, the salt has many related names such as baking soda, bread soda, cooking soda, bicarbonate of soda. Colloquially, its name is shortened to sodium bicarb.

4. Role of Sodium Bicarbonate during High-Intensity Exercise

During high-intensity exercise, muscles keep up with the demand for energy by converting some carbohydrate to lactic acid. A build-up of acid inside the muscle cells is one of the factors responsible for fatigue. This system's total capacity is limited by the progressive increase in acidity within the muscles caused by the accumulation of lactate and H^+ ions. Increased acidity ultimately inhibits energy transfer and the ability of the muscles to contract, leading to a decrease in exercise performance. Reducing the build-up of acid should reduce fatigue and allow the athlete to go faster or further. The body's defences against an increase in acidity are the bicarbonate "buffers", which help to neutralize the acid produced by intense exercise. The natural bicarbonate supply, part of the body's buffering system, provides a rapid first line of defence against this increased acidity.

5. Acting Mechanism

Sodium bicarbonate is an alkalising agent and therefore reduces the acidity of the blood (known as a buffering action). By buffering acidity in the blood, bicarbonate may be able to draw more of the acid produced within the muscle cells out into the blood and thus reduce the level of acidity within the muscle cells themselves. This in turn could delay the onset of fatigue [8]. Studies [9,10] have confirmed that increased extracellular pH and higher bicarbonate raise the H^+ and lactic acid efflux from active muscles. This is due to an increase in the activity of the Lactic acid/H^+ co-transporter, which becomes more active as the intracellular/extracellular H^+ gradient increases, during contraction as well as during recovery. It has been suggested that this mechanism causes a decrease in muscular fatigue, delaying the decrease in pH level and leading to a greater contractile capacity of the muscular tissue involved by means of enhanced muscle glycolytic ATP production. It has been proven that all of these metabolic perturbations imply a shift in muscle metabolism toward anaerobic energy production, which is especially advantageous during high-intensity exercise [5].

Taking a sufficient quantity of sodium bicarbonate (baking soda) before high-intensity event makes the muscles and blood less acidic during the event and can enhance physical performance [11].

6. Athletes Benefiting from Sodium Bicarbonate Loading

Athletes have been practising "soda loading" or "bicarbonate loading" for over 70 years in an attempt to delay the onset of muscular fatigue during prolonged anaerobic exercise. The specific athletes who might stand to benefit from bicarb supplementation are those who typically compete in events that last between 1 and 7 minutes, *i.e.* 400 m - 1500 m running, 100 m - 400 m swimming, sprint cycling, kayaking, rowing and canoeing events at intensities that fall between 80 and 125% of peak maximal oxygen uptake, and many team sports with their repeated nature of high intensity exercise. All these events stress the anaerobic glycolysis system significantly and produce a lot of acidity. Sports that are dependent on repeated anaerobic bursts may also benefit from bicarbonate loading [6,8].

7. Evidences for Improvement in Performance

A study done by McNaughton *et al*. [9], among moderately trained female athletes, showed significant improvement in both work and power output, during high intensity exercise of 60 second duration. The ingestion of sodium bicarbonate in the experimental trial had the desired effect of raising blood bicarbonate levels by 60% above the resting bicarbonate value.

According to Stellingwerff [12], a meta-analysis of 29 studies on the performance effects of sodium bicarbonate, featuring predominately untrained individuals, found that bicarbonate supplementation resulted in a performance effect that was 0.44 standard deviations better than in the control trial. An improvement of 0.44 of the standard deviation would bring the 2006 average men's 800m Golden League time of 1:46.36 down to 1:45.52, which is a worthwhile improvement. In summary, most data suggest that the ingestion of 0.3 g/kg body weight of sodium bicarbonate administered in solution approximately 1-2 hours before exercise offers a small, but significant, effect on middle-distance race performance.

In one study [7] designed to simulate athletic competition, trained non-elite middle-distance runners performed a simulated 800 m race. In the alkalotic condition, they ran almost 3 s faster than in the placebo or control trials. Another report by Maughn *et al*. [13] indicated similar improvements (3-4 s) over a distance of 1500 m in runners who completed simulated races in about 4 min 15s. Although these effects on performance might appear small, they are of considerable significance to the athlete, for whom an improvement of even a fraction of a second in these events is considered to be a major achievement. In a study by VanMontfoort *et al*. [10], ingestion of sodium bicarbonate was found to increase the sprint performance in elite male athletes compared to citrate, lactate and chloride ingestion.

Studies by McNaughton *et al.* [14] have also found that sodium bicarbonate can be used as an ergogenic aid to offset the fatigue process in high-intensity, competitive cycle ergometry of 1 hour duration.

8. Conflicting Evidence

However, even though many of the studies have shown that sodium bicarbonate administration modifies the blood acid-base balance, its effects on performance are not always positive [5]. For instance, no improvements have been registered following 90 seconds of maximal cycle exercise by untrained men [15].

No differences were found between the experimental or placebo trials in female cyclists pedalling at 95% VO2 max [9]. This suggested that bicarbonate buffering does not improve performance in female athletes during repeated bouts of high intensity exercise.

The reasons for the conflicting effects are not altogether clear, but are at least in part due to variations in the intensity and duration of the exercise tests used, the nature of the exercise task, the dosage of bicarbonate administered and the time delay between bicarbonate administration and the beginning of the exercise test (*i.e.* the amount of metabolic alkalosis induced). Performance has been monitored over exercise durations ranging from a few seconds to more than 1 hour, and during continuous, incremental and intermittent dynamic exercise as well as during sustained isometric contractions [13].

There is no clear pattern of exercise duration between those studies where a positive effect was observed and those where no effect was seen. In most studies, a dose of 0.3 g of sodium bicarbonate/kg of body weight has been used to induce alkalosis, and this has usually been administered orally in solution or in capsule form. Such a dose has usually resulted in an increase of 4-5 $mmol.L^{-1}$ in the plasma buffer base 2-3 hours after administration, although the time-course of changes in acid-base status was not carefully followed in most of these studies [16].

Those studies in which high-intensity and short-term exercises (less than 2 minutes) were used, and in which the doses of sodium bicarbonate given were lower than 0.3 g/kg body weight, did not generally produce an enhancement of performance, which might be due to the use of an insufficient dose or due to the short duration of the effort [5]. Therefore, it has been suggested that exercises lasting less than 1 minute may not be of sufficient duration for the glycolytic metabolism to activate completely, in that the capacity of the intracellular buffer is exceeded and a positive gradient between the intracellular and extracellular medium is established. The main reason for the lack in performance improvement may consequently be due more to the exercise duration than to the doses used.

9. Recommended Dosage

Many studies indicate an effective dose of 300mg of sodium bicarbonate per kg body weight taken 1-2 hours prior to short-term maximal, high intensity performance lasting 60 sec shows 30% improvement in performance [6,11]. Sodium bicarbonate appears to be safe when taken in the recommended dose of 140mg/pound of body weight. For a 150 pound athlete this translates to a dose of 5 teaspoons of baking soda that provides 21,000mg or 21g of bicarbonate. Safety should not be confused with side effects. Baking soda should be consumed with plenty of water, (eg. a litre or more), when taken 1 or 2 hours prior to exercise [4].

10. Side Effects

There are of course potential problems of severe alkalosis associated with the use of increased doses of bicarbonate. Additionally side effects like vomiting, gastrointestinal discomfort, bloating, and diarrhea may occur particularly if sufficient water is not taken with sodium bicarbonate. Vomiting and diarrhea are frequently reported as a result of ingestion of even relatively small doses of bicarbonate, and this may limit any attempt to improve athletic performance by this method, certainly among those individuals susceptible to gastrointestinal problems. There have been reports of athletes using this intervention, which is not prohibited by the rules of the sport, being unable to compete because of the severity of these symptoms. Although unpleasant and to some extent debilitating, these effects are not serious and there are no long term adverse consequences [17].

11. Acute vs Chronic Loading

Given that some individuals exhibit urgent gastrointestinal distress with bicarbonate, such as vomiting and diarrhoea, it is important for athletes to experiment with bicarbonate in training that features daily consecutive races, since much of the gastrointestinal distress seems to occur after a race (semi-finals), which could limit performance in any subsequent race (finals) [12].

Studies [18,19] have shown more favourable gastrointestinal tolerance effects after chronic multiday bicarbonate supplementation as compared to acute pre-exercise single-dose administration. Performance in high-intensity exercise may be enhanced for a full 2 days after cessation of chronic bicarbonate supplementation which might alleviate many of the severe gastrointestinal side-effects found with acute bicarbonate loading.

Not withstanding these results, more research is needed to show performance efficacy for chronic bicarbonate ingestion protocols in elite athletes, and to better elucidate the dosing and time-course effects between the cessation of dosing and exercise performance testing.

12. Conclusions

In conclusion, there are sufficient data to suggest that sodium bicarbonate can be used as a nutritional ergogenic aid or dietary supplement for improving performance in short term, high intensity exercise, provided it is taken in the recommended dosage of 300 mg (0.3g)/kilogram

body weight. Continued use of bicarbonate may help athletes become less susceptible to the side effects and may give even larger improvements in performance.

13. Further Research

Since the time elapsed from the ingestion of bicarbonate to the beginning of exercise (time of absorption) varies considerably, until further research can clarify the time course and cessation of dosing that that can lead to performance enhancement, the individual athlete is advised to experiment in training to judge their own case. The athlete needs to discover not only the potential for performance improvement, but also the likelihood of unwanted side-effects. Further research is also needed to find out the longest duration of endurance exercise that benefits from the use of sodium bicarbonate.

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Characteristics of Antioxidant Isolated from Some Plant Sources

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ABSTRACT

Antioxidant characteristics of ginger roots, guava leaves, guava seeds, orange peel, sesame coat, rice bran and wheat germ as affected by ethanol, ethyl acetate, chloroform, hexane and petroleum ether were evaluated. Petroleum ether extract of ginger roots, ethanol extracts of guava leaves, guava seeds, orange peel and sesame coat and ethyl acetate extracts of rice bran and wheat germ appeared to possess higher antioxidant activity than those from other solvents. Ginger roots, orange peel and guava leaves exhibited higher antioxidant activity than that of α-tocopherol, while guava seeds, sesame coat, rice bran and wheat germ had lower antioxidant activity than that of α-tocopherol. Guava leaves extract had the highest total phenolics content among the other plant material extracts followed by ginger roots, sesame coat and orange peel extracts. However, total flavonoids content was the highest in ginger roots extract followed by guava leaves extract. Ferulic was the highest phenolic compounds in guava leaves and sesame coat extracts. However, chlorogenic acid was the highest phenolic compounds in ginger roots extract. Antioxidants in ginger roots, guava leaves and sesame coat extracts as well as α-tocopherol were heat stable with 73.1, 73.8, 66.7 *and* 71.6*% activity, respectively, after heating at* 100*°C for* 180 *min. Induction periods of sunflower oil containing* 2*% guava leaves and* 2*% ginger roots extracts were increased by* 230.6*% and* 226.7*%, respectively. However, induction period of sunflower oil containing sesame coat was increased by* 174.1*%, at* 0.5*% concentration. Similar increment was found for the protection factor. Ginger roots, guava leaves and sesame coat might be promising sources of natural antioxidant to be used in food products.*

***Keywords*:** *Antioxidative Activity*, *Ginger Roots*, *Guava Leaves*, *Sesame Coat*, *Rancimat*

1. Introduction

The shelf–life of foods is often limited as their stability is restricted due to reactions such as oxidative degradation of lipids. Oxidation of lipids not only produces rancid odours and flavours, but also can decrease the nutritional quality and safety by the formation of secondary products in food after cooking and processing [1-3]. Antioxidants are used as food additives in order to prevent the oxidative deterioration of fats and oils in processed food. Addition of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) can control lipid oxidation in foods [4,5]. Since some of synthetic antioxidants had toxigenic, mutagenic, and carcinogenic effects and some natural antioxidants were effective in enhancing the shelf life of food but less effective than synthetic antioxidants, there is a great demand for the use of new natural antioxidants in food [5,6]. Therefore, there is a strong need for effective antioxidants from natural sources to prevent deterioration of foods. Some components of extracts isolated from oil seeds, spices, fruits and vegetables have been proven in model systems to be as effective antioxidants as synthetic antioxidants [5,7-9].

The present study was conducted to utilize some plant materials such as ginger roots, guava leaves, guava seeds, orange peel, rice bran and wheat germ as natural sources of antioxidants.

2. Materials and Methods

2.1 Materials

Sesame (*Sesamum indicum* L.) coat was obtained from El Rashidi El Mizan Company, 6 of October city, second industrial zone, Egypt. Rice bran (*Oryza sativa*), guava

seeds and leaves (*Psidium guajava* L.), ginger roots (*Zingiber officinale* L.), sour (bitter) orange peel (*Citrus aurantium*) and wheat germ (*Triticum aestium*) were obtained from El-Tahanoon El-Masrion, number 16 in the third industrial zone, 6 of October city, Egypt. Sunflower oil was obtained from Integrated Oil Industries Company, Al Adabia, Suez city, Egypt.

2.2 Reagents and Solutions

Ethanol, ethyl acetate, chloroform, hexane and petroleum ether were obtained from El-Nasr Pharmaceutical Chemicals, El-Ameriea, Cairo, Egypt. Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), tannic acid (phenolic standard), quercetin (flavonoid standard), α-tocopherol mixed isomers type v from vegetable oil were obtained from Sigma Chemical Co., St. Louis, MO, USA.

2.3 Preparation of Plant Samples

Rice bran, wheat germ and ginger roots were dried in an electric air draught oven (Nüve San. Malz, Model FN 500, Akyuri, Ankara, Turkey) at 40ºC for 1hr, however (orange peel, guava leaves and guava seeds) were dried at the same temperature for 12hr. The dried samples were ground using a laboratory electric mill (Braun, Model 2001DL, Germany) then packed in the polyethylene and stored at –20ºC in the deep freezer (Ultralow, Revco, Inc., Sweden) until use.

2.4 Preparation of Antioxidant Extracts

Fifty grams of each ground samples (ginger roots, guava leaves, guava seeds, rice bran, orange peel, wheat germ, sesame coat) were extracted three times with 500 ml of each solvent (ethyl acetate, petroleum ether, ethanol, hexane, and chloroform) using a Teflon- coated magnetic stir bar and stir plate (Framo-Geratetechnik, Germany) for 6 hr at room temperature. Extracts were filtrated through Whatman No. 1. The combined filtrates from each material were concentrated in a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany) at 40°C to a final volume of 100 ml of crude extracts and stored at –20°C until used.

2.5 Determination of Antioxidant Activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method according the procedure described by Brand-Williams *et al.* [10]. Fifty µl from each extract (stock solution was 20.0 g/l) was placed in a cuvette, and 2 ml of 6×10^{-5} M methanolic solution of DPPH was added. Absorbance was measured immediately at 517 nm (UNICO 2802 C/PCS Series Spectrophotometer, USA). The decrease in absorbance was measured every 5 min for 1 h. Alpha-tocopherol was used for comparative purposes. The absorbance of the DPPH radical without antioxidant (control) was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution [11]. The percentage of inhibition of the DPPH radical by the extracts (antioxidant activity) was calculated according to the equation of Yen and Duh [12]:

$$\% \text{ inhibition} = [(AC(o)_{517} - AA(t)_{517}) \div AC(o)_{517}] \times 100$$

where: $AC(o)_{517}$ is the absorbance of the control at t = 0 min.

$AA(t)_{517}$ is the absorbance of the antioxidant at t = 1 h.

2.6 Determination of Total Phenolic Compounds

Total phenolic compounds were determined with Folin–Ciocalteu reagent using tannic acid as standard according the method described by Taga *et al.* [13]. Absorbance was measured at 750 nm using spectrophotometer (UNICO 2802 C/PCS Series Spectrophotometer, USA) and compared with tannic acid calibration curve.

2.7 Determination of Total Flavonoids

Aluminum chloride colorimetric method was used for the determination of total flavonoids [14].

2.8 Separation and Identification of Phenolic Compounds

Separation and identification of phenolic compounds were carried out as described by Ricardo *et al.*, [15] using HPLC (Thermo Separation Products Inc.) system consisting of Consta METRIc 4100 series pump, spectra series AS-100 auto sampler, spectra system FL 3000 fluorescence detector (Ex: 250 nm – Em: 400 nm) and interfaced with IBM computer equipped with PC 1000 Chromatography software version 3.5. Methanol: Ammonium acetate (12: 88; v/v at pH = 5.4) was used as mobile phase with a flow rate of 1 ml / min. Column ODC-2 (3 µM; 150 mm × 4.6 mm I.d., Alltech. USA).

2.9 Heat Stability of Antioxidant

2.9.1 Heating

Antioxidant extracts (2 ml) were incubated at different temperatures in the range of 40-100°C for 30 min. Antioxidant activity was determined as previously mentioned using DPPH method. The extracts were also boiled in water bath for 0, 30, 60, 90, 120, 150 and 180 min, and the residual antioxidant activity was determined [9].

2.9.2 Rancimat

The extracts and α-tocopherol were added to sunflower oil at the concentrations of 0.5%, 1% and 2%. The mixture was kept at 40ºC for 30 min and then in vacuum rotary evaporator (Buchi 011, Buchi, Switzerland) below 40ºC for 1 h for complete removal of solvent. A 679 Rancimat (Metrohm, Herisan, Switzerland) was used. A

5 g portion of each test sample was loaded into the reaction vessel cylinder. Six different samples were conducted in one batch. The air supply was maintained at 20 ml/min and the heating temperature was kept at 110ºC throughout the experiment as described by Antolovich *et al.* [16].

The induction period (IP) (h) was recorded automatically. The protection factor (PF) was calculated according to the following equation:

PF = IP extract / IP control

2.9.3. Statistical Analysis

The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system [17]. Duncan's multiple range tests were used to determine the differences among means at the level of 5%.

3. Results and Discussion

3.1 Antioxidant Activity

Data presented in **Table 1** showed the effect of solvent types on the antioxidant activity of different plant materials. Petroleum ether extract of ginger roots and ethyl acetate extracts of rice bran and wheat germ had the highest ($p \leq 0.05$) antioxidant activity among other solvent extracts.

Guava leaves, guava seeds, orange peel and sesame coat exhibited the highest ($p \leq 0.05$) antioxidant activity when extracted with ethanol. Ethyl acetate extract of guava leaves showed similar ($p > 0.05$) antioxidant activity to ethanol extract.

Chang *et al.* [14] reported that sesame coat extracts displayed similar antioxidant activity. Qian and Nihorimbere [18] reported that the extracts of guava leaves with 50% aqueous ethanol showed higher antioxidant activities than that with water extract.

Solvent extracts of the plant materials, which showed the highest antioxidant activity, were selected for further studies.

Ginger roots, orange peel and guava leaves extracts exhibited higher ($p \leq 0.05$) antioxidant activity than that of α-tocopherol while, guava seeds, sesame coat, rice bran and wheat germ extracts had lower ($p \leq 0.05$) antioxidant activity compared to *α*-tocopherol (**Table 2**).

Ginger roots extract had higher ($p \leq 0.05$) antioxidant activity than those of other plant material extracts. The antioxidant activity value of ginger roots extract was similar to that reported by Hussein *et al*. [5], however, it was higher than that obtained by Mansour and Khalil [9]. This difference might be due to the interspecies variation.

Chang *et al.* [14] reported that sesame coat extracts (methanol, ethanol and acetone) exhibited strong antioxidant activity ranging from 91.8% to 92.6%.

3.2 Total Phenolics and Total Flavoniods

Total phenolics, total flavonoids and the ratio of flavonoids/phenolics in plant material extracts are summarized in **Table 3**. Guava leaves extract had the highest ($p \leq 0.05$) total phenolics content among the other plant material extracts followed by ginger roots, sesame coat and orange peel extracts. On the other hand, guava seeds extract had the lowest ($p \leq 0.05$) total phenolics content. Total phenolic contents were relatively correlated with the antioxidant activity previously mentioned in **Table 2**.

These results are in good agreement with those obtained by Dasgupta and De [19] and Dorman and Hiltunen [20] who found highly positive relationship existed between total phenolics and antioxidant activity in many plant species. Huang and Zhang [21] found that the major components in the ethanol extract of guava leaves were polyphenols.

Ginger roots extract showed the highest ($p \leq 0.05$) total flavonoids content among all plant material extracts followed by guava leaves extract. However, guava seeds extract had the lowest ($p \leq 0.05$) total flavonoids content.

It is important to note that the total flavonoid contents in ginger roots extract and guava leaves extract were

Table 1. Antioxidant activity of plant material extracts as affected by different solvents

Solvent	Antioxidant activity (%)						
	Ginger roots	Guava leaves	Guava seeds	Orange peel	Sesame coat	Rice bran	Wheat germ
Ethanol	$93.3^{b} \pm 0.37$	$86.7^{a} \pm 0.39$	$31.2^{a} \pm 0.77$	$90.8^{a} \pm 0.74$	$75.9^{a} \pm 2.8$	$41.0^{b} \pm 1.0$	$41.9^{b} \pm 0.49$
Ethyl acetate	$93.7^{b} \pm 0.54$	$85.6^{a} \pm 0.91$	$20.2^{b} \pm 0.21$	$69.8^{b} \pm 0.63$	$67.8^{b} \pm 0.90$	$61.6^{a} \pm 0.88$	$59.1^{a} \pm 1.7$
Chloroform	$91.2^{c} \pm 0.89$	$55.5^{c} \pm 1.0$	$3.1^{c} \pm 0.09$	$48.7^{c} \pm 0.46$	$51.7^{c} \pm 1.7$	$30.4^{c} \pm 0.87$	$9.7^{c} \pm 0.50$
Hexane	$93.7^{b} \pm 0.99$	$50.3^{d} \pm 0.20$	$3.5^{c} \pm 0.60$	$43.4^{d} \pm 0.14$	$15.3^{e} \pm 1.1$	$10.0^{e} \pm 0.40$	$8.5c^{d} \pm 0.90$
Petroleum ether	$95.2^{a} \pm 0.22$	$64.7^{b} \pm 0.58$	$2.0^{d} \pm 0.03$	$24.4^{e} \pm 0.20$	$27.8^{d} \pm 0.95$	$12.2^{d} \pm 1.2$	$6.9^{d} \pm 0.40$
LSD	1.222	1.309	0.816	0.897	3.006	1.647	1.690

Means in the same column with different letters are significantly different ($p \leq 0.05$).

Table 2. Antioxidant activity of plant material extracts compared to *α*-tocopherol

Plant material extracts	Antioxidant activity[4] (%)
Ginger roots[1]	95.2[a] ± 0.22
Guava leaves[2]	86.7[c] ± 0.39
Guava seeds[2]	31.2[h] ± 0.77
Orange peel[2]	90.8[b] ± 0.74
Sesame coat[2]	75.9[e] ± 2.8
Rice bran[3]	61.6[f] ± 0.88
Wheat germ[3]	59.1[g] ± 1.70
α-tocopherol	84.0[d] ± 0.54
LSD	2.235

[1]Petroleum ether; [2]Ethanol; [3]Ethyl acetate; [4]Means in the same column with different letters are significantly different ($p \leq 0.05$)

correlated with the antioxidant activity, which indicates that flavonoids might be the main compound responsible for its activity (**Table 2**). The total flavonoids content of orange peel was much lower than that reported by Wang *et al.* [22] for citrus peel.

Total flavonoids and their antioxidant activity as well as their effects on human nutrition and health have been reported by Kessler *et al.* [23]. The mechanisms of action of flavonoids are through scavenging or chelating process.

Ginger roots extract had the highest ($p \leq 0.05$) flavonoids/phenolics ratio among all other plant material extracts followed by guava leaves extract (**Table 3**). No significant ($p > 0.05$) difference was found among guava seeds, orange peel, sesame coat, rice bran and wheat germ extracts in flavonoids/phenolics ratio.

Marinova *et al.* [24] attributed the high flavonoids/phenolics ratio to the rich spectrum of phenolic acids, while attributed the low ratio to the rich spectrum of flavonoids compounds.

3.3 Separation and Identification of Phenolic Compounds

Data in **Table 4** showed that guava leaves extract contained the highest total phenolic compounds content among all other plant material extracts followed by sesame coat extract and orange peel extract. Guava seeds extract contained the lowest total phenolic compounds.

Table 3. Total phenolics and total flavoniods contents in plant material extracts

Plant material extracts	Total phenolics (mg tanic /1 g dried extract)	Total flavonoids (mg quercetin /1 g dried extract)	Flavoniods / phenolics
Ginger roots	39.49[b] ± 2.9	55.10[a] ± 1.4	1.40[a] ± 0.12
Guava leaves	48.83[a] ± 1.0	35.46[b] ± 1.9	0.73[b] ± 0.03
Guava seeds	10.48[f] ± 0.33	1.10[d] ± 0.23	0.10[c] ± 0.02
Orange peel	34.87[c] ± 0.74	4.24[c] ± 0.13	0.12[c] ± 0.01
Sesame coat	37.46[bc] ± 0.77	2.40[d] ± 0.12	0.06[c] ± 0.01
Rice bran	29.03[d] ± 2.3	4.10[c] ±0.09	0.14[c] ± 0.02
Wheat germ	18.5[e] ± 0.66	1.14[d] ± 0.23	0.06[c] ± 0.01
LSD	2.652	1.566	0.083

Means in the same column with different letters are significantly different ($p \leq 0.05$)

Table 4. Phenolic compounds of plant material extracts as analyzed by HPLC

Phenolic compound contents (ppm)	Plant material extracts						
	Ginger roots	Guava leaves	Guava seeds	Orange peel	Sesame coat	Rice bran	Wheat germ
Caffeic	–	–	–	–	19.6	–	2.7
Caffein	–	39.15	11.69	26.65	–	11.3	–
Catechol	–	–	–	92.69	–	–	–
Chlorogenic	102.49	36.7	–	–	29.68	–	–
Chrisin	4.09	–	–	11.77	–	–	–
Cinnamic	29.43	60.26	40.82	36.54	18.3	2.7	–
Coumarin	–	–	–	8.8	32.93	–	9.63
Ferrulic	–	4129.74	–	–	770.05	160.9	45.17
Protocatchic	–	–	–	1022.3	550.44	–	311.13
Syringic	–	–	–	10.62	43.73	5.73	–
Vanillic	–	–	21.13	35.45	–	–	3.99
Total phenolic compounds	136.01	4265.85	73.64	1244.82	1464.73	180.63	372.62

– Not Detected

Chlorogenic acid content was the highest phenolic compounds in ginger roots extract. This result was differed from those reported by Hussein *et al.* [5] who found that pyrogallic, hudroqunion; phenol and resorcinol were the main phenolic compounds in the methanol extract of ginger roots. This difference may be due to the interspecies variation. However, ferrulic was the highest phenolic compounds in guava leaves, sesame coat and rice bran extracts. Also protocatchic represented the highest phenolic compounds in orange peel and wheat germ extracts.

Liang *et al.* [25] reported that the polyphenolic compounds in guava leaves were gallic acid, quercetin, procatechuic acid, chlorogenic acid, caffeic acid, kaempferol and ferulic acid. Bocco *et al.* [26] found that the total content of phenolic acids (caffeic acid, q-coumaric acid, ferulic acid and sinapinic acid) in sour orange peel was twenty times that found in bergamot peel.

3.4 Heat Stability

Antioxidant activity was constant for all plant material extracts when incubated at 40°C for 30 min. Incubating all plant material extracts at a temperature higher than 40°C for 30 min, resulted in a significant ($p \leq 0.05$) decrease in antioxidant activity (**Table 5**). Mansour and Khalil [9] and Hussein *et al.* [5] reported that the antioxidant activity of ginger roots extract was stable when incubated at a temperature ranging from 40 to 60°C for 30 min.

Increasing the heating temperature from 40°C to 100°C for 30 min resulted in significant ($p \leq 0.05$) decrease in the antioxidant activity of ginger roots, guava leaves, guava seed, orange peel, sesame coat, rice bran, wheat germ extracts and α-tocopherol by 18.1, 15.6, 56.6, 32.9, 16.3, 28.9, 51.1% and 11.8%, respectively. The reduction in antioxidant activity of α-tocopherol (11.8%) was lower ($p \leq 0.05$) than that of all plant material extracts. Higher reduction value was reported by Mansour and Khalil [9] for ginger roots (25%) when heated to 100°C and comparable reduction value (17.9%) was reported by Hussein *et al.* [5].

Ginger roots extract had the highest ($p \leq 0.05$) antioxidant activity followed by guava leaves extract and α-tocopherol. Alpha-tocopherol had a higher ($p \leq 0.05$) antioxidant activity than those of guava seeds extract, orange peel extract, sesame coat extract, rice bran extract and wheat germ extract.

Increasing the boiling time resulted in a significant ($p \leq 0.05$) decrease in the antioxidant activity of all plant material extracts (**Table 6**). These results agree well with those reported by Mansour and Khalil [9] and Hussein *et al.* [5] for ginger roots extract.

Boiling the plant material extracts and α-tocopherol for 180 min reduced ($p \leq 0.05$) the antioxidant activity of ginger roots, guava leaves, guava seeds, orange peel, sesame coat, rice bran and wheat germ extracts and α-tocopherol by 26.9, 26.2, 80.1, 54.7, 33.3, 54.7 and 72.7 and 28.5%, respectively. These results indicated that antioxidants in ginger roots, guava leaves, sesame coat extracts and α-tocopherol were relatively heat stable (remaining activity ranging from 66.7 to 73.8 %). However, the remaining antioxidant activity in the other plant material extracts ranged from 19.9 to 45.3%.

Good thermal stability of ginger extract has been attributed to the inhibition of peroxidation of linoleic acid when the extract was heated to 185°C for 120 min [27]. However, Hussein *et al.* [5] found that the remaining antioxidant activity of ginger roots extract was 29.04% when boiled for 120 min.

Induction periods of sunflower oil containing different levels of plant material extracts as well as α-tocopherol were higher than that of control (**Table 7**). Induction period of sunflower oil increased by increasing the concentration of ginger roots, guava leaves, orange peel and rice bran extracts. An opposite trend was observed with sesame coat, guava seeds and wheat germ extracts.

Table 5. Effect of temperature on the antioxidant activity of plant material extracts

Temperature (°C)	Antioxidant activity (%)								Mean[1]
	Ginger roots	Guava leaves	Guava seeds	Orange peel	Sesame coat	Rice bran	Wheat germ	α-tocopherol	
0	94.9 ± 1.3	85.5 ± 0.2	30.1 ± 1.7	89.7 ± 0.8	75.1 ± 0.5	60.7 ± 1.0	58.5 ± 0.8	84.7 ± 0.3	72.40[a]
40	93.9 ± 0.8	84.5 ± 0.1	29.5 ± 0.2	89.4 ± 0.4	75.4 ± 0.5	60.2 ± 0.6	57.2 ± 0.3	83.7 ± 0.5	71.72[a]
60	92.9 ± 0.4	83.9 ± 0.7	23.5 ± 0.1	80.5 ± 0.1	75.1 ± 0.6	54.0 ± 0.1	47.7 ± 0.7	82.0 ± 0.5	67.45[b]
80	84.8 ± 0.3	80.0 ± 0.5	17.8 ± 0.6	67.7 ± 0.1	71.6 ± 0.5	47.2 ± 0.4	36.0 ± 0.9	78.7 ± 0.5	60.48[c]
100	76.9 ± 0.4	71.3 ± 0.8	12.8 ± 0.5	60.0 ± 0.3	63.1 ± 0.3	42.8 ± 0.5	28.0 ± 0.5	73.8 ± 0.4	53.59[d]
Mean[2]	88.67[a]	81.03[b]	22.74[g]	77.47[c]	72.07[d]	52.97[e]	45.49[f]	80.57[b]	

[1]Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.897; [2]Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.958

Table 6. Effect of boiling for different time on the antioxidant activity of plant material extracts

Time (min)	Antioxidant activity (%)								Mean[1]
	Ginger roots	Guava leaves	Guava Seeds	Orange peel	Sesame coat	Rice bran	Wheat germ	α-tocopherol	
0	94.9 ± 1.3	85.5 ± 0.2	30.1 ± 1.7	89.7 ± 0.8	75.1 ± 0.5	60.7 ± 1.0	58.5 ± 0.8	84.7 ± 0.3	72.40[a]
30	76.9 ± 0.4	71.3 ± 0.8	12.8 ± 0.5	60.0 ± 0.3	63.1 ± 0.3	42.8 ± 0.5	28.0 ± 0.5	73.8 ± 0.4	53.58[b]
60	73.1 ± 0.6	68.4 ± 0.9	10.0 ± 0.2	52.5 ± 0.2	59.7 ± 0.4	36.9 ± 0.6	25.0 ± 0.4	68.0 ± 0.5	49.20[c]
90	72.0 ± 0.4	67.2 ± 0.8	8.8 ± 0.2	47.9 ± 0.1	57.5 ± 0.1	34.0 ± 0.9	21.6 ± 0.1	64.7 ± 0.4	46.71[d]
120	71.3 ± 0.2	66.0 ± 0.3	7.8 ± 0.3	44.0 ± 0.7	55.0 ± 0.5	30.9 ± 0.8	18.8 ± 0.2	62.5 ± 0.1	44.53[e]
150	70.0 ± 0.5	64.3 ± 0.6	6.9 ± 0.8	42.0 ± 0.4	52.2 ± 0.4	29.4 ± 0.1	16.9 ± 0.4	61.5 ± 0.1	42.90[f]
180	69.4 ± 0.1	63.1 ± 0.5	6.0 ± 0.3	40.6 ± 0.4	50.1 ± 1.5	27.5 ± 0.4	16.0 ± 0.5	60.6 ± 0.6	41.66[g]
Mean[2]	75.36[a]	69.40[b]	11.77[h]	53.82[e]	58.96[d]	37.45[f]	26.41[g]	67.96[c]	

[1]Means in the same column with different letters are significantly different ($p \leq 0.05$), LSD = 0.720; [2]Means in the same row with different letters are significantly different ($p \leq 0.05$), LSD = 0.883

The highest induction period of sunflower oil was obtained by the addition of 2% guava leaves extract followed by the addition of 2% ginger roots extract. However, the addition of guava seeds extract at 2% level showed the lowest induction period. The induction period of sunflower oil increased by 230.6 and 226.7% by the addition of 2% guava leaves extracts and 2% ginger roots extract respectively.

Alpha-tocopherol had a higher induction period (11.86 h) than all plant material extracts at 1% concentration. Induction period of sunflower oil containing *α*-tocopherol was increased by 232.5%. On the other hand sesame coat extract had the highest induction period (8.88 h) at 0.5% concentration. Induction period of sunflower oil containing sesame coat was increased by 174.1%.

Elleuch *et al*., [28] found that raw sesame seed oil had a higher induction period (28.2 h) then followed by dehulled sesame seed oil (25.7 h). Thus, the dehulling processes decrease the stability of the oils.

Data in **Table 8** showed the protection factor of sunflower oil containing plant material extracts and *α*-tocopherol. All protection factors of plant material extracts had a positive trend similar to the induction period (**Table 7**). The highest protection factor of sunflower oil was obtained by the addition of 2% guava leaves extract followed by ginger roots and orange peel extracts at the same level. Protection factor of sunflower oil containing guava leaves, ginger roots and orange peel extracts was increased by 231, 227 and 164%, respectively. Similar increment was found for the induction period (**Table 7**). While, guava seeds, rice bran and wheat germ extracts had lower stabilizing effect than that of *α*-tocopherol. Garau *et al*., [29] reported that the average protection factor value for orange peel samples was 1.52.

Table 7. Induction period (hr) of sunflower oil containing different levels of plant material extracts and *α*-tocopherol assessed by rancimat at 100°C

Plant material extracts	Level of addition (%)			
	0	0.5	1	2
Ginger roots	5.1	6.94	8.54	11.56
Guava leaves	5.1	8.46	9.54	11.76
Guava seeds	5.1	6.36	5.14	5.11
Orange peel	5.1	7.95	8.13	8.36
Sesame coat	5.1	8.88	6.87	6.81
Rice bran	5.1	5.26	5.44	5.98
Wheat germ	5.1	6.30	6.04	5.34
α-tocopherol	5.1	10.00	11.86	11.10

Table 8. Protection factor of sunflower oil containing different levels (%) of plant material extracts and *α*-tocopherol assessed by rancimat at 100°C

Plant material extracts	Level of addition (%)			
	0	0.5	1	2
Ginger roots	1	1.36	1.67	2.27
Guava leaves	1	1.66	1.87	2.31
Guava seeds	1	1.25	1.01	1.00
Orange peel	1	1.56	1.59	1.64
Sesame coat	1	1.74	1.35	1.34
Rice bran	1	1.03	1.07	1.17
Wheat germ	1	1.24	1.18	1.05
α-tocopherol	1	1.96	2.33	2.18

Alpha-tocopherol (at 1% concentration) had a higher protection factor than all plant material extracts. On the other hand, sesame coat extract (at 0.5% concentration) had the highest protection factor as compared to the other plant material extracts.

From the above results, it could be concluded that the antioxidant activities of ginger roots, guava leaves and sesame coat were comparable to *α*-tocopherol. Ginger roots, guava leaves and sesame coat showed good thermal stability in comparison with *α*-tocopherol. Ginger roots, guava leaves and sesame coat might be promising sources of natural antioxidant to be used in food products.

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Effect of Calcium and Phosphorus on Nonhaeme Iron Absorption and Haematogenic Characteristics in Rats

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ABSTRACT

The objectives of this study were to use the dry thyme leaves as source of nonhaeme iron and evaluate the effects of calcium, phosphorus and calcium + phosphorus on nonhaeme iron absorption and haematogenic characteristics in rats. Thirty adult male albino rats, weighing 150 ± 5 *g were divided into five groups. The first group fed basal diet, the second group fed thyme diet, the third group fed thyme diet + calcium, the fourth group fed thyme diet + phosphorus and the fifth group fed thyme diet + calcium + phosphorus. All groups fed experimental diets for six weeks. Hemoglobin* (*Hb*)*, haematocrit* (*Ht*)*, red blood cell* (*RBC*)*, mean corpuscular volume* (*MCV*)*, serum iron* (*SI*)*, serum ferritin* (*SF*)*, total iron–binding capacity and transferrin saturation were determined at the beginning and the at end of the experiment. Iron in diet, Fe intake, Fe feces and Fe absorption were also evaluated. The results indicated that the lowest Fe absorption was observed in rats fed the thyme diet + calcium and thyme diet + calcium + phosphorus. Supplementation the thyme diet with calcium or calcium + phosphorus decreased the values of Hb, Ht, RBC, SI and SF. However, supplementation the thyme diet with phosphorus did not affect in Ht, RBC and MCV but Hb, SI and SF increased. The results suggest that supplementation the diet with calcium or calcium + phosphorus interfere with iron absorption.*

Keywords**:** *Nonhaeme Iron*, *Thyme*, *Iron Absorption*, *Hemoglobin*

1. Introduction

The most common nutritional deficiencies now affecting all ages involve iron and calcium [1]. In recent years, Ca-enriched foods have come to be a habitual part of daily diet [2]. On the other hand, Fe deficiency is the most common nutritional disorder worldwide, affecting people of all ages in the both industrialized and developi93ng countries [3]. Nutritional Fe deficiency arises when physiological requirement cannot be met by Fe absorption from the diet. The efficiency of iron absorption depends on the both bioavailability of dietary iron and iron status. Iron absorption is influenced by many factors. Body need, vitamin C, protein and carbohydrate intakes enhance absorption [4,5]. On the other side, binding agents such as phytate, oxalate and phosphate, dietary fiber, calcium, coffee, tea and gastrointestinal diseases inhabit iron absorption [6-8].

Several studies with animals have clearly shown that Ca interferes with dietary absorption of Fe and that addition of Ca to the diet may even induce Fe deficiency [9]. Increased Ca supplementation may have an adverse effect on the metabolism of some micronutrients such as iron and zinc [10].

Thyme is source of protein and iron [11]. The iron content in dry thyme leaves was 117.2 mg/100g dry matter [12]. It is increasingly recognized that simultaneous provision of iron, calcium, phosphorous in supplements may decrease benefit of one or three. These complex micronutrient interactions and their implications for nutritional interventions are incompletely understood. The absorption and bioavailability of nonheme iron have not been adequately studied. Therefore, the objectives of this study were to use the dry thyme leaves as source of nonheme iron and evaluate the effects of calcium, phosphorus and calcium + phosphorus on nonhaeme iron absorption and haematogenic characteristics in rats.

2. Materials and Methods

Dry thyme leaves used in this study was purchased from Shibin El-Kom, Egypt. The thyme leaves were ground, sieved and stored at – 4°C until use. Calcium carbonate,

calcium phosphate and sodium phosphate mono hydrogen were obtained from Gomhouria Company, Cairo, Egypt.

2.1 Experimental Design

Thirty adult male albino rats, Sprague drawly strain, weighing 150 ± 5 g were purchased from Helwan farm. The rats were housed individually in cage and fed basal diet for one week for adaptation. The basal diet consisted of 100 g/kg corn oil; 126.3 g/kg casein; 40 g/kg mineral mixture, USP XIV; 10 g/kg vitamin mixture; 3 g/kg DL-methionine and 2 g/kg choline chloride and 50 g/kg fiber and corn starch 668.7 g/kg [13].

At the beginning of experiment, A 5 ml blood sample were taken to determine hemoglobin, haematocrit serum iron, serum ferritin, red blood cell, and total iron-binding capacity. As the data obtained basis, the rats were divided into five groups, 6 rats per group. The first (control group) fed basal diet, the second group fed thyme diet (21 g dry thyme leaves/kg basal diet), the third group fed thyme diet + double amount of the recommended dietary allowance of Ca (10 g/kg diet) from $CaCO_3$, the fourth group fed thyme diet + double amount of the recommended dietary allowance of P (8 g/kg diet) from sodium phosphate mono hydrogen (Na H PO_4) and the fifth group fed thyme diet + double amount of the recommended dietary allowance of Ca and P from calcium phosphate (Ca PO_4) as described by [14]. Feed intake was recorded daily. Faces were collected of each animal daily. Body weight was recorded at the beginning and at the end of experimental period. At the end of experimental period (6 weeks), the rats fasted overnight and were anaesthetized. Blood sample were collected and aliquots were analyzed to measure the hematological parameters. The remaining blood was centrifuged to obtain serum for determination serum iron, serum ferritin and total iron binding capacity.

2.2 Analytical Methods

Total nitrogen content, crude fiber, fat, moisture, and ash were determined according to [15]. The carbohydrate was calculated by difference. The concentration of Fe in the diets and faces were determined by atomic absorption spectrophotometer (Perkin Elmer 1100B, Norwalk, and Ct, USA). Hemoglobin (Hb) red blood cell (RBC) and haematocrit (Ht) in heparinized blood samples were measured using automated hematology analyzer (Sysmex, Kobe, Japan). Total iron-binding capacity (TIBC), serum iron and serum ferritin levels were determined calorimetrically and enzymatically, using sigma diagnostics iron, ferritin and TIBC reagents, (sigma diagnostics, st. Louis, MI, USA). Transferrin saturation (%) was calculated using the following equation: Transferring saturation (%) = (Serum iron concentration ÷ TIBC) × 100. Mean corpuscular volume was calculated as described by [16] using the following equation:

$$MCV = \frac{HT}{RBC} \times 10$$

2.3 Statistical Analysis

The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system [17]. Duncan's multiple range tests were used to determine the differences among means at the level of 95%.

3. Result and Discussion

The proximate chemical composition and iron content of dry thyme leaves were presented in **Table 1**. Data showed that the protein (20.5%), carbohydrate (45.2%), fiber (12.6%) and iron content (122.7 mg/g dry matter) were high in dry thyme while moisture (4.95%) and fat (4.6%) were low. These results are agreement with [12] who reported that the dry thyme was high content in protein (18.9g/100 dry matter), carbohydrate (49.6g/100g), fiber (15g/100g dry matter) and iron (117.2mg/100 dry matter) but low in moisture and fat.

Data in **Table 2** showed that rats fed basal diet had the

Table 1. Proximate chemical composition and iron content of dry thyme leaves

Moisture (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Fiber (%)	Ash (%)	Fe (mg/100g)
4.95 ± 0.25	20.5 ± 1.2	4.6 ± 0.36	45.2 ± 0.56	12.6 ± 0.81	12.6 ± 0.81	122.7

Table 2. Fe diet, Fe intake, Fe feces and Fe absorption in rats fed basal diet, thyme diet and thyme diet supplemented with minerals

Diet	Fe diet (mg/kg)	Fe intake (mg/kg)	Fe feces (mg/kg)	Fe absorption (%)
Basal diet	46^{b} ± 1.0	32.03^{b} ± 1.74	15.90^{c} ± 1.40	50.35^{a} ± 1.73
Thyme diet	70.67^{a} ± 2.1	48.48^{a} ± 2.42	30.43^{b} ± 0.98	37.10^{b} ± 1.50
Thyme diet + Ca	69^{a} ± 1	48.55^{a} ± 1.61	33.57^{a} ± 1.80	31.25^{c} ± 1.98
Thyme diet + P	68.67^{a} ±1.53	46.76^{a} ± 1.36	29.33^{b} ± 1.03	37.63^{b} ± 1.32
Thyme diet + Ca + P	70^{a} ± 1.0	48.90^{a} ± 1.46	33.68^{a} ± 1.70	31.15^{c} ± 1.51
LSD	2.53	3.2	2.48	2.95

Means in the same column with different letters are significantly different ($p \leq 0.05$)

lowest ($P \leq 0.05$) iron diet and iron intake as compared to rats fed thyme diet and thyme diet supplemented with minerals. There was no significantly ($P > 0.05$) difference in Fe intake between rats fed thyme diet and rats fed thyme diet supplemented with minerals. Rats fed basal diet had lower ($P \leq 0.05$) Fe feces and higher Fe absorption than those fed thyme diet and thyme diet supplemented with minerals. The lowest Fe absorption was observed in rats fed the thyme diet + calcium and thyme diet + calcium + phosphorus. Although the Fe intake was lower ($P \leq 0.05$) in rats fed basal diet than those fed thyme diet and thyme diet supplemented with minerals, the Fe absorption was the highest ($P \leq 0.05$). This is due to the low Fe fecal excretion in rats fed basal diet and polyphenol compound in thyme diet which had adverse effect on Fe absorption. These results are agreement with those reported by [18,19] they reported that polyphenols inhibited the absorption of nonheme iron. As suggested by [20] the percentage of iron absorbed decreases as iron intake increases. Similar results were reported by [21] who found in humans that those fed bread fortified with increasing amounts of iron (1, 3 and 5 mg) had lower percentages of iron absorbed, but their absolute absorption increased in response to increasing iron intakes.

Fe absorption was lower ($P \leq 0.05$) in rats fed thyme diet + calcium and thyme diet + calcium + phosphorus than those fed thyme diet and thyme diet + phosphorus. This may be due to the losses of Fe in feces and the presence of calcium or and phosphorus in the diet which inhibit nonhaeme iron absorption. Similar results were reported by [22,23]. However these results were differed from those reported by [2,24] they found that feeding rats high calcium diet for two weeks do not inhibit iron absorption.

Effect of calcium and phosphorous on the hemoglobin (Hb), haematocrit (Ht), red blood cell (RBC) and mean corpuscular volume(MCV) in rat fed basal diet, thyme diet and thyme diet supplemented with minerals are shown in **Table 3**. There was no significant ($P > 0.05$) change in Hb between rats fed basal diet. However, Hb was significantly ($P \leq 0.05$) affected in rats fed thyme diet, thyme diet supplemented with calcium, thyme diet supplemented with phosphorus and thyme diet supplemented with calcium + phosphorus. Hemoglobin was significantly ($P \leq 0.05$) increased in rats fed thyme diet and thyme diet supplemented with phosphorus. However, hemoglobin was significantly ($P \leq 0.05$) decreased in rats fed thyme diet supplemented with calcium and thyme diet supplemented with calcium + phosphorus.

Haematocrit and red blood cell did not significantly ($P \leq 0.05$) affect in rats fed basal diet, thyme diet and thyme diet supplemented with phosphorus. However, haematocrit and red blood cell were significantly ($P \leq 0.05$) decreased in rats fed thyme diet supplemented with calcium and thyme diet supplemented with calcium + phosphorus.

Mean corpuscular volume was significantly ($P \leq 0.05$) decreased in rats fed basal diet and thyme diet. Mean corpuscular volume was significantly ($P \leq 0.05$) increased in rats fed thyme diet supplemented with calcium. However, mean corpuscular volume did not significantly ($P \leq 0.05$) affect in rats fed thyme diet supplemented with phosphorus and thyme diet supplemented with calcium + phosphorus.

These data indicated that supplementation the diet with calcium decreased the values of Hb, Ht and RBC. Supplementation the diet with phosphorus did not affect in Ht, RBC and MCV but Hb increased. However, supplementation the diet with calcium + phosphorus decreased the values of Hb, Ht and RBC but MCV did not affect. Increased Fe intake response with increment of Hb concentration [25]. Hemoglobin concentration was negative-

Table 3. Effect of calcium and phosphorous on the hemoglobin, haematocrit, red blood cell and mean corpuscular volume in rat fed basal, thyme diet and thyme diet supplemented with minerals

	Basal Diet	LSD	Thyme diet	LSD	Thyme Diet + Ca	LSD	Thyme Diet + P	LSD	Thyme Diet + Ca + P	LSD
Hb (g/dl)										
Initial	$11.9^{a} \pm 0.35$	0.599	$11.9^{b} + 0.35$	0.47	$15.2^{a} \pm 0.58$	1.27	$11.76^{b} \pm 0.23$	0.35	$14.25^{a} \pm 1.1$	1.35
Final	$12.2^{a} \pm 0.34$		$12.45^{a} \pm 0.17$		$10.55^{b} \pm 0.87$		$12.45^{a} \pm 0.17$		$11.9^{b} \pm 0.12$	
Ht (%)										
Initial	$33.5^{a} \pm 1.7$	2.2	$35.5^{a} \pm 0.57$	1.57	$45^{a} \pm 1.2$	3.15	$38.5^{a} \pm 0.58$	0.998	$41.5^{a} \pm 2.88$	3.6
Final	$35.5^{a} \pm 0.6$		$34^{a} \pm 1.2$		$37^{b} \pm 2.3$		$37.5^{a} \pm 0.57$		$35.5^{b} \pm 0.58$	
RBC (mil/cmm)										
Initial	$3.87^{a} \pm 0.2$	0.32	$4.1^{a} \pm 0.12$	0.16	$5.1^{a} \pm 0.23$	0.83	$4.4^{a} \pm 0.35$	0.43	$4.75^{a} \pm 0.29$	0.38
Final	$4.18^{a} \pm 0.1$		$4.05^{a} \pm 0.1$		$3.75^{b} \pm 0.64$		$4.25^{a} \pm 0.1$		$4^{b} \pm 0.12$	
MCV (fl)										
Initial	$86.6^{a} \pm 1$	1.53	$86.6^{a} + 1$	1.96	$88.2^{b} \pm 1.7$	3.03	$87.5^{a} \pm 5.6$	6.85	$87.36^{a} \pm 0.75$	1.63
Final	$84.9^{b} \pm 0.7$		$84.31^{b} \pm 1.2$		$98.7^{a} \pm 1.8$		$88.2^{a} \pm 0.17$		$88.75^{a} \pm 1.1$	

Hb: Hemoglobin; Ht: Haematocrit; RBC: Red blood cell; MCV: Mean corpuscular volume. Means in the same column for each variable with different letters are significantly different ($p \leq 0.05$)

ly and significantly correlated with the intake of calcium [26]. Calcium supplementation reduced heme and total iron without significantly affecting nonheme-iron absorption [23].

The effect of calcium and phosphorus on the serum iron (SI), serum ferritin (SF), total iron binding capacity (TIBC) and transferrin saturation (TS) in rat fed basal diet, thyme diet and thyme diet supplemented with minerals are shown in **Table 4**. Serum iron, serum ferritin and transferrin saturation did not significantly ($P \leq 0.05$) affect in rats fed basal diet. Serum iron, serum ferritin and transferrin saturation were significantly ($P \leq 0.05$) increased in rats fed thyme diet and thyme diet supplemented with phosphorus. However, SI, SF and TS were significantly ($P \leq 0.05$) decreased in rats fed thyme diet supplemented with calcium and thyme diet supplemented with calcium + phosphorus. The improvement in SI, SF and TS for rats fed thyme diet and thyme diet + P may be due to low iron stores in these groups.

Similar results were obtained by [27] who reported that high calcium supplementation at doses 500 and 1000 mg/day for 3 months reduce serum ferritin concentration in women. In an extensive study in France (n = 1108), serum ferritin concentration was negatively and significantly correlated with the intake of calcium [26]. Similar findings were made in a study on French students (n = 476) [28]. Nonheme iron can enhance levels of serum iron and serum ferritin [29].

Total iron binding capacity was significantly ($P \leq 0.05$) decreased in rats fed thyme diet and thyme diet supplemented with phosphorus. Total iron binding capacity did not significantly ($P \leq 0.05$) affect in rats fed basal diet and thyme diet supplemented with calcium + phosphorus. However, total iron binding capacity was significantly ($P \leq 0.05$) increased in rats fed thyme diet supplemented with calcium.

The effect of calcium and phosphorus on the feed intake, body weight gain and feeding efficiency ratio in rat fed basal diet; thyme diet and thyme diet supplemented with minerals are shown in **Table 5**. There were no significant ($P > 0.05$) changes in feed intake, body weight gain and feeding efficiency ratio among rats fed basal diet; thyme diet and thyme diet supplemented with calcium and thyme diet supplemented with calcium and phosphorus.

This finding was in agreement with [30] who reported

Table 4. Effect of calcium and phosphorous on serum iron, serum ferritin, total iron binding capacity and transferrin saturation in rat fed basal, thyme diet and thyme diet supplemented with minerals

	Basal Diet	LSD	Thyme Diet	LSD	Thyme Diet + Ca	LSD	Thyme Diet + P	LSD	Thyme Diet + Ca + P	LSD
SI (μ g/dl)										
Initial	$69.5^{a} \pm 2.9$	4.52	$67^{b} \pm 2.3$	3.16	$110^{a} \pm 3.5$	5.99	$102^{b} \pm 2.3$	4.52	$99.5^{a} \pm 1.7$	2.5
Final	$73^{a} \pm 2.3$		$94^{a} \pm 1.2$		$94^{b} \pm 3.6$		$114.5^{a} \pm 2.8$		$96^{b} \pm 1.2$	
SF (μ g/dl)										
Initial	$29.75^{a} \pm 1.4$	2.7	$18.6^{b} \pm 0.92$	2.72	$35.15^{a} \pm 0.4$	1.9	$26.8^{b} \pm 0.92$	2.04	$31.5^{a} \pm 2.9$	3.98
Final	$32.05^{a} \pm 1.7$		$23.75^{a} \pm 2$		$24.2^{b} \pm 1.5$		$31.7^{a} \pm 1.4$		$27^{b} \pm 1.5$	
TIBC (μ g/dl)										
Initial	$310^{a} \pm 17.3$	28	$336.5^{a} \pm 1.7$	14.3	$293^{b} \pm 6.9$	9.18	$319^{a} \pm 11.5$	15.79	$311^{a} \pm 1.2$	3.8
Final	$302^{a} \pm 15$		$305^{b} \pm 11.5$		$317.5^{a} \pm 2.9$		$300^{b} \pm 5.8$		$312.5^{a} \pm 2.88$	
T.S (%)										
Initial	$22.42^{a} \pm 2.1$	3.6	$19.91^{b} \pm 0.585$	2	$37.54^{a} \pm 2$	3	$32.6^{b} \pm 0.46$	2.15	$32^{a} \pm 0.44$	0.55
Final	$24.17^{a} \pm 2$		$30.86^{a} \pm 1.54$		$29.61^{b} \pm 1.4$		$38.2^{a} \pm 1.7$		$30.72^{b} \pm 0.1$	

SI: Serum iron, SF: Serum ferritin, TIBC: Total iron binding capacity, ST: Transferrin saturation. Means in the same column for each variable with different letters are significantly different ($p \leq 0.05$)

Table 5. Feed intake, body weight gain and feeding efficiency ratio in rat fed basal, thyme diet and thyme diet supplemented with minerals

Diet	Feed intake (g)	Body weight gain (g)	FER
Basal diet	$696^{a} \pm 22.6$	$15.5^{a} \pm 2.29$	$2.23^{a} \pm 0.38$
Thyme diet	$690^{a} \pm 25.98$	$14.5^{a} \pm 1.15$	$2.1^{a} \pm 0.1$
Thyme diet + Ca	$703.5^{a} \pm 15.8$	$15.25^{a} \pm 1.56$	$2.17^{a} \pm 0.21$
Thyme diet + P	$681^{a} \pm 10.39$	$15.25^{a} \pm 1.66$	$2.24^{a} \pm 1.32$
Thyme diet + Ca + P	$698.8^{a} \pm 30.34$	$16.75^{a} \pm 1.15$	$2.4^{a} \pm 0.23$
LSD	40.41	2.94	0.47

Means in the same column with different letters are significantly different ($p \leq 0.05$)

that there no differences in food intake and body weight gain among groups fed flours supplemented with reduced and increased iron.

From the above results, it could be concluded that supplementation the diets with calcium carbonate (as a source of Ca) and calcium phosphate (as a source of Ca + P) reduced the iron absorption in rats fed these diets, which must be continuous to have a long – term influence on serum ferritin, total iron binding capacity, transferrin saturation, hemoglobin, haematocrit, red blood cell and mean corpuscular volume.

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Scientific Research

Effect of Technological Treatments on Cassava (*Manihot Esculenta Crantz*) Composition

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ABSTRACT

The composition of cassava roots and those of its derived food (*attiéké and semolina*), *were determined. The comparative study of the cassava roots composition with those of the semolina and attiéké has shown that the technological applied treatments in the preparation of cassava meal and attiéké influenced its composition. Thus, apart from the lipids content and energy values which slightly increased, all the components* (*protein, ash, cellulose, carbohydrates, starch and hydrocyanic acid*) *decreased in food derived from cassava.*

Keywords: *Cassava, Semolina, Attiéké, Composition, Technological Treatment*

1. Introduction

Cassava has been widespread in all tropical regions of the globe, because of the ease of its culture [1]. However on the nutritional point of view that plant is toxic in all its parts [2]. Indeed the crude cassava roots contain some cyanogenic glycosides. These glycosides are converted in prussic acid (hydrogen cyanide, HCN) when the cells of cassava roots are ruptured. Good methods of preparation and cooking reduce the levels of cyanide and acute poisoning occurs very rarely. Contrariwise, a chronic toxicity due to cyanide appears in case of a high consumption of cassava. Especially when the consumption of iodine and/or proteins is very low [1].

Cassava roots are rich in energy and contain mainly starch and soluble carbohydrates [3]. Although they are low in protein, it is a staple food for about 200 to 300 million people worldwide [4]. Cassava is consumed in various forms [5]. The aim of our work is to study the roots composition of two varieties of cassava and to show the influence of technology on the cassava food value compared to two derived food, semolina and attiéké.

2. Material and Methods

For this study we used as plant material two varieties of cassava roots, a bitter variety "bouanga Koutouan" and a sweet variety "Bonoua red" and also samples of foods derived from cassava : Attiéké bought in Abidjan market and semolina prepared from studied cassava roots.

3. Chemical Analysis

Water content determined by drying at 105°C with constant weight [6], the protein content determined by the method of Kjeldahl with 6.25 as conversion factor, the lipid content determined by Soxhlet extraction with ether, and the ash content determined by incineration at 650°C in muffle furnace [7]; the cellulose content determined by the method of Weender [8], the carbohydrate content determined by difference; the starch content calculated by multiplying the carbohydrate content by the conversion factor 0.9, the energy value calculated by equation (4 × protein content) + (9.75 × lipid content) + (4.03 × glucids content) [9]; hydrocyanic acid (HCN) content determined by alkaline titration [10].

4. Results

4.1 The Composition of Cassava Roots is Given in Table 1

The indicated values represent the average of three determinations. The water content varies from 48.02 ± 0.80% (bitter cassava) to 68.84 ± 1.50% (sweet cassava). The carbohydrate content varies from 94.62% ± 1.34 dm (sweet cassava) to 94.70 ± 2.67% dm (cassava). The starch is the dominant fraction of carbohydrates; it represents 85.16 ± 2.21 - 85.23 ± 2.42% of these carbohydrates. The protein content varies from 1.80% ± 0.02 dm (bitter cassava) to 1.84% ± 0.13 dm (sweet cassava). The lipid

Table 1. **Composition of cassava roots**

Eléments / Cassava roots	Water % m f	Protein % m.s	Lipid % m.s	Ash % m.s	Cellulose % m.s	Carbohydrates % m.s	Starch % m.s	Energy value (cal/100g m.s)	HCN % m.s
sweet specie	68.84 ± 1.50	1.84 ± 0.13	1.00 ± 0.09	2.53 ± 0.11	2.95 ± 0.33	94.62 ± 1.34	85.16 ± 2.21	371.49 ± 37.59	0.1×10^{-2} ±0.00
bitter specie	48.02 ± 0.80	1.80 ± 0.02	1.01 ± 0.02	2.49 ± 0.14	3.66 ± 0.01	94.70 ± 2.67	85.23 ± 2.42	371.72 ± 20.22	0.13×10^{-2} ± 0.00

The indicated values represent the average of three determinations.

content varies from 1.00% ± 0.09 dm (sweet cassava) to 1.01 ± 0.02% dm (bitter cassava). The ash content varies from 2.49% ± 0.14 dm (bitter cassava) to 2.53 ± 0.11% dm (sweet cassava). The cellulose content varies from 2.95% ± 0.33 dm (sweet cassava) to 3.66 ± 0.01% dm (bitter cassava).

The Hydrocyanic acid content varies from 0.001 ± 0.00% dm (sweet cassava) to 0.0013 ± 0.00% dm (bitter cassava). The energy value varies from 371.49 ± 37.59 to 371.72 ± 20.22 cal/g 100 dm (bitter cassava).

The composition of foods derived from cassava for instance semolina and attiéké are respectively in **Tables 2** and **3**. The values in each table represent the average of three determinations.

4.2 Composition of the Semolina (Table 2)

The carbohydrate content varies from 94.53 ± 1.79% dm (sweet cassava) to 94.57 ± 2.14% dm (better cassava). The protein content varies from 1.77% ± 0.02 dm (bitter cassava) to 1, 79% ± 0.57 dm (sweet cassava). The lipid content varies of1, 20% ± 0.01 dm (bitter cassava) to 1.22% ± 0.06 dm (sweet cassava). The ash content varies from 2.46% ± 0.06 dm (bitter cassava) to 2.50% ± 0.28 ms (sweet cassava). The cellulose content varies from 2.52% ± 0.09 ms (sweet cassava) to 2.96% ± 0.41 dm (bitter cassava). The energy value of the semolina varies from 372, 88 ± 21.59 cal/100g dm) (bitter cassava) to 372.92 ± 8.41 cal / 100g dm (sweet cassava).

Hydrocyanic acid which is an anti-nutritional factor in the semolina is in the residual state in the semolina, it varies from 0.0001% dm (sweet cassava) to 0.0002% dm (bitter cassava).

4.3 Composition of Attiéké (Table 3)

The carbohydrate content varies from 94.50% ± 1.41 dm (sweet cassava) to 94.53% ± 1.41 dm (bitter cassava). The protein content varies from 1.70 ± 0.00% dm (bitter cassava) to 1.75 ± 0.01% dm (sweet cassava). The lipid content varies from 1, 25% ± 0.03 dm (sweet cassava) to 1, 40% ± 0.05 dm (bitter cassava). The ash content varies from 2.37% ± 0.01 dm (bitter cassava) to 2.49 ± 0.02% dm (sweet cassava). The cellulose content varies from 0.91% ± 0.04 dm (sweet cassava) to 1.85 ± 0.07% dm (bitter cassava). The energy value of attiéké is high, varying from 373.00 ± 25.4 cal/100g dm (sweet cassava) to 374.31 ± 28.15 cal/100g dm (bitter cassava).

Hydrocyanic acid which is an antinutritional factor in attiéké is in the residual state, it corresponds to 0.0001% dm.

5. Discussion

The moisture content of cassava roots is equal to average 58.43% pf, it is the same level of magnitude as the values

Table 2. Composition of cassava semolina

elements / Semoule	Water % ps	Protein % m. s	Lipid % m. s	Ash % m. s	Cellulose % m. s	Carbohydrate % m. s	Starch % m. s	Energy value (cal/100g m.s)	HCN % m. s
sweet specie	13.50 ± 1.40	1.79 ± 0.57	1.22 ± 0.06	2.50 ± 0.28	2.52 ± 0.09	94.53 ± 1.79	85.06 ± 1.46	372.92 ± 8.41	0.01×10^{-2} ± 0.00
bitter specie	13.20 ± 0.30	1.77 ± 0.02	1.20 ± 0.01	2.46 ± 0.06	2.96 ± 0.41	94.57 ± 2.14	85.11 ± 0.30	372.88 ± 21.59	0.02×10^{-2} ± 0.00

The indicated values represent the average of three determinations.

Table 3. Composition of attiéké bought in the market

elements / Attiéké	Water % p f	Protein % m. s	Lipid % m.s	Ash % m.s	Cellulose % m.s	Carbohydrate % m.s	Starch % m.s	Energy value (cal/100g m.s)	HCN % m.s
sweet specie	55.21 ± 0.06	1.75 ± 0.01	1.25 ± 0.03	2.49 ± 0.02	0.91 0.04	94.50 ± 1.41	84.65 ± 0.09	373.00 ± 25.4	0.01×10^{-2} ± 0.00
bitter specie	48.00 ± 0.10	1.70 ± 0.00	1.40 ± 0.05	2.37 ± 0.01	1.85 ± 0.07	94.53 ± 1.26	84.99 ±0.90	374.31 ± 28.15	0.01×10^{-2} ± 0.00

The indicated values represent the average of three determinations.

(54-58 pf) found by Favier [11]. The dry matter of cassava roots is mainly composed of carbohydrates with 94.62 to 94.70% dm, its values are close to that (94.1% dm) found by Woolfe [12].

These carbohydrates are composed mainly of starch, about 85%. Bornet *et al* [13] found rates ranging from approximately 84 to 87%.

The energy value of cassava roots is equal here average about 372 dm cal/100g. The high calorific value of cassava starch is an energy food.

Cassava roots have a protein content equal to 1.81% dm average; Favier [11] found a protein rate of the same order of magnitude (2.0% dm).

The lipid content of cassava roots is average of 1.01% dm. That percentage is close to the value of 0.99% dm found by Aboua and Kamenan [14].

The ash content is average of 2.51% dm, this result is consistent with the value of 2.50% dm found by Meuser and Smolnik [15].

These different results indicate that cassava is a rich food in carbohydrates but low in protein, lipid and mineral salts as indicated by Lingani *et al* [16].

The cellulose content is average equal to 3.31% dm. This value is less than the minimum percentage of 5% which would be tolerated in foods [17]. So cassava is a digestible food.

The hydrocyanic acid content varies from 0.001 to 0.13 × $\mathbf{10^{-2}}$% dm, either an average grade of 0.11 × $\mathbf{10^{-2}}$% dm. Hydrocyanic acid stays the main anti- nutritional factor to be reduced or removed before eating cassava.

The technological applied treatments to cassava roots during the preparation of the semolina (**Figure 1**) and attiéké (**Figure 2**) influence on its composition. The variation differences of cassava composition elements compared to the semolina and attiéké are respectively shown in **Tables 4** and **5**.

Thus the protein content decreased in the semolina by 0.05% compared to sweet cassava and by 0.03% compared to the bitter cassava. In attiéké, the protein content decreased by 0.09% compared to sweet cassava and by 0.10% compared to bitter cassava. The decrease in the protein content would be due in part to a loss of nitrogenous matter by solubilization during the roots cooking [18].

The lipid content increased in semolina by 0.22% compared to sweet cassava and by 0.19% compared to bitter cassava. By cons in attiéké, the lipid content increased by 0.25% compared to sweet cassava and by 0.39% compared to bitter cassava. Note that only the lipid content increased in the semolina as in attiéké. This could be explained by a condensation phenomenon of lipids in the dry matter during the drying operation.

The ash content decreased in the semolina by 0.03%

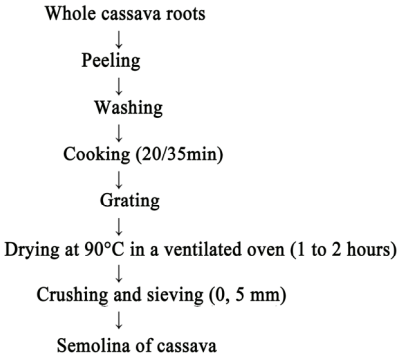

Figure 1. Diagram of semolina preparation

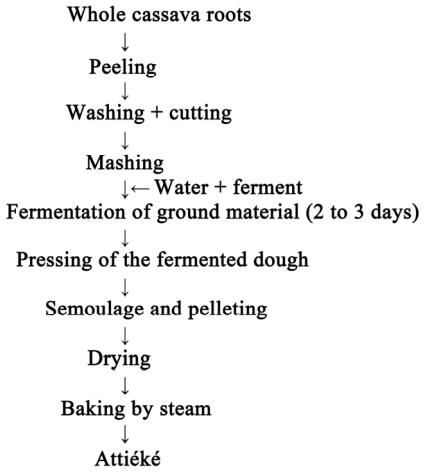

Figure 2. Diagram of traditional preparation of attiéké

compared to sweet cassava and by 0.03% compared to bitter cassava. In attiéké, the ash content decreased by 0.04% compared to sweet cassava and by 0.12% compared to bitter cassava. The decrease in ash could be explained by the phenomenon of dissolution [19], the mineral elements pass in eliminated water during the process of preparing food.

The cellulose content decreased by 0.43% compared to sweet cassava and by 0.70% compared to bitter cassava.

There is a cellulose decrease in attiéké; its level has dropped by 2.04% compared to sweet cassava and 1.81% from bitter cassava.

The low rate of cellulose promotes digestibility of its foodstuffs.

In semolina, carbohydrate content, decreased by 0.5 × $\mathbf{10^{-2}}$% compared to sweet cassava and by 0.13% compared to bitter cassava; in attiéké, carbohydrate content decreased by 0.12% compared to sweet cassava and by 0.17% compared to the bitter cassava.

The hydrocyanic acid content in semolina decreased by 0.09 × $\mathbf{10^{-2}}$% compared to sweet cassava and by

Table 4. Differences between the compositions of the roots and semolina

Nutrients / Samples	Protein % m. s	Lipid % m.s	Ash % m.s	Cellulose % m.s	Carbohydrate % m.s	Starch % m.s	Energy Value (cal/100g m.s)	HCN % m.s
Sweet specie roots/ semolina	– 0.05	+ 0.22	– 0.03	– 0.43	– 0.05	– 0.10	+ 1.43	-0.09×10^{-2}
Bitter specie roots/ semolina	– 0.03	+ 0.19	– 0.03	– 0.70	– 0.13	– 0.12	+ 1.16	-0.11×10^{-2}

Table 5. Differences between the composition of the roots and attiéké

Nutrients / Samples	Protein % m. s	Lipid % m.s	Ash % m.s	Cellulose % m.s	Carbohydrate % m.s	Starch % m.s	Energy Value (cal/100g m.s)	HCN % m.s
Sweet specie roots/ attiéké	– 0.09	– 0.25	– 0.04	– 2.04	– 0.12	– 0.51	– 0.51	-0.09×10^{-2}
Bitter specie roots/ attiéké	– 0.10	– 0.39	– 0.12	–1.81	– 0.17	–0.14	– 0.24	-0.12×10^{-2}

$0.11 \times \mathbf{10^{-2}}$% compared to bitter cassava. In attiéké the hydrocyanic acid content decreased by $0.09 \times \mathbf{10^{-2}}$% compared to sweet cassava and by $0.12 \times \mathbf{10^{-2}}$% compared to bitter cassava.

The loss of this element would be linked in part to its soluble and volatile nature at room temperature.

The technological applied treatments to cassava could also explain the decrease in the content of some elements of its composition compared to semolina and attiéké. These derived foods are poorer in protein, lipid and mineral salt than the roots of cassava. The increase of lipids rate in the semolina as in attiéké could explain the increase in the energy value of semolina and attiéké. One gram of burned lipid increases the energy value of 9.3 calories according to Atewater and Rosa [20].

6. Conclusions

The technological processing of cassava, in the preparation of their derived food such as semolina and attiéké, has influenced its composition. Thus, the hydrocyanic acid content, antinutritional factors characteristic of cassava decreased in semolina and attiéké making their consumption less hazardous. Regarding nutrients, except the lipid rate and energy value that have increased in semolina and attiéké, the rates of carbohydrate, protein, mineral salt and cellulose decreased. Note however that the main nutrient of cassava and its derived foods are carbohydrates which are composed mainly of starch whose high calorific value makes these commodities to be energy foods. However, the poverty of those foods in protein, lipid and mineral salt strongly lowers their nutritional value.

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Effects of Mixing Canola and Palm Oils with Sunflower Oil on the Formation of *Trans* Fatty Acids during Frying

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ABSTRACT

*GLC analysis was conducted to indicate the formation of trans- C*18 *fatty acids of sunflower, canola and palm oils during frying. Blends of sunflower oil and palm oil or canola oil were obtained by mixing sunflower oil with palm or canola oils at the volume ratios of* 60: 40, 40: 60 *and* 20: 80 (*v/v*), *then heated at* 180˚C ± 5˚C *for* 5, 10, 15 *and* 20 *h in the atmospheric oxygen. GLC results demonstrate that the formation of trans C*18*-fatty acids was generally dependent upon the frying time and oil mixing ratios. Furthermore, mixing sunflower oil with oils rich in monounsaturated fatty acids* (*palm or canola oils*) *lowered the formation of trans-C*18 *fatty acids during frying.*

***Keywords*:** *Sunflower Oil*, *Canola Oil*, *Palm Oil*, *Trans Fatty Acids*, *Frying Process*, *GLC Analysis*

1. Introduction

Trans fats or *trans* fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. *Trans* fatty acids occur naturally in small amounts in foods produced from ruminant animals. However, most of *trans* fatty acids in the diet are produced during the process of partial hydrogenation of vegetable oils into semi-solid fats [1]. Also, *trans*-fatty acids can be formed from *cis*-unsaturated fatty acids by the effect of several bacteria, especially parasitic bacteria in the rumen of cattle which convert unsaturated lipids from *cis to trans*-isomers through a *cis/trans* isomerase [2].

Trans fatty acids have an adverse effect on blood lipids and have been shown to increase the risk of heart disease. *Trans* fatty acids increase LDL-cholesterol (the bad cholesterol) and decrease HDL-cholesterol (the good cholesterol) and overall, *trans* fatty acids increase LDL cholesterol (bad cholesterol) similarly to saturated fat, but, unlike saturated fat, they also decrease HDL cholesterol. As a result, the net effect of *trans* fat on the LDL/HDL cholesterol ratio is approximately double that of saturated fat. It is worth noting that the risk of cardiovascular disease is increased if *trans* fatty acids are consumed in high amounts [1,3]. In addition, *trans* fatty acids appear to have an adverse effect on some lipoproteins and apoproteins when fed at relatively high levels [4]. In contrast, lower levels of *trans* fatty acids did not appear to be deleterious [5].

Deep-fat frying is one of the most processes used in all food preparations. Basically, this process includes immersing a food item in a large quantity of heated oil and reused several times before being disposed. Deep-fat frying produces a product with desired sensory characteristics, including fried food flavour, golden brown colour and a crisp texture [6]. This process has also been considered a source for the production of *trans* fatty acids. Formation of *trans* fatty acids during frying has been shown to be closely related to process temperature and time. In sunflower oil, the amount of *trans* isomers were found to be 1.10 % when heated at 200˚C for 40 min as compared to 11.45% at 300˚C for the same duration of heating [7]. Conversely, Romero *et al.*, [8] reported a very minimal production of eliadic acid in extra virgin olive oil, high oleic sunflower oil and sunflower oil. On the other hand, commercial frying and baking oils contain significant amount of *trans* fatty acids. In general, baked goods, fries snack foods (e.g., dough nuts, French fries) and fried fast-food items (e.g., fried chicken and fried fish) contain significant amounts of *trans* fatty acids [9].

It seems that *cis/trans*-isomerisation process depends on the most prominent fatty acids present in an oil. For instance, eliadic acid was the most abundant *trans* fatty acid in extra virgin olive oil fried potatoes while *trans* linoleic isomers were more abundant in sunflower oil. High oleic sunflower oil was in between [8].

In this context, the Food and Drug Administration (FDA) [10] ruled that the nutrition labels for all conventional foods and supplements must indicate the content of *trans* fatty acids. In addition, the Department of Agriculture made a limited intake of *trans* fatty acids a key recommendation of the new food-pyramid guideline subsequent to the recommendations of the Dietary Guidelines Advisory Committee [11] that the consumption of *trans* fatty acids be kept below 1% of total energy intake. Furthermore, the UK Faculty of Public Health and Royal Society for Public Health [12] proposed that consumption of *trans* fatty acids should be virtually eliminated.

A set of experiments was conducted in the present study where oils rich in monosaturated fatty acids (canola and palm oils) were mixed with sunflower oil at various volume ratios in an attempt to extent its shelf-life [13]. The major objective of the present work was to see if blending oils rich in monosaturated fatty acids with sunflower oil might lower the formation of *trans* fatty acid content of the binary oil blends during frying at 180˚C for 5 h daily for 4 consecutive days.

2. Materials and Methods

2.1 Oils

1) Refined sunflower oil was obtained from Sila Edible Oil company (Kom Osheim, El-Fayoum governorate, Egypt). The oil peroxide and acid values were 1.2 (meq. active oxygen Kg^{-1} oil) and 0.08 (mg KOH/g^{-1} oil), respectively.

2) Refined palm oil was obtained from Savola Sime company, 10th of Ramdan city , Sharkia governarate, Egypt . The oil peroxide and acid values were 0.14 (meq. active oxygen Kg^{-1} oil) and 0.043 (mg KOH g^{-1} oil), respectively.

3) Canola seeds (*Brassica species*, Serwi 4 variety) were obtained from Oil Crops Department, Field Crops Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. The seeds were ground and packed in cheese cloth, pressed by hydraulic Carver (model 2759 S/N 2759-584 Freds. Carver Inc., U.S.A.). The produced oil was filtered through Whatman filter paper No.1 and kept in a brown glass bottle at 7˚C.

2.2 Frying Process

A known amount (2 L) of each of refined sunflower, palm and canola oils were separately placed in a Cordon Bleu deep fat-fryer (Model DZ-05D, China) equipped with a temperature controller. Portions of sunflower oil were mixed with palm oil or canola oil at ratios of 60: 40, 40:60 and 20: 80, (v/v). Potato chips (2 mm thickness × 40 mm width × 50 mm length) were soaked in a sodium chloride solution (10%, w/v) for 0.5 h and fried in different oil samples at 180ºC ± 5ºC. The frying process was conducted 4 times each day and the frying period was 5 h day^{-1}. This process was repeated for 4 consecutive days. Oil samples were taken every 5 h and the total continuous heating period was 20 h. The oil samples were left to cool down then stored at – 10ºC for fatty acid analysis.

2.3 Fatty Acid Compositions of Fried Sunflower, Palm and Canola Oils and Their Binary Mixtures

Capillary gas chromatography (HP 6890) was used for the qualitative and quantitative determinations of fatty acids of the oil samples and reported in relative area percentages. Fatty acids were transesterified into their corresponding fatty acid methyl esters (FAMEs) by shaking a solution of oil (ca. 0.1 g) in heptane (2 ml) with a solution of methanolic potassium hydroxide (0.2 ml, 2N). The FAMEs were identified using a gas chromatograph equipped with DB-23 (50%- cyano propyl)-methylpoly siloxane) capillary column (60m × 0.32 mm × 0.25 µm film thickness) and a flame ionization detector. Nitrogen flow rate was 0.6 ml/min, hydrogen and air-flow rates were 45 and, 450 ml/min, respectively. The oven temperature was isothermally heated at 195˚C. The injector and the detector temperatures were 230˚C and 250˚C, respectively. MEFAs were identified by comparing their retention times with known fatty acid standard mixture. Peak areas were automatically computed by an integrator. All GC measurements for each oil sample were made in triplicate and the average values were reported.

3. Results and Discussion

3.1 *Trans* C18 Fatty Acids of Individual Oils

In this set of experiments, the major concern is focused on the formation of *trans*-fatty acids (eliadization) during heating at 180˚C ± 5˚C for various periods. It is worth mentioning that the fatty acid profile of fresh sunflower oil was discussed earlier in detail [13]. **Table 1** shows the changes in heated sunflower oil during frying. No *trans*-oleic acid (elaidic) was found after the first 5 h heating period. Afterwards, its content was slightly and gradually increased towards the end of heating period (20 h). Similar results were noticed with *trans* linoleic acid (linoelaideic acid). It is worth mentioning that the amount of *trans* linoleic acid was much greater than that of *trans*-oleic acid (**Figure 1**). Similar findings were reported by Hunter and Applewhite [9] who reported that the *trans* amounts of fatty acids increased gradually by increasing the heating period.

Looking at *trans* fatty acid profile of heated palm oil at 180˚C ± 5˚C for various periods, one can deduce the following remarks. The amount of trans-oleic acid was formed at 5 h and its amount become constant throughout the entire heating period (20 h). It is worth mentioning that the amount of *trans*-oleic acid was much lower than

Table 1. *Trans* and their parent fatty acid compositions (area %) of heated sunflower, palm and canola oils for various periods

Oil blend	Heating period (h)							
	5	10	15	20	5	10	15	20
	Trans-oleic acid (elaidic) acid				*Cis*-oleic acid			
Sunflower oil	ND	0.03	0.13	0.15	29.16	28.33	29.13	29.22
Palm oil	0.03	0.04	0.04	0.05	40.25	39.58	39.57	41.20
Canola oil	0.03	0.06	0.11	0.14	46.25	46.71	47.15	49.14
	Trans-linoleic (linolaidic) acid				*Cis*-linoleic acid			
Sunflower oil	ND	1.09	1.22	1.5	57.52	57.99	56.40	55.42
Palm oil	0.08	0.08	0.08	0.09	12.09	10.20	9.40	9.31
Canola oil	0.02	0.05	0.05	0.05	18.33	17.76	17.88	16.41
	Total *trans* C18 fatty acids							
Sunflower oil	ND	1.12	1.35	1.65				
Palm oil	0.11	0.12	0.12	0.14				
Canola oil	0.14	0.11	0.16	0.19				

ND refers to non detected fatty acid

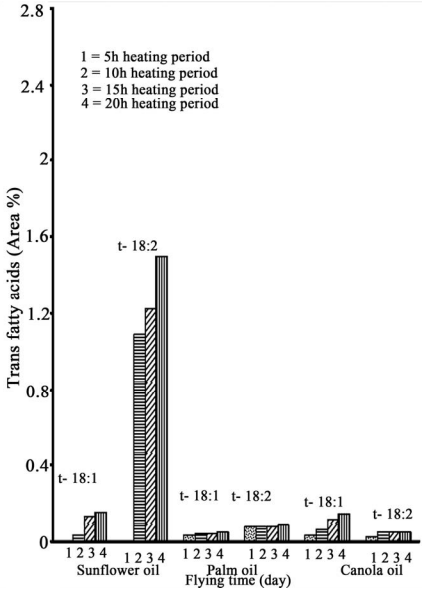

Figure 1. *Trans* fatty acids of sunflower, palm and canola oils heated for various periods. t- 18: 1 and t- 18: 2 acids refer to *trans*-oleic and *trans*-linoleic fatty acids, respectively

that found in sunflower oil. However, palm oil contains high level of oleic acid (parent acid) than that of sunflower oil. On the contrary, the level of linoleic acid in sunflower oil was much greater than that present in palm oil and the level of *trans* linoleic acid was about 16 times as great as that present in palm oil at the end heating period (20 h) (**Table 1**).

Dealing with canola oil, the amount of *trans*-oleic was found after heating for 5 h and gradually increased throughout at the entire heating period (20 h). The amount of *trans*-oleic acid at the end of the heating period was about the same as that of sunflower oil. The amount of *trans* oleic acid increased in the following order depending on the oil type: sunflower oil > canola oil > palm oil. This sequence may be stem from the level of *cis*-oleic which can be arranged in the following order: according to oil type: canola oil > palm oil > sunflower oil.

The amount of *trans*-linoleic acid in canola oil was very low compared with *trans*-oleic of other two oils during the various heating periods. This may be due the low level of *cis*-linoleic acid compared with *cis*-oleic acid.

In general, the total amounts of *trans* C18 fatty acids at the end of this series of experiments can be arranged in the following order: sunflower oil > canola oil > palm oil. These findings are in contrast with the results of the nutritional experiments reported by Farag *et al.*, [13] where the major alteration induced to rat organ tissues (liver, kidney, heart) when rats administered the heated sunflower oil, palm oil and canola oil as part of their diet. The changes on rat organ tissues were related to the type of fatty acids (erucic acid in canola oil and palmitic acid in palm oil). Several reports indicated that *trans* acids induced deleterious effects on human health [14]. This fact was not found in the present work. This is might be due to the presence of *trans*-acids in very low amounts (lower than 2% in sunflower oil and 0.2% in canola and palm oils). Similar results were reported by Nestel *et al.* [5].

3.2 *Trans* Fatty Acids of Sunflower Oil and Canola Oil Blends

Table 2 shows the effect of blending different ratios of canola oil with sunflower oil and heated for various periods. Generally speaking, the levels of elaidic increased with increasing the heating period and mixing

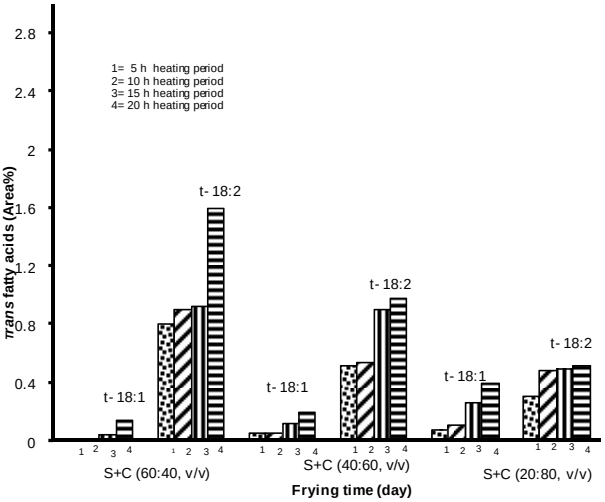

Figure 2. *Trans* fatty acids of fried sunflower oil (S) mixed with various levels of canola oil (C) heated for different periods. t- 18: 1 and t- 18: 2 indicate *trans*-oleic and *trans*-linoleic acids, respectively

ratios. In the mean time, the level of *cis*-oleic was slightly increased with heating time. It is noticeable that its amount increased by increasing the blending ratio and in particular at the volume ratio of 20:80 (v/v) (**Figure 2**).

Here again, the amount of *trans*-linoleic increased at all volume ratios with prolonging the heating period. On the contrary, its level was progressively decreased with increasing the blending ratios. Concerning *cis*-linoleic acid, its levels were remarkably decreased with extending the heating period and increasing with increasing the level of canola in the blending ratios. The results with the total *trans*-oleic and linoleic acids are greed quite well with the previous findings (**Table 2**, and **Figure 2**).

3.3 Trans Fatty Acids of Sunflower Oil and Palm Oil Blends

The amounts of *trans*-oleic acid and its parent acid of heated sunflower oil and mixed with heated palm oil at volume ratio of 60: 40 (v/v) for various periods are shown in **Table 3**. In general, the results demonstrate a progressive increase in the amount of elaidic with prolonging the heating period. The amount of linolaidic acid formation was more pronounced than that of elaidic acid with

Table 2. *Trans* and their parent fatty acid compositions (area %) of heated binary mixtures of sunflower oil (S) and canola oil (C) for various periods

Oil blend	Heating period (h)							
	5	10	15	20	5	10	15	20
		Trans-oleic acid (elaidic) acid				*Cis*-oleic acid		
S + C (60: 40, v/v)	ND	ND	0.04	0.14	33.89	34.56	34.61	33.61
S + C (40: 60, v/v)	0.05	0.05	0.12	0.20	40.74	43.07	42.59	43.57
S + C (20: 80, v/v)	0.07	0.11	0.26	0.40	42.19	42.50	42.49	42.47
		Trans-linoleic (linolaidic) acid				*Cis*-linoleic acid		
S + C (60: 40, v/v)	0.80	0.90	0.92	1.60	47.89	45.89	44.91	42.42
S + C (40: 60, v/v)	0.52	0.54	0.90	0.98	34.65	32.14	31.78	29.74
S + C (20: 80, v/v)	0.31	0.48	0.49	0.52	27.22	27.35	26.65	24.81
		Total *trans* fatty acids						
S + C (60: 40, v/v)	0.80	0.90	0.96	1.74				
S + C (40: 60, v/v)	0.59	0.65	1.16	1.38				
S + C (20: 80, v/v)	0.36	0.53	0.61	0.72				

ND refers to non detected fatty acid

Table 3. *Trans* and their parent fatty acid compositions (area %) of heated binary mixtures of sunflower oil (S) and palm oil (P) for different periods

Oil blend	Heating period (h)							
	5	10	15	20	5	10	15	20
		Trans-oleic acid (elaidic) acid				*Cis*-oleic acid		
S + P (60: 40, v/v)	0.03	0.04	0.09	0.13	31.80	31.48	32.40	32.98
S + P (40: 60, v/v)	0.03	0.05	0.18	0.20	33.64	34.80	34.77	34.96
S + P (20: 80, v/v)	0.04	0.05	0.21	0.48	35.36	34.91	34.85	34.40
		Trans-linoleic (linolaidic) acid				*Cis*-linoleic acid		
S + P (60: 40, v/v)	0.77	0.79	0.90	0.92	37.19	38.28	35.71	34.96
S + P (40: 60, v/v)	0.53	0.54	0.64	0.73	28.96	26.54	23.19	17.17
S + P (20: 80, v/v)	0.33	0.33	0.36	0.40	19.19	18.77	16.14	15.38
		Total *trans* C18 fatty acids						
S + P (60: 40, v/v)	0.80	0.83	0.99	1.05				
S + P (40: 60, v/v)	0.56	0.61	0.82	0.93				
S + P (20: 80, v/v)	0.37	0.38	0.57	0.88				

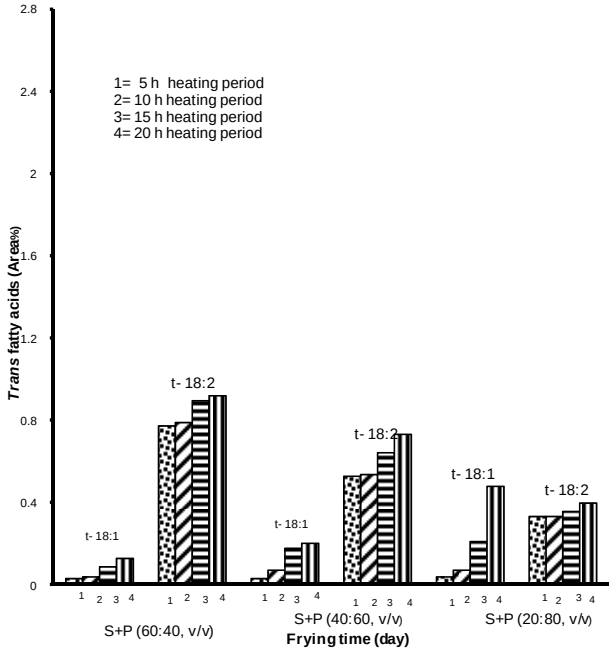

Figure 3. Trans fatty acids of fried sunflower oil (S) mixed with various levels of palm oil (P) heated for different periods. t- 18:1 and t- 18:2 indicate trans-oleic and trans-linoleic acids, respectively

increasing the heating time (**Figure 3**). This might be due to the fact that the amount of *cis*-linoleic is higher than *cis*-oleic acid. Also, one has to mention that cis-linoleic acid is much labile than *cis*-oleic acid and rate of steromutation is in the favour of linoleic acid.

With increasing the mixing ratios of heated palm oil with heated sunflower oil (60: 40 and 20: 80, v/v) led to an increase of the amount of elaidic acid. It is worth mentioning that the levels of *cis*-oleic acid slightly increased with increasing the ratio of blending palm oil with sunflower oil. On the contrary, the level of linolaidic acid decreased with increasing the admixture ratios of palm oil (**Figure 3**). This is logic since the amount of *cis*-18: 2 was decreased with the admixture ratio.

The results in **Table 3** indicate that the amount of total *trans* C18-fatty acids decreased with increasing the volume ratio of palm oil with sunflower oil during frying. Similar findings were seen by the increasing the mixing of canola oil with sunflower oil during frying. These findings led to deduce that mixing sunflower oil with canola or palm oils induced lowering effect on the total-*trans* fatty acids during frying. In this case one would say mixing sunflower oil with other oils rich in monounsaturated acid might reduce the formation of *trans* fatty acids during frying which is in the favor of human health.

The ratio of linoleic acid to palmitic acid (C18: 2/C16: 0) has been suggested by Normand *et al.*, [15] as a valid indicator of the level of polyunsaturated fatty acid deterioration. The results of the present work showed a decrease in this ratio from 9.02 to 6.79, 3.82 to 2.93 and 0.24 to 0.22 at the end frying period for sunflower, canola and palm oils respectively. The ratios indicate the incidence of deterioration of polyunsaturated fatty acids of the oils under frying process.

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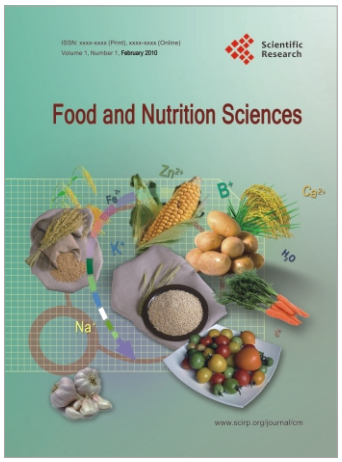

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