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Original Article

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# Physicochemical Analysis of Margarine, Butter and Butter Oil Samples of Iran'sMarkets



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## Abstract

Butter, margarine and butter oil are three products which consume daily by Iranian community. Detection of type and existence of synthetic antioxidants (SAs) quantities in the margarine, butter, and butter oil samples is so important in point of view of the safety. For this purpose, peroxide value (PV), oxidative stability (OS), iodine value (IV), fatty acid analysis (FA), type and amount of SAs, and its antioxidant activity (AA%) of the samples was determined as described methods of the International Organization for Standardization (ISO) documents including ISO numbers: 3960, 6886, 3961, 5509, AOCS Ce 6-86, and DPPH assay, respectively. The direct relationship between the quantities of OS, SA, and AA% was observed in the samples. It was observed that many of butter oil samples were without SA. In margarine samples, the amount of IV was higher than that of in butter and butter oil samples ($P<0.05$). The SAs quantities in margarine samples were estimated higher than those of butter and butter oil samples ($P<0.05$). The AA% was observed in the most of butter and butter oil samples that SA was not detected in them by HPLC which may be depending on the existence of natural bioactive materials including phospholipids having antioxidant activity in butter and butter oil samples.Results showed that propyl gallate (PG) was detected in all samples of margarine and the amount of SA in all samples was lower than that of specified in Iranian National Standards Organization (INSO).

**Keywords:** Antioxidant Activity, Butter, Margarine, Stability, Synthetic Antioxidants

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## Introduction

Butter is a water-in-oil emulsion consisting of fat (80–82%) and an aqueous phase (18–20%) containing salt and milk-solids-not-fat. The upper legal limit for water is 16%, according to INSO 162 (2008). Butter is made from cow milk (3–4% fat) that is converted first to cream (30–45% fat) by centrifuge. Butter or cream is converted into butter oil (ghee) by controlling temperature with over 99.3% milk fat and less than 0.5% moisture according to descriptions of INSO, 1254 (2002). Margarine is made from refined vegetable oils (80% fat); water and dry materials according to descriptions of INSO, 143 (2011). Butter, margarine and butter oil products can be spoiled like many other dairy products including cheese, cream, and yogurt and so on. It was reported that because of the higher fat content of butter, margarine and butter oil samples, they are susceptible products for the deterioration (Homero-Mendez et al., 2001). Therefore, the high oxidative stability of lipids is a very important reason for human health, storage of products and has economic importance. The peroxide value (PV) and oxidative stability (OS) are widely used as a measure to extend of these unwanted reactions not only in foodstuffs (Homero-Mendez et al., 2001). In the margarine emulsion (water and oil type), oxygen diffuses from air into the continuous oil phase, where the oxidation takes place (Pokorny et al., 2001). It is reported that margarine is a cholesterol free product and has less saturated fatty acids than butter and butter oil and for this reason, manufacture and consumption of this product are growing worldwide, therefore attention to keep and storage condition is interested. The existence of water in oils affects negatively the performance and shelf-life of oil. Oxidation of food can be prevented by synthetic antioxidants (SAs), including butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG). European Food Safety Authority (EFSA) established acceptable daily intakes (ADIs) of 0.25, 1.0, and 0-0.7 mg per kg body weight per day for BHT, BHA, and TBHQ respectively, but noted that the exposure of adults and children was unlikely to exceed these intakes (Carocho and Ferreira, 2013; EFSA, 2012; EFSA, 2004; EFSA, 2011). If SAs used in higher concentrations, it could be dangerous. According to the reports of Lanigan and Yamarik (2002), BHT had a bad effect on the rat liver, kidney, and lung because of their potential action as carcinogens. Some studies have reported that BHA and BHT could be cytotoxic because of the carcinogenicity of BHA in the forestomach of rodents (Satio et al., 2003), thus, some decisions have been made by governments to reduce the use of SAs in food. There are several methods for the determination of SAs, including gas chromatography (GC) (Yang et al., 2002), high-performance liquid chromatography (HPLC) (Perrin and Meyer, 2003), gel permeation chromatography (Doeden et al., 1979), and voltammetry methods (Ceballos and Fernandez, 2000). Other important parameters for determination of quality control of oils and fats are included IV and fatty acid analysis by GC (Thomas, 2002). The aim of this study is the determination of PV, OS, IV and AA%for investigation of function, and quality of added SAs and study of the correlation between these quality parameters in butter, butter oil, and margarine samples. In addition, the kind and amount of SAs in these products were obtained and compared with specified standard limits in Iranian National Standards Organization (INSO).

## Materials and Methods

### Materials

BHT, BHA, TBHQ and PG with high purity were purchased from the Merck Company (Germany). Other materials, including, hexane, n-propanol, acetonitrile (ACN), reagents, glacial acetic acid, chloroform, potassium iodide (KI), thiosulfate sodium, starch and Hanus solution were also purchased from the Merck Company (Germany).

### Sampling

20 samples, including margarine (10 samples, with 80% fat and 18% moisture, 2% salt and dry material), butter (5 samples, with minimum 82% fat, 16% moisture), butter oil (5 samples, with 99.5% fat and 0.5% moisture), were purchased from Tehran's markets. Sampling was performed in October 2015. All samples were heated at 35 to 40 ºC and then centrifuged. The

oil phase of the samples was separated and kept for performing of next analysis, including PV, IV, OS, SAs, FA and AA%.

#### Determination of PV

Determination of PV was performed by the iodometric method as described in INSO No. 3960 (2017).

#### Determination of IV

Determination of the IV was performed as the described method in 1SO No. 3961 (2013). For each sample, three replicates were recorded.

#### Determination of oxidative stability using Rancimat

Determination of the OS was performed as the described method in ISO No.6886 (2006). Rancimat (Metrohm 743, Switzerland) was used for OS quantification in samples.

#### Determination of fatty acids by GC

The fatty acid composition has commonly been determined by GC (equipped with a flame ionization detector (FID)). In the most common methods, lipids and edible oils must be modified into methyl esters before the analysis by GC (Craske, 1993). Preparation of methyl esters was done as the described method in ISO 5509 (1978) and analyzed by GC as the described method in ISO 5508 (1990). Hydrogen and air flow rate were adjusted at 30, and 300 ml min-1, respectively. A capillary column (CP-SIL 88) with dimensions of 0.25 mm × 100 m, 0.2$\mu$m was used. The temperature of the injector and detector was adjusted in 270 and 300°C, respectively. The oven was programmed at 50 ºC (1 min) with a heating rate of 4 ºC/min and reached to 190ºC (40 min).

#### Determination of quantity of SAs by HPLC

The Preparation of standard solutions was determined as the described method in AOCS CE 6-86 (1997). A stock standard solution (1mg ml-1) was prepared. Briefly, 50 mg of each SA (PG, TBHQ, BHA, and BHT) accurately weighted in a volumetric flask (50 ml), dissolved, and diluted to the volume with 2-propanol: acetonitrile (1:1 (v: v)). A prepared stock solution covered with an aluminum foil and stored in a freezer (4ºC) (AOCS CE 6-86 (1997)).The extraction and quantification of added SAs in test samples were performed as the described method in AOCS CE 6-86 (1997). For each sample, three replicates were recorded.Separation of selected SAs was carried out using HPLC, equipped with a reversed-phase C18 analytical column (150 mm × 100 mm, 0.53 $\mu$m), and UV detector (at 280 nm). Mobile phases consisted of deionized water with 5% glacial acetic acid (solvent A), acetonitrile with 5% glacial acetic acid (solvent B). The following gradient was applied: linear increase from solution 30% B to 100 % B in 10 min, hold at 100% B at a flow rate of 2 ml min-1 for 4 min. The volume of injection was 20 $\mu$L (AOCS CE 6-86 (1997)).

#### Determination of antioxidant activity (AA %)

For determination of AA% of the extracted SA of test samples, the method of DPPH assay was used as described by Robert et.al. (1999). For this purpose, 1.5 ml of a prepared methanolic solution of DPPH (0.135 mM) was added in 0.5 ml of the solution containing extracted SA and then mixed. A blank solution was also prepared. Then test sample and blank were incubated in a dark place at room temperature for 30 minutes. The absorbance of test sample and blank was recorded at room temperature at 517 nm. For each sample, three replicates were recorded.

#### Statistical analysis

Data analysis was obtained at least in triplicates averaged. Statistical analysis was carried out using one-way analysis of variance (ANOVA). A value of $p \leq 0.05$ was considered statistically significant. The results are shown as the mean ± standard deviation (SD). The SPSS and excel software for statistical calculations was used.

## Results

#### PV, OS, and IV

PV is a measure of the concentration of peroxides and hydro- peroxides formed in the first stages of lipid oxidation. The measured quantity of PV in the samples is shown in the Table. 1. For margarine samples, the maximum standard limit of 5 meq kg-1 set for PV in the National Standards of Iran. Results showed that the measured PV in all margarine samples was lower than 5 meq kg-1. For butter and butter oil, the maximum standard limit of 1meq kg-1 set for PV in INSO Nos. 162 and 1254, respectively. Results showed that the

**Table:. PV, OS and IV in the margarine, butter and butter oil samples**

| Sample | No | PV (milli equivalent O2/kg of oil) | OS (h) | IV |
|---|---|---|---|---|
| Margarine | 1 | $0.09 \pm 0.008^{b}$ | $29.67 \pm 0.42^{a}$ | $70.97 \pm 0.02^{g}$ |
| Margarine | 2 | $0.09 \pm 0.001^{b}$ | $28.77 \pm 0.27^{ab}$ | $72.68 \pm 0.09^{g}$ |
| Margarine | 3 | $0.11 \pm 0.008^{b}$ | $28.28 \pm 0.09^{b}$ | $78.81 \pm 0.03^{e}$ |
| Margarine | 4 | $0.12 \pm 0.014^{b}$ | $19.33 \pm 0.09^{f}$ | $79.61 \pm 0.02^{d}$ |
| Margarine | 5 | $0.11 \pm 0.014^{b}$ | $21.90 \pm 0.21^{d}$ | $80.43 \pm 0.02^{c}$ |
| Margarine | 6 | $0.13 \pm 0.012^{b}$ | $18.73 \pm 0.00^{f}$ | $81.22 \pm 0.08^{b}$ |
| Margarine | 7 | $0.13 \pm 0.008^{b}$ | $17.66 \pm 0.11^{g}$ | $81.88 \pm 0.02^{ab}$ |
| Margarine | 8 | $0.14 \pm 0.001^{b}$ | $17.05 \pm 0.12^{h}$ | $81.21 \pm 0.09^{b}$ |
| Margarine | 9 | $0.15 \pm 0.002^{b}$ | $16.66 \pm 0.02^{h}$ | $82.85 \pm 0.01^{a}$ |
| Margarine | 10 | $0.15 \pm 0.005^{b}$ | $17.78 \pm 0.02^{g}$ | $82.91 \pm 0.02^{a}$ |
| Butter | 1 | $0.12 \pm 0.064^{b}$ | $14.28 \pm 0.21^{j}$ | $25.13 \pm 0.01^{l}$ |
| Butter | 2 | $0.12 \pm 0.031^{b}$ | $14.04 \pm 0.28^{j}$ | $26.15 \pm 0.02^{k}$ |
| Butter | 3 | $0.08 \pm 0.015^{b}$ | $22.61 \pm 0.43^{c}$ | $26.12 \pm 0.01^{k}$ |
| Butter | 4 | $0.09 \pm 0.022^{b}$ | $21.35 \pm 0.22^{d}$ | $28.28 \pm 0.02^{i}$ |
| Butter | 5 | $0.19 \pm 0.013^{b}$ | $13.32 \pm 0.06^{k}$ | $30.12 \pm 0.01^{h}$ |
| Butter oil | 1 | $0.09 \pm 0.006^{b}$ | $15.36 \pm 0.31^{i}$ | $26.21 \pm 0.03^{j}$ |
| Butter oil | 2 | $0.19 \pm 0.008^{b}$ | $14.26 \pm 0.44^{j}$ | $30.62 \pm 0.09^{h}$ |
| Butter oil | 3 | $0.20 \pm 0.006^{a}$ | $10.96 \pm 0.23^{l}$ | $31.22 \pm 0.06^{h}$ |
| Butter oil | 4 | $0.20 \pm 0.002^{z}$ | $10.91 \pm 0.22^{l}$ | $31.42 \pm 0.05^{h}$ |
| Butter oil | 5 | $0.20 \pm 0.012^{z}$ | $10.67 \pm 0.27^{l}$ | $31.87 \pm 0.02^{h}$ |

obtained PV for all the samples is in a permissible range. The obtained OS for each sample is shown in the Table. 1. OS for each sample was determined at a temperature between 110 and 130 °C. For each sample with high PV, the OS was low. Results showed that the maximum OS belonged to margarine samples of No. 1, 2 and 3. Results of IV for the samples with three replicates are shown in the Table. 1. The range of the obtained IV in margarine samples was 70-82 but the limit of the IV is not specified in INSO No.143 or Codex STAN 32 (1981). The obtained IV in butter and butter oil samples was between 25- 31. It was observed that the IV in butter No. 1 was not in the range of 26-40 as specified in INSO 162 (2008).

### Fatty acids composition

The common unsaturated FAs present in the sample oils can be quantified by GC. The results of the FAs analysis of the samples are shown in Table.2. It was observed that in the margarine samples, the amount of stearic acid (C18:0), linoleic acid (C18:2) and linolenic acid (C18:3) was higher than that of butter and butter oil samples, but the short-saturated FAs such as butyric (C4:0), and caprylic (C8:0) were just observed in the butter and butter oil samples. Capric (C10:0), lauric (C12:0), and myristyl (C14:0) acids in butter and butter oil samples were higher than margarine samples.The limit of specified saturated fatty acid in INSO NO. 143 is 48(wt%)

**Table 2: Fatty acid profiles of the samples**

| Fatty acids (%) | C4:0 | C6:0 | C8:0 | C10:0 | C12:0 | C14:0 | C14:1 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20;0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Margarine1 | - | - | - | - | 0.10±0.21 | 0.80±0.12 | 0.10±0.09 | 27.30±0.11 | 0.10±0.09 | 55.10±0.12 | 29.80±0.13 | 21.10±0.12 | 2.40±0.21 | 0.40±0.12 |
| Margarine2 | - | - | - | - | 0.10±0.12 | 0.90±0.23 | 0.10±0.12 | 27.20±0.21 | 0.10±0.03 | 55.00±0.23 | 29.90±0.13 | 21.20±0.13 | 2.50±0.12 | 0.40±0.15 |
| Margarine3 | - | - | - | 0.02±0.20 | 0.10±0.21 | 1.00±0.15 | 0.10±0.13 | 28.20±0.24 | 0.16±0.09 | 55.30±0.17 | 30.40±0.21 | 20.30±0.21 | 2.50±0.23 | 0.30±0.04 |
| Margarine4 | - | - | - | - | 0.11±0.03 | 0.90±0.22 | 0.10±0.21 | 28.30±0.09 | 0.13±0.08 | 55.40±0.13 | 31.20±0.17 | 20.90±0.22 | 2.30±0.08 | 0.40±0.22 |
| Margarine5 | - | - | - | - | 0.12±0.10 | 0.93±0.21 | 0.11±0.11 | 28.20±0.28 | 0.1±0.04 | 55.50±0.12 | 29.98±0.18 | 20.90±0.13 | 2.30±0.23 | 0.30±0.11 |
| Margarine6 | - | - | - | - | 0.14±0.03 | 0.78±0.10 | 0.12±0.24 | 27.90±0.23 | 0.11±0.09 | 54.60±0.22 | 30.30±0.21 | 21.70±0.16 | 2.40±0.15 | 0.30±0.14 |
| Margarine7 | - | - | - | - | 0.15±0.21 | 0.79±0.11 | 0.10±0.14 | 27.30±0.12 | 0.10±0.12 | 54.44±0.01 | 30.20±0.06 | 21.90±0.03 | 2.50±0.10 | 0.40±0.06 |
| Margarine8 | - | - | - | - | 0.11±0.12 | 0.83±0.13 | 0.09±0.08 | 27.70±0.12 | 0.10±0.09 | 55.10±0.12 | 29.90±0.16 | 22.00±0.12 | 2.20±0.17 | 0.40±0.14 |
| Margarine9 | - | - | - | - | 0.13±0.12 | 0.98±0.21 | 0.10±0.12 | 27.90±0.12 | 0.12±0.19 | 54.20±0.14 | 31.20±0.09 | 21.00±0.09 | 2.400±0.05 | 0.50±0.21 |
| Margarine10 | - | - | - | - | 0.15±0.04 | 0.92±0.12 | 0.14±0.19 | 27.10±0.12 | 0.11±0.18 | 55.10±0.09 | 29.80±0.14 | 21.00±0.15 | 2.50±0.15 | 0.40±0.13 |
| Butter1 | 2.00±0.11 | 2.20±0.12 | 1.30±0.06 | 3.30±0.23 | 3.90±0.15 | 12.00±0.12 | 1.6±0.06 | 31.10±0.13 | 1.60±0.23 | 10.40±0.15 | 21.60±0.14 | 2.50±0.23 | 0.10±0.13 | 0.10±0.21 |
| Butter2 | 2.60±0.23 | 2.30±0.18 | 1.50±0.23 | 3.30±0.18 | 4.90±0.12 | 12.70±0.13 | 2.00±0.12 | 29.10±0.12 | 2.40±0.09 | 9.70±0.16 | 27.90±0.22 | 1.20±0.3 | 0.10±0.21 | 0.20±0.11 |
| Butter3 | 1.00±0.12 | 3.60±0.17 | 1.40±0.05 | 3.20±0.15 | 3.70±0.21 | 10.90±0.09 | 1.60±0.22 | 26.30±0.14 | 3.20±0.11 | 9.70±0.26 | 24.60±0.23 | 2.10±0.11 | 0.10±0.06 | 0.05±0.02 |
| Butter4 | 2.00±0.07 | 2.50±0.1 | 1.50±0.16 | 3.50±0.05 | 3.80±0.12 | 12.00±0.08 | 1.70±0.42 | 31.00±0.12 | 1.50±0.14 | 10.30±0.22 | 25.20±0.12 | 2.10±0.21 | 0.10±0.06 | 0.10±0.02 |
| Butter 5 | 2.70±0.08 | 3.10±0.12 | 1.00±0.09 | 2.70±0.09 | 3.10±0.12 | 12.20±0.09 | 1.30±0.13 | 2.20±0.17 | 1.80±0.03 | 15.50±0.12 | 22.90±0.12 | 1.80±0.21 | 0.05±0.03 | 0.20±0.09 |
| Butter oil1 | 1.30±0.08 | 1.70±0.12 | 1.20±0.11 | 3.20±0.17 | 4.50±0.15 | 12.30±0.09 | 1.80±0.15 | 28.70±0.14 | 3.10±0.03 | 9.60±0.12 | 20.30±0.12 | 0.70±00.12 | 0.60±0.09 | 0.10±0.11 |
| Butter oil2 | 2.50±0.20 | 1.40±0.09 | 1.50±0.06 | 3.30±0.14 | 4.50±0.12 | 12.10±0.12 | 1.84±0.09 | 29.10±0.09 | 2.40±0.06 | 9.80±0.12 | 22.60±0.06 | 1.40±0.09 | 0.10±0.03 | 0.10±0.21 |
| Butter oil3 | 1.00±0.33 | 3.40±0.23 | 1.50±0.09 | 3.20±0.15 | 3.70±0.66 | 10.90±0.05 | 1.50±0.05 | 26.20±0.15 | 3.20±0.23 | 9.70±0.12 | 24.50±0.13 | 2.60±0.27 | 0.10±0.11 | 0.10±0.09 |
| Butter oil4 | 1.80±0.34 | 3.10±0.13 | 1.40±0.05 | 3.30±0.11 | 3.90±0.14 | 10.10±0.11 | 1.10±0.02 | 25.30±0.13 | 3.10±0.21 | 9.90±0.11 | 23.60±0.02 | 2.40±0.11 | 0.10±0.06 | 0.20±0.19 |
| Butter oil5 | 1.90±0.53 | 2.40±0.29 | 1.60±0.19 | 3.20±0.12 | 3.40±0.23 | 11.90±0.12 | 1.60±0.09 | 24.30±0.16 | 3.20±0.29 | 8.70±0.16 | 23.80±0.16 | 2.10±0.08 | 0.20±0.07 | 0.10±0.06 |

for a table or breakfast margarine while results showed all studied margarine samples had a higher amount of saturated fatty acids (83.67-85.11 (wt%)) which were out of specified limits of INSO NO. 143. In addition, the minimum limit of linoleic acid is specified 15 for a table or breakfast margarine in the standard while the amount of C18:2C was in the acceptable range of 20.7 (wt %) to 22 (wt %) in margarine sample. Results showed all margarine samples had a high amount of saturated fatty acids.

For butter samples, each of fatty acid composition was according to specified limits in INSO NO. 162. In addition, for butter oil samples, all fatty acid compositions (except C4:0 and C6:0) was according to specified limits in INSO NO. 1254.

**The Contents of BHA, BHT, TBHQ and PG and AA%**

The results of antioxidant activity and amount of the extracted SAs are shown in the Table. 3. The amount of SAs was measured in mg kg-1 (ppm). For margarine samples, the maximum standard limit for using SAs (PG, TBHQ, BHT and BHA) is specified ≤200 ppm. In margarine samples of numbers 1 and 2, the use of SAs was higher than that of a specified range in the international standard (INSO 143, 2011). For butter oil, the maximum standard limits for the use of PG, TBHQ, BHT, and BHA are specified in the range of 100, 200, 75 and 175 ppm, respectively. The amount of added SAs into butter oil samples was smaller than that of the specified range in INSO

**Table 3: The quantity and type of theextracted SA from the samples and their AA%**

| sample | No | Amount of synthetic antioxidant (mg kg-1) | Type of antioxidant | Antioxidant activity (%) |
|---|---|---|---|---|
| Margarine | 1 | 240.2 ± 1.06$^{a}$ | PG | 46.57 ± 0.02a |
| Margarine | 2 | 212.1 ± 2.07$^{ab}$ | PG | 45.46 ± 0.01$^{b}$ |
| Margarine | 3 | 160.22 ± 1.11$^{b}$ | TBHQ | 45.65 ± 0.02$^{b}$ |
| Margarine | 4 | 123.85 ± 2.08$^{bc}$ | TBHQ+PG | 39.45 ± 0.01$^{c}$ |
| Margarine | 5 | 120.67 ± 2.28$^{bc}$ | TBHQ+PG | 35.65 ± 0.04$^{e}$ |
| Margarine | 6 | 103.55 ± 1.60$^{bc}$ | TBHQ+PG | 37.02 ± 0.01$^{d}$ |
| Margarine | 7 | 85.84 ± 1.90$^{c}$ | TBHQ+PG | 35.06 ± 0.02$^{e}$ |
| Margarine | 8 | 75.33 ± 1.24$^{c}$ | TBHQ+PG | 33 ± 0.01$^{f}$ |
| Margarine | 9 | 140.24 ± 1.34$^{bc}$ | PG | 29.58 ± 0.02$^{hg}$ |
| Margarine | 10 | 122.12 ± 0.98$^{bc}$ | PG | 25.72 ± 0.02$^{i}$ |
| Butter | 1 | 0 ± 0.00$^{d}$ | - | 17.5 ± 0.01$^{l}$ |
| Butter | 2 | 0 ± 0.00$^{d}$ | - | 22.52 ± 0.02i |
| Butter | 3 | 112.25 ± 0.61$^{bc}$ | TBHQ+PG | 30.06 ± 0.00$^{g}$ |
| Butter | 4 | 200.3 ± 0.61$^{ab}$ | PG | 29.01 ± 0.01$^{h}$ |
| Butter | 5 | 0 ± 0.00$^{d}$ | - | 18.3 ± 0.02$^{k}$ |
| Butter oil | 1 | 36.67 ± 0.13$^{d}$ | TBHQ | 29.54 ± 0.01$^{hg}$ |
| Butter oil | 2 | 0 ± 0.00$^{d}$ | - | 20.7 ± 0.00$^{j}$ |
| Butter oil | 3 | 0 ± 0.00$^{d}$ | - | 20.59 ± 0.01$^{j}$ |
| Butter oil | 4 | 0 ± 0.00$^{d}$ | - | 20.54 ± 0.01$^{j}$ |
| Butter oil | 5 | 0 ± 0.00$^{d}$ | - | 20.51 ± 0.01$^{j}$ |

1254 (2002). For butter samples, there is not the specified standard limit for using of SAs in the INSO 162 but in the butter samples of 3 and 4, a kind of the SA was detected. It was observed that among of the SAs, PG was the most applied SA in the margarine samples. Results showed PG and TBHQ were the most of the SAs which used in the most of the samples. The results of the determined AA% of test samples are also shown in the Table. 3. The determined AA% of all samples was increased with the amount of the extracted SAs. The use of SAs was not observed in most of butter and butter oil samples (except butter of 3, 4 and butter oil of 1). The AA% was observed in the most of butter and butter oil samples, even in the samples without SA. This AA% depends on the existence of the natural bioactive materials such as phospholipids having antioxidant activity which were existed in the butter and butter oil samples.

## Discussions

Butter samples may be due to the high amount of existing unsaturated fatty acids, moisture, and no SA had high PV. Results showed that in butter and butter oil samples, the amount of short and medium saturated fatty acids is high and PV was slightly higher than that of the margarine sample. Margarine samples had lower PV because of having higher SA content. Margarine samples contain higher amounts of saturated fatty acids such as stearic acid and unsaturated fatty acid with one double bond. The order of samples is arranged based on their production date in the tables.

The results indicated that PV increased significantly during the storage period. Crapiste et al., (1999) investigated the variations of oil PV over storage. They stated that PV increased significantly as the storage period proceeds. Maskan et al. (1993) stored two types of margarine at 14ºC and examined the variations of PV. In addition, the determined OS in margarine samples was more than that of in butter and butter oil samples. The maximum resistance was observed in the margarine samples because of the sufficient SA content. Zaeroomali et al., (2014) reported that the PV in margarine samples increased over the storage period and resistance to oxidation was significantly decreased. It was observed that the OS of butter and butter oil samples was approximately high even in butter without SA. It depends on the bioactive components existing in the taken fats from milk, such as phospholipids and triglycerides (Contarini and Povolo, 2013). Moreover, the amount of the short saturated fatty acids in butter is high and it causes to reducing the risk of oxidation. In butter oil samples, the amount of water (0.5%) and unsaturated fatty acid was low (having many short - saturated fatty acids) which causes to increase their resistance (Contarini and Povolo, 2013). Comparison of OS and PV showed an indirect relationship of these two factors in all test samples. The results showed that the margarine samples had a high IV due to increasing of the amount of long unsaturated fatty acids such as linoleic acid. It was found that the content of SA in the margarine samples was higher than that of in other samples because of the higher amount of unsaturated fatty acids such as linoleic and linolenic acids. It was clear that during the hydrogenation process of vegetable oils to produce of margarine, the content of natural antioxidants will be decomposed (Zhang et al., 2015). Therefore, margarine samples need more SA. It was observed that in the margarine samples of No. 4, 5, 6, 7, and 8, the mixture of PG and TBHQ was used. For margarine samples of No. 1, 2, 9 and 10, PG was just used, and the amount of the mixture of PG and TBHQ was lower than PG lonely. Margarine containing the high content SA was leading to lower PV and higher resistance than butter and butter oil against the light, oxygen, air, moisture and decomposition of triglyceride. The result of AA% showed that if the content of SA was increased, the antioxidant activity was also increased. Thus, there was a direct relationship between the content of SA and AA%. Another important result was the relationship between the kind and quality of SA in the fats and oils. For example, in both of margarine samples No. 1, and 2, PG was used but the amount of PG in the samples 1 and 2 was 240.2 and 212.1 mg kg-1, respectively. Both of margarine samples were produced on the same day, but the PG was purchased from two companies with different purity grade. The determined AA% of the sample No. 2 was obtained close to the No. 1. Another important factor was the synergistic effect of the SAs when two or more of SAs applied together to produce an effect greater than one SA. For example, in margarine No. 8, the

mixture of TBHQ and PG (about 75.33 mg kg-1) was used and the AA% was obtained about 33%, but it was observed that PG was used in margarine No. 9 (about 140.24 mg kg-1) and its AA% was about 29.58%. This result was observed in the butter No. 3 which the mixture of PG and TBHQ (about 112.25 mg kg-1) has been added and its AA% was obtained 30.06%, but in butter No.4, about 200.3 mg kg-1 of PG was used which its AA% was obtained 29.01%. Ozturk and Cakmakci (2006) investigated the effect of antioxidants on butter in relation to storage temperature and duration. The addition of 50-100 ppm of BHA, BHT, and tocopherol provided the protection against autoxidation during storage of butter at 4 °C for more than 180 days. Table 4 shows a statistical relationship between two sets of data for each sample type, including margarine, butter, and butter oil samples, separately. In this table, correlation coefficients (r) were calculated for two sets of obtaining data including OS and PV or PV and AA or AA and OS for each sample type. Kaya (2000) studied physicochemical properties and stability of butter oil obtained from milk or yogurt and found that oxidative changes in comparison with hydrolytic changes are of greater significance in the thermal stability of butter oil samples. They found that by an increase of temperature, Peroxide and thiobarbituric acid values increased and the percent loss of linolenic acid was about 3 times faster than that of linoleic acid. In addition, oxidative and storage stability of margarine samples were studied by Maskan et al., (1993). During long-term storage at 4°C (96 days), PV and free fatty acids slightly increased.

**Table 4: Statistical relationship for each sample type**

| Sample Type | Statistical Relationship | r |
|---|---|---|
| **Margarine** | PV vs OS | -0.9088 |
| | PV vs IV | 0.9029 |
| | PV vs AA | -0.9173 |
| | OS vs IV | -0.8859 |
| | OS vs AA | 0.8837 |
| | IV vs AA | -0.8384 |
| | AA vs SA | 0.8191 |
| **Butter and Butter oils** | PV vs OS | -0.7959 |
| | PV vs IV | 0.8418 |
| | PV vs AA | -0.7636 |
| | OS vs IV | -0.40824 |
| | OS vs AA | 0.7768 |
| | IV vs AA | -0.3464 |
| | AA vs SA | 0.8191 |

*If r≥0, the direct relationship is between two variables.*

## Conclusion

There was an indirect relationship between OS with PV and storage period when the SAs with high quality were used. It should be considered that using high quantities of SAs could also have harmful

effects on the body. This study showed that margarine sample which contained the mixture of SAs, including PG and TBHQ, indicated a suitable antioxidant activity. It caused to decrease using a higher amount of SA in these samples. Results showed that PG alone or in combination with TBHQ was used in the most of the margarine samples. The use of SAs was lower than that of the specified limits in the national standard of Iran. In the future, It is necessary to replace synthetic antioxidants with natural antioxidants (with suitable cost and stability) and also reduction of saturated fatty acids in producing of margarine in the industry.

**Conflicts of interest:** There is no conflict interest for this project.

## References

AOCS (1997).Antioxidants – Liquid chromatographic method".6-86 American Oil Chemists' Society.

Carocho M., and Ferreira ICFR.(2013). A review of antioxidants, prooxidants, and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. J. Food Chem Toxic.51, 15–25.

Ceballos C, and Fernandez H (2000). Synthetic antioxidants in edible oils by square- wave voltammetry on ultramicroelectrodes. J. Am Oil Chem Soc. 77, 731-5.

CrapisteGH., Brevedan MIV, and Carelli AA (1999). Oxidation of sunflower oil during storage.J. Am Oil Chem Soc. 76,1437-43.

Contarini G and Povolo M (2013) Phospholipids in Milk Fat: Composition, Biological and Technological Significance, and Analytical Strategies. Int J Mol Sci. 14, 2808–2831.

Craske JD (1993). Separation of instrumental and chemical errors in the analysis of oils by gas chromatography—A Collaborative Evaluation.J. Am Oil Chem Soc. 70, 325-34.

Doeden WG, Bowers RH, Ingala AC (1979). Determination of BHA, BHT, and TBHQ in edible fats and oils.J. Am Oil Chem Soc.56, 12-4.

EFSA (2004). The opinion of the scientific panel on food additives, flavourings, processingaids and materials in contact with food on a request from the commission related to tertiary-butylhydroquinone (TBHQ)" Euro Food Safety Authority.J. EFSA.84,:1–50.

EFSA (2011).Scientific opinion on the reevaluation of butylated hydroxyl anisole–BHA (E 320) as a food additive.Euro Food Safety Authority.J.EFSA.9, 2392.doi: 10.2903/j.efsa.2011.2392.

EFSA (2012). Scientific opinion on the reevaluation of butylated hydroxyl toluene BHT (E 321) as a food additive" European Food Safety Authority.J. EFSA.10, 2588;

Homero-Mendez D, Perez-Galvez A, Minguez-Mosquera IA (2001).rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. J. Am Oil Chem Soc. 78,1151-55.

INSO.Iranian national standard organization.1th ed. No. 1254 (2002).Edible fats and oils- Butter oil-specifications. Institute of Standards and Industrial Research of Iran .

INSO.Iranian national standard organization.1th ed. No. 1997(2015). Animal and vegetable fats and oils -Determination of peroxide Iodometricvalue- (visual) endpoint determination.

INSO.Iranian national standard organization.4th ed. No.143 (2011).Vegetable butter (margarine)-Specifications" 4th.revision. Institute of Standards and Industrial Research of Iran .

INSO.Iranian national standard organization.5th ed. No. 162 (2008).Pasteurized butter-Specification and test method.Institute of Standards and Industrial Research of Iran.

ISO 3961 (2013).Animal and vegetable fats and oils - Determination of iodine value" International Organization for Standardization.

ISO 5508 (1990).Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids.

ISO 5509 (1978). Animal and vegetable fats and oils – preparation of methyl esters of fatty acids" .International Organization for Standardization.

ISO 6886 (2006). Animal and vegetable fats and oils- determination of oxidation stability (accelerated oxidation test) .International Organization for Standardization.

Kaya A (2000) Properties and stability of butter oil obtained from milk and yoghurt. Nahrung 44, 126–129

Lanigan RS, and Yamarik TA (2002).Final report on the safety assessment of BHT.J. Toxicol. 21,19–94.

Maskan M., Oner M.D., Kaya A. (1993). Storage stability and accelerated shelf life testing of margarine samples. J. Food Quality. 16,175-86.

Mohadjerani M, and Pakzad KH.(2013). Evaluation of total phenolic content andantioxidant activity of Nelumbonucifera seed from north of Iran.J. Appl Chem. 7,45-48.

Ozturk S. and Cakmakci S (2006).The effect of antioxidants on butter in relation to storage temperature and duration.Euro. J. Lipid Sci. Technol. 108, 951-959.

Perrin C, and Meyer L (2003). Simultaneous determination of ascorbylpalmitate and nine phenolic antioxidants in vegetable oils and edible fats by HPLC.
J. Am Oil Chem Soc. 80,115-118.

Pokorny J, Yanishlieva NV, and Gordon M (2001).Antioxidants in food.Practical applications.First published. Cambridge, England.ISBN 9780849312229 - CAT# WP1222. 1-42.

Robert RE, Pellegrini N, Proteggente A, Pannala A, Yang M, and Rice-Evans C (1999).Antioxidant activity applying an improved ABTS radical cation decolorization assay.J. Free RadicBiol Med. 26, 1231-7.

Saito M., Sakagami H, and Fujisawa S (2003). Cytotoxicity and apoptosisinduction by butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT).J. Anticancer Res. 23, 4693–01.

Thomas A (2002). Fats and fatty oils.Ullmann's Encyclopedia of Industrial Chemistry.Weinheim: Wiley-VCH. DOI: 10.1002/14356007.a10_173.1-173.

Yang MH, Lin HJ, and Choong MC (2002).A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats.J. Food Res. Inter. 35, 627-33.

Zaeroomali M, Maghsudlou Y, and Aryaey P (2014).The changes of table margarine characterization during storage time.Exp Biol.4,185-7.

Zhang Q, Qin W, Li M, Shen Q, and Saleh A.S.M (2015) Application of Chromatographic Techniques in the Detection and Identification of Constituents Formed during Food Frying: A Review. Comprehensive Rev Food Sci Food Safety, 14, 601-633.