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# Monitoring of Fat Content, Free Fatty Acid and Fatty Acid Profile Including *trans* Fat in Pakistani Biscuits

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**Abstract** The fat contents of 12 brands of biscuits were extracted and evaluated for free fatty acids (FFA) and their fatty acid composition (FAC). The oil content and FFA varied from 13.7 to 27.6% and 0.2 to 1.0%, respectively. The FAC was analyzed by gas chromatography–mass spectroscopy with particular emphasis on *trans* fatty acids (TFA). Total saturated, unsaturated, *cis*-monounsaturated and polyunsaturated fatty acids were determined in the range of 37.9–46.9, 53.0–62.0, 12.3–43.7 and 0.1–9.2%, respectively. The high amount of TFA was observed in all biscuit samples and varied from 9.3 to 34.9%. The quantity and quality of the lipid fraction of the biscuits indicated that the all analyzed biscuits are a rich source of fat, saturated fatty acids and *trans* fatty acids, consequently not suitable for the health of consumers. The high content of *trans* fatty acids and palmitic acid also indicated that blends of RBD palm oil and partially hydrogenated oil had been used in the biscuit manufacturing.

**Keywords** Biscuits fat · *trans* Fatty acids · GC–MS · Free fatty acid (FFA)

## Introduction

The quality of fats plays a very important role in food processing technology. Fat oxidation is the main reason of deterioration in the quality of foods and can directly affect many quality characteristics such as flavor, color, texture, nutritive value, and safety of the food. The quality and dietary character of the edible oil have been a topic of concern among food scientists, nutritionists and consumers. Flour, sugar, fat, water and salt are the fundamental components in a soft dough biscuit formulation [1]. Fat is the principle ingredient responsible for adding a rich quality to cookies [2] and in a biscuit formulation has numerous roles. It interacts with other ingredients to develop texture, mouthfeel and overall sensation of lubricity of the product, thereby affecting the rheological properties of baked biscuits [3]. For biscuits manufacturing, the choice of a better kind of lipid is often dependent on the basis of technological and economic parameters, without considering the nutritional implications. For this reason it is necessary to evaluate the quality and quantity of fat. High fat intake and *trans* fats are associated with various health disorders such as obesity, cancer, high blood cholesterol, and coronary heart disease [4, 5]. There is very limited data available on the quality of fat used in Pakistani foodstuffs. Although some previous studies [6, 7] have shown the presence of significant amounts of *trans* fatty acids in Pakistani margarines and butters, while the data on other foods are lacking. In Pakistan, biscuit production has increased rapidly in the last few years because of increasing consumer demand. No study has been found on the quality of fat in biscuits consumed in Pakistan. The increasing pressure to decrease the calories intake from fat, removal of *trans* fat from foods and to supply products with much lower saturated fat contents have become the global concern of many international food organizations such as the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and Food and Drug Administration (FDA), etc. Therefore, the present work reports the results of oil content, free fatty acids and gas chromatography–mass spectroscopy (GC–MS) fatty acid profile of the most

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consumed biscuit brands to explore the quality of fat used in the manufacturing of the biscuits.

## Materials and Methods

### Samples and Reagents

Twelve biscuit samples of different brands were purchased in duplicate, i.e., from two different lots. The local supermarkets of Jamshoro and Hyderabad, Pakistan were selected for the sampling. The choice of the brands was based on the highest consumption among those available in the market. The Biscuits were coded as B-1, B-2, B-3, B-4, B-5, B-6, B-7, B-8, B-9, B-10, B-11 and B-12. All reagents, chemicals and solvents used were from E. Merck (Darmstadt, Germany). Both *trans* and *cis* fatty acids methyl esters (FAMEs) standards (GLC 481-B and 607) were purchased from Nu-Check-Prep, Inc (Elysian, MN).

### *Methods*

*Extraction of the Lipids.* Total lipid extraction from the biscuit samples was carried out by hexane extraction under the operating conditions specified by the International Association for Cereal Chemistry [8], and expressed as a percentage by mass of the product as received. Fat obtained from biscuit samples was transferred into 10 mL glass vials. The decanted samples were all frozen at −18 °C until further analysis.

*Determination of Free Fatty Acids.* Free fatty acid content as a percentage of the oleic acid, was determined by the titration of a solution of oil dissolved in hot neutral ethanol with sodium hydroxide in the presence of phenolphthalein indicator using standard AOCS method Ca 5a-40 [9].

*Determination of Fatty Acid Composition.* For the determination of fatty acids composition FAMEs were prepared using standard IUPAC method 2.301 [10]. An Agilent GC–MS was used with ChemStation (version D.02.00) Scale Mode software. GC–MS chromatograms obtained were compared with two libraries (NIST & Wily) which provided the best information about the identification of fatty acids present in biscuit samples.

*GC–MS Conditions.* The GC–MS analysis of FAMEs was performed on an Agilent 6890 N gas chromatography instrument coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). A capillary column HP-5MS (5% phenyl methylsiloxane) with dimensions of 30 m × 0.25 mm i.d × 0.25 $\mu$ film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of fatty acid methyl esters. The initial temperature of 150 °C was maintained for 2 min, raised to 230 °C at the rate of 4 °C/min, and kept at 230 °C for 5 min. The split ratio was 1:50, and helium was used as a carrier gas with a flow rate of 0.8 ml/min. The injector and detector temperatures were 240 and 260 °C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV; with an ion source temperature of 230 °C, a quadrupole temperature of 150 °C, and a translating line temperature of 270 °C. The mass scan ranged from 50 to 550 *m/z* with an Em voltage, 1,035 V.

*Calculations and Statistical Analyses.* Peak identification of the fatty acids in the analyzed biscuit samples were carried out by comparison with the retention times and mass spectra of known standards. Two samples of each brand were collected and each sample was analyzed three times. The data obtained were put into Origin 7 program and reported as the means ($n = 2 \times 3 \pm$ standard deviation).

## Results and Discussion

The total fat contents and free fatty acids present in oil extracted from the biscuit samples are given in Table 1. Total fat contents of the samples ranged from 13.7 to 27.6% with a mean of 21.8%. The highest amount of fat was found in sample B-11 27.6% and the lowest in sample B-5 13.7%; these levels were comparable to Turkish biscuits which were reported to have from 8.5 to 26.0% [11]. Although the biscuits are considered to be a digestive food [12] all analyzed samples have fat contents above 12.0%. Free fatty acid content is one of the main criteria for checking the quality of oil. Its value varied from 0.2 to 1.0%. The highest amount of FFA, 1.0%, was observed in sample B-3 and was lowest in sample B-7. These levels

**Table 1** Mean fat content and free fatty acid (FFA) content of biscuits consumed in Pakistan (g/100 g)

| Samples | Fat content (Mean ± SD) | FFA (Mean ± SD) |
|---|---|---|
| B-1 | 20.70 ± 1.01 | 0.43 ± 0.05 |
| B-2 | 25.69 ± 1.11 | 0.29 ± 0.04 |
| B-3 | 17.14 ± 0.54 | 1.01 ± 0.07 |
| B-4 | 21.90 ± 0.62 | 0.29 ± 0.03 |
| B-5 | 13.69 ± 0.34 | 0.65 ± 0.04 |
| B-6 | 23.70 ± 0.56 | 0.24 ± 0.04 |
| B-7 | 20.60 ± 0.78 | 0.22 ± 0.03 |
| B-8 | 15.82 ± 0.79 | 0.52 ± 0.04 |
| B-9 | 20.59 ± 0.65 | 0.27 ± 0.03 |
| B-10 | 26.73 ± 0.61 | 0.28 ± 0.04 |
| B-11 | 27.64 ± 0.92 | 0.26 ± 0.03 |
| B-12 | 26.85 ± 0.89 | 0.42 ± 0.04 |

were comparable to the reported values (0.2–1.4%) for Italian biscuits [13]. The level of FFA indicates a higher level of oil hydrolysis and usually freshly processed edible oils contain less than 0.1% FFA.

Table 2 shows the average saturated fatty acids (SFA) composition expressed in g/100 g total fatty acids. The main SFA in the analyzed samples were myristic (C14:0), palmitic (C16:0) and stearic acid (C18:0), while a small quantity of pentadecanoic (C15:0), margaric (C17:0), arachidic (C20:0) and lignoceric acids (C24:0) were also determined in a few biscuit samples. In relation to the individual saturated fatty acids, the major contribution was from palmitic acid, 30.0 to 41.2%. The highest amount of palmitic acid was present in B-1 and lowest in B-9. Stearic acids ranged from 4.6 to 8.9% and was lowest in B-1 and highest in B-5. Myristic acid ranged from 0.6 to 1.4%, with the highest level in B-3 and the lowest in B-10. Pentadecanoic, margaric, arachidic and lignoceric acids were present in small amounts of less than 1.0%, except lignoceric acid which was at 1.2% in sample B-10. These results indicate that palm oil was used to make the biscuits. Various studies [14, 15] have suggested that saturated fatty acids with a chain length of C12:0–C16:0 are atherogenic, stearic acid is neutral, and oleic and polyunsaturated fatty acids have a lipid lowering effect. Among the monounsaturated fatty acids, the major contributor was oleic acid (C18:1n-9) in all biscuit samples and it was found at a range of 11.7–43.6%. The maximum amount of oleic acid was determined in sample B-1 and minimum amount in B-4 with a mean value of 25.5%. Palmitoleic (C16:1) and eicosenoic (C20:1n-11) acids were the other members of MUFA determined at less than 1%. Most naturally occurring vegetable oils are rich in unsaturated fatty acids which contain only non-conjugated double bonds in the *cis* configuration. The unsaturated constituents can be isomerized to the *trans* form during extraction and processing or as a result of oxidation, conversion during heating and by partial hydrogenation [16] for the manufacture of margarines, shortenings and a large variety of food products. Compared with *cis* unsaturated fatty acids, the structure, physical properties, chemical stability and the physiological effects (atherogenic effects) of *trans* fatty acids resemble those of the saturated fatty acids [17]. The *trans* polyunsaturated fatty acids were only found in trace amounts. The amount of *trans* fatty acids in the biscuits ranged from 9.3 to 34.9% with a mean value of 26.7%. A higher value of *trans* fat was determined in sample B-4 and a lower level in sample B-1. These values are higher than those reported in the literature [11, 13, 18–20]. None of the analyzed samples were free from *trans* fatty acids. The presence of the *trans* fat at high amounts in the biscuit samples indicate that hydrogenated oil was used in the manufacturing process.

**Table 2** Mean fatty acid composition of biscuits consumed in Pakistan (g/100 g)

| Samples | B-1 | B-2 | B-3 | B-4 | B-5 | B-6 | B-7 | B-8 | B-9 | B-10 | B-11 | B-12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C14:0 | 1.13 ± 0.05 | 0.78 ± 0.03 | 1.35 ± 0.05 | 0.76 ± 0.02 | 1.05 ± 0.05 | 0.79 ± 0.02 | 0.81 ± 0.005 | 0.6 ± 0.02 | 0.95 ± 0.04 | 0.56 ± 0.02 | 0.85 ± 0.04 | 0.96 ± 0.04 |
| C15:0 | – | – | – | – | 0.05 ± 0.002 | – | – | – | – | – | 0.04 ± 0.001 | 0.03 ± 0.001 |
| C16:0 | 41.16 ± 1.1 | 34.35 ± 1.15 | 38.23 ± 1.15 | 36.95 ± 1.25 | 30.31 ± 1.02 | 34.52 ± 1.15 | 33.28 ± 1.25 | 35.33 ± 1.25 | 29.97 ± 0.95 | 32.12 ± 0.95 | 32.55 ± 1.25 | 32.71 ± 0.95 |
| C17:0 | – | 0.07 ± 0.003 | – | 0.12 ± 0.005 | 0.14 ± 0.002 | 0.07 ± 0.003 | 0.08 ± 0.003 | 0.26 ± 0.01 | – | 0. 19 ± 0.01 | 0.09 ± 0.004 | 0.1 ± 0.002 |
| C18:0 | 4.64 ± 0.15 | 5.72 ± 0.15 | 5.11 ± 0.15 | 5.89 ± 0.25 | 8.86 ± 0.34 | 5.58 ± 0.26 | 6.43 ± 0.3 | 6.41 ± 0.31 | 6.96 ± 0.2 | 5.56 ± 0.24 | 6.82 ± 0.24 | 6.39 ± 0.21 |
| C20:0 | – | 0.35 ± 0.01 | 0.63 ± 0.025 | 0.39 ± 0.015 | 0.73 ± 0.03 | 0.34 ± 0.01 | 0.37 ± 0.01 | 0.46 ± 0.01 | | 0.32 ± 0.01 | 0.43 ± 0.02 | 0.51 ± 0.02 |
| C24:0 | – | – | – | 0.48 ± 0.015 | 0.35 ± 0.015 | – | – | 0.56 ± 0.02 | – | 1.24 ± 0.05 | – | – |
| C16:1n9c | – | 0.16 ± 0.005 | – | 0.27 ± 0.013 | – | 0.14 ± 0.005 | 0.21 ± 0.005 | 0.22 ± 0.01 | – | 0.37 ± 0.01 | 0.22 ± 0.01 | 0.15 ± 0.005 |
| C18:1n9t | 9.26 ± 0.40 | 28.83 ± 1.2 | 29.26 ± 1.24 | 34.88 ± 1.24 | 14.21 ± 0.51 | 27.45 ± 1.05 | 32.6 ± 1.34 | 33.97 ± 1.64 | 29.88 ± 1.09 | 26.92 ± 0.57 | 27.29 ± 0.95 | 25.51 ± 0.65 |
| C18:1n9c | 43.62 ± 2.10 | 20.39 ± 0.95 | 20.59 ± 0.92 | 11.74 ± 0.51 | 43.34 ± 1.35 | 26.93 ± 1.03 | 17.15 ± 0.81 | 14.58 ± 0.54 | 23.52 ± 1.08 | 24.81 ± 0.64 | 27.02 ± 0.84 | 29.34 ± 0.87 |
| C18:2n9,12*c-c* | – | 8.5 ± 0.2 | 4.77 ± 0.15 | 8.17 ± 0.24 | – | 2.84 ± 0.08 | 8.64 ± 0.41 | 6.99 ± 0.21 | 8.6 ± 0.4 | 7.39 ± 0.23 | 3.49 ± 0.14 | 3.32 ± 0.08 |
| C18:2n9,12*t-t* | – | – | – | – | 0.25 ± 0.005 | 0.19 ± 0.005 | – | – | – | – | 0.28 ± 0.01 | 0.14 ± 0.005 |
| C18:2n8,11*c-c* | 0.1 ± 0.004 | 0.58 ± 0.02 | – | – | 0.21 ± 0.005 | – | 0.12 ± 0.004 | – | – | 0.16 ± 0.01 | 0.32 ± 0.012 | 0.31 ± 0.009 |
| C18:3n9,12,15 | – | 0.15 ± 0.005 | – | – | 0.09 ± 0.003 | 1.04 ± 0.05 | – | 0.22 ± 0.01 | – | 0.26 ± 0.006 | 0.21 ± 0.01 | 0.24 ± 0.005 |
| C20:1n11 | – | – | – | 0.24 ± 0.005 | 0.35 ± 0.01 | – | 0.23 ± 0.005 | 0.31 ± 0.01 | – | – | 0.32 ± 0.01 | 0.22 ± 0.007 |

*n* Indicates the position of a double bond

**Table 3** Groups and ratio between the types of fatty acids from the composition of biscuit samples

| Samples (%) | B-1 | B-2 | B-3 | B-4 | B-5 | B-6 | B-7 | B-8 | B-9 | B-10 | B-11 | B-12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SFA | 46.93 | 41.27 | 45.32 | 44.59 | 41.49 | 41.3 | 40.97 | 43.62 | 37.88 | 39.99 | 40.78 | 40.7 |
| UFA | 52.98 | 58.61 | 54.62 | 55.3 | 58.45 | 58.59 | 58.95 | 56.29 | 62.0 | 59.91 | 59.15 | 59.23 |
| Total MUFA | 52.88 | 49.38 | 49.85 | 47.13 | 57.9 | 54.52 | 50.19 | 49.08 | 53.4 | 52.1 | 54.85 | 55.22 |
| *cis* MUFA | 43.62 | 20.55 | 20.59 | 12.25 | 43.69 | 27.07 | 17.59 | 15.11 | 23.52 | 25.18 | 27.56 | 29.71 |
| Total PUFA | 0.10 | 9.23 | 4.77 | 8.17 | 0.55 | 4.07 | 8.76 | 7.21 | 8.60 | 7.81 | 4.30 | 4.01 |
| *cis* PUFA | 0.10 | 9.23 | 4.77 | 8.17 | 0.3 | 3.88 | 8.76 | 7.21 | 8.6 | 7.81 | 4.02 | 3.87 |
| Total TFA | 9.26 | 28.83 | 29.26 | 34.88 | 14.46 | 27.64 | 32.6 | 33.97 | 29.88 | 26.92 | 27.57 | 25.65 |
| SFA + TFA | 56.19 | 70.10 | 74.58 | 79.47 | 55.95 | 68.94 | 73.57 | 77.59 | 67.76 | 66.91 | 68.35 | 66.35 |
| SFA/UFA | 0.89 | 0.70 | 0.83 | 0.81 | 0.71 | 0.70 | 0.69 | 0.77 | 0.61 | 0.67 | 0.69 | 0.69 |
| MUFA + PUFA | 52.98 | 58.61 | 54.62 | 55.3 | 58.45 | 58.59 | 58.95 | 56.29 | 62.0 | 59.91 | 59.15 | 59.23 |
| *cis* MUFA + *cis* PUFA | 43.72 | 29.78 | 25.36 | 20.42 | 43.99 | 30.95 | 26.35 | 22.32 | 32.12 | 32.99 | 31.58 | 33.58 |
| *cis* PUFA/SFA | 0.002 | 0.22 | 0.11 | 0.18 | 0.01 | 0.10 | 0.21 | 0.17 | 0.23 | 0.20 | 0.11 | 0.10 |
| *trans*/*cis* | 0.21 | 0.97 | 1.15 | 1.71 | 0.33 | 0.89 | 1.24 | 1.52 | 0.93 | 0.82 | 0.87 | 0.76 |
| *cis* PUFA/(SFA + TFA) | 0.002 | 0.13 | 0.06 | 0.10 | 0.01 | 0.06 | 0.12 | 0.09 | 0.13 | 0.12 | 0.06 | 0.06 |
| *cis* MUFA + PUFA/SFA + TFA | 0.78 | 0.42 | 0.34 | 0.26 | 0.79 | 0.45 | 0.36 | 0.29 | 0.47 | 0.49 | 0.46 | 0.51 |

The polyunsaturated fatty acids (PUFA) are very important for biological and nutritional values, because essential fatty acids (EFA) are included in this group. The content of PUFA in the biscuit samples ranged from 0.1 to 9.2%, individually higher in sample B-2 and B-7, 9.2 and 8.8%, respectively. Among the PUFA the major contribution was from linoleic acid (C18:2). Some different isomers of linoleic acid like C18:2n-9, 12 *t-t* and C18:2n-8, 11 *c-c* were also determined and found to be less than 1%. Only a partial hydrogenation process that can change both the geometrical configuration and the double bond shift [21]. Out of twelve samples, six contained less than 0.4% linolenic acid and only one sample B-6 contained 1.0%. An alarming feature was observed that all analyzed biscuits samples, contained high levels of TFA and low levels of essential fatty acids.

Table 3 shows the groups, fatty acid ratios and nutritive values of the biscuits. The mean value of saturated fatty acids in biscuit samples, 42.1%, was higher than Turkish and Brazilian biscuits [11, 20] and lower than Italian biscuits [13]. The ratio of saturated/unsaturated FA showed the relation between the two major FA groups of the fat composition; its value varied from 0.61 to 0.89. The Pakistani biscuits products have a larger mean ratio 0.73 of saturated/unsaturated FA than the Turkish mean ratio 0.62 [11], which indicate a higher proportion of saturated FA. The British Department of Health, UK [22] recommended a 0.45 minimal *cis* PUFA/SFA ratio. In this study the mean value ratio of the investigated biscuits was 0.14 which is much lower than the recommended value and also compared to those reported by Martin et al., for Brazilian biscuits [20]. A lower value indicates foods that are not good for health, with regard to cardiovascular disease. The ratio of *trans*-FA/*cis*-FA expresses the degree of conversion of the *cis*-forms to *trans*-forms and also a higher ratio indicates the greater mixing of hydrogenated oils. The ratio varied between 0.21 and 1.71 with a mean value of 0.95 for the biscuits studied, which corresponds to a higher ratio than Brazilian biscuit ratio of 0.39. The highest ratio of *trans* to *cis* fatty acid of 1.71 was found in sample B-4 while the lowest was in B-1, 0.21. The mean value of the indices (*cis* PUFA/SFA + TFA) and (*cis* MUFA +*cis* PUFA/SFA + TFA), which were most commonly used to express nutritional value of edible oils and fats [23], were 0.08 and 0.47, respectively. However, the values of these indices of biscuit samples from Pakistan could not be compared with those in the literature as there were no previously available reports of the values of these indices.

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