Development Of Soft Dough Biscuits With High Unsaturated Fatty Acids

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Abstract—The food industry is primarily driven by consumer health trends. This study was conducted to select high unsaturated edible grade fat types for soft dough biscuits. While Sunflower, soybean, corn and peanut oils were used as treatments, palm oil was used as the standard/control. Chemical and physical parameters of oils were determined and they were within the acceptable range of regulatory requirement.

While initial Free fatty acids levels of sunflower, palm, corn, Soya bean and peanut oils were 0.31, 0.03, 0.41, 0.20 and 0.36 respectively, Iodine values (g per 100g) of the same order of oils were 130, 55, 127, 130 and 95. Chemical and physical properties of the prepared soft dough biscuits using aforesaid oils were within the regulatory requirements of the SLS 251:2010. While there was no significant different between nutritional compositions of prepared biscuit, high level of unsaturated fatty acids were also reported against control treatment.

Unsaturated fat content (from total fat) of biscuits, prepared with Palm, sunflower, soya bean, corn and peanut oils were 56.40%, 83.00%, 79.57%,78.60% and 81.04% respectively. Sensory evaluation revealed that there was no significant different between the attributes of the biscuits against control except the texture. While biscuits of all treatments were recorded more than 70% unsaturated fatty acids against control, sunflower was the best amongst them. If biscuits having with more than 70% unsaturated fatty acids can go for ‘nutrition and health claims’. Therefore, sunflower oil incorporated biscuits are an ideal product for the health conscious consumers in the market.

Keywords—Soft dough biscuits, Sunflower oil, Soybean oil, Corn oil and Peanut oil, unsaturated fatty acids

I. INTRODUCTION

Biscuits are small baked products made principally from flour, sugar and fat. They typically have a less moisture content and when packaged in moisture proof containers has a long shelf life, perhaps six months or more. The appeal to consumers is determined by the appearance and eating qualities. The food industry is primarily driven by consumer health trends. A present day dietary concern is the consumption of a large amount of fat and sugar. With the growing incidence of obesity and diabetes, low calorie foods have gained immense popularity. Most well-maintained strategies in terms of fat reduction diets involve either the use of low-fat foods or fat substitutes or modifications such as trimming of fat from foods.

Fat is an essential component for some food products because it can lays many functional and sensory roles. Fat provides creaminess, lubricity, and a good appearance to a product. Structure and volume are two other critical roles of fat, particularly in baked goods. During the creaming of fat and sugar, leavening gases are evenly distributed and air cells are created. The leavening gases cause the cells to expand during baking, which results in a product with increased volume (Penfield and Campbell, 1990). Fat is an important contributor to the sensory qualities of food products too. Although the fat itself may not have a strong or distinctive flavor, it is able to distribute, release, enhance, and affect the intensity of other ingredient’s flavors (Bennett, 1992; Giese, 1996). Flavor sensation is reduced in the absence of fat because fat-soluble flavors are released all at one time. Although fat-soluble volatiles are perceived through the nose or mouth when fat is first consumed, textural qualities and fat-soluble flavors are gradually perceived in the mouth upon chewing and warming of the food (Drewnowski 1992). Therefore, the flavor profile of a reduced-in-fat product may be altered due to the decreased amount of fat available to contribute to these sensations.

Fats perform a textural function in dough. During the mixing of a dough there is competition for the flour surface between the aqueous phase and the fat. The water or sugar solution interacts with the flour protein to create gluten which forms a cohesive and extensible network. When fat coats the flour this network is interrupted and the eating properties after baking are less hard, shorter and more inclined to melt in the mouth. If the fat level is high the lubricating function in the dough is so pronounced that little or no water is required to achieve a desired consistency, little gluten is formed and starch swelling and gelatinization is also reduced giving a very soft texture. The dough breaks easily when pulled, it is short. This is the origin of the term ‘shortening’ for a dough fat. (Duncan, 2000)

Therefore this study was conducted to develop high unsaturated fat biscuits using four types of oils as Corn oil, Sunflower oil, Soybean oil and Peanut oil.
II. METHODOLOGY

The research was conducted to determine the possibility of development of soft dough biscuits with high unsaturated fatty acids for health food industry. Four types of edible grade unsaturated fatty acids were used with the reference (control) fatty acid which is conventionally used in biscuit industry. Physical, chemical and organoleptic properties of soft dough biscuit produced were analyzed using standard research protocols. Individual research methodologies used for the study are discussed in details below.

Determination of Characteristics properties of selected oils

Saturated and unsaturated fatty acids content of oils were determined by using standard Shimatzu protocols.

2-3 drops of oil was added into the screw caped tube. 3mL of toluene was added in to it. 2mL of 0.5M sodium methoxide was added which was the transesterification agent. Sample was Incubated at 70-80°C for 45 minutes. Tubes were cooled to room temperature and 5mL of 5% sodium chloride solution and 2mL of hexane were added. Tubes were let stand overnight. (Urgent sample analysis can be performed after 5 hours.) 1-2ml from the organic layer was transferred to a GC vial to analysis. FAMEs (Fatty acid methyl esters) were analyzed using the GCMS, then corresponding fatty acids were identified. (Identification can be performed using standards and their retention times. Furthermore the GCMS library search can also be used for identification purposes.)

To convert the FAMEs to corresponding fatty acids a conversion factor was used. It was called the fatty acid conversion factor. (FACF). Quantifying fatty acids using individual fatty acid conversion factors can be a tedious and time consuming process. Instead a food specific fatty acid conversion factor was used to quantify fatty acids in a food sample as a whole.

Calculation

Identify individual percentages of each fatty acid in relation to the total fatty acids in the injected sample. This was done automatically using GCMS software. Manual calculations were also being done using below equation.

$$\text{FAME}(X) \text{ %} = \frac{\text{Peak area of FAME } (X)}{\text{Total peak area}} \times 100$$

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$$\text{FAME}(X) \text{ %} = \frac{\text{Peak area of FAME } (X)}{\text{Total peak area}} \times 100$$

Total saturated fatty acids = Sum of all saturated FAMESs X FACF X Total fat % of the sample

Food specific conversion factor (FACF) for biscuits was 0.95

Free fatty acid levels were determined using standard protocol Association of Analytical Chemists (AOAC) 940.28.

Iodine value of selected oils were determined using standard protocol AOAC 993.20A.

Specific gravity was determined by using standard protocol AOAC 920.212.

Determination of physical and chemical properties of Soft Dough Biscuits

Moisture content was determined by using the method specified in AOAC 2000.

pH values of the prepared biscuits were determined by the standard protocol using pH meter.

Protein content of prepared biscuits was determined using standard protocol kjeldhal method (AOAC-960.52).

The standard method of (Soxhlet method) AOAC 945.16 were used for fat content analysis.

Fat samples were extracted by using Soxhlet method. Saturated and unsaturated fatty acid contents of biscuits were determined by using standard Shimatzu protocols.

Determination of total sugar content (Reducing and non reducing sugar content) of the biscuits sample. The standard method of AOAC 923.09 was used for the study.

Total Carbohydrate contents and energy values were determined by standard calculation methods.

Evaluation of organoleptic properties of biscuits

Organoleptic properties of biscuits were determined with respect to four (4 types of biscuits prepared with sunflower, soya bean, peanut & corn oils) treatments along with a control. Evaluation was carried out with 30 members of untrained sensory panel by using 5 point hedonic scale, as given below.

Very Poor 1, Poor 2, Average 3, Good 4, Very good 5

Therein, the members were asked to indicate their choice on 5 types of biscuits by a numerical number on the ballot paper pertaining to five sensory stimuli such as Texture/softness, Aroma, Taste, After taste and Overall acceptability. The data collected were analyzed using MINITAB 14 Statistical Analysis Software.

III. RESULTS AND DISCUSSION

Saturated and unsaturated fatty acids content of selected oils
Fatty acid content of the soybean oil, corn oil, peanut oil, sunflower oil and palm oil samples were determined, using GCMS spectroscopic method and results are given in figures 1, 2, 3, 4 and 5.

According to results shown in the figure 1, saturated fat content was only 10.866% (sum of the Palmitic acid and Stearic acid) and the rest 89.114% is the unsaturated fatty acids (Sum of the Oleic acid and Linoleic acid). Saturated fat content of soybean oil was only 16.118% (sum of the Palmitic acid and Stearic acid 11.410+4.708) and 83.882% is the unsaturated fatty acids (Fig. 2). Major unsaturated fatty acid of the soybean oil was Linoleic acid (53.493%) and other unsaturated fatty acids were Oleic and Linolenic acids. According to the figure 3, saturated fat content was only 13.389% (sum of the Palmitic acid and Stearic acid 11.259+2.130) and the rest 86.611% is the unsaturated fatty acids. Major unsaturated fatty acid of the corn oil is Linoleic acid (55.919%) and unsaturated fatty acids were Oleic and Linolenic acids. According to the figure 4, saturated fat content of peanut oil (Fig. 4) is about 15.475% (sum of the Palmitic acid, Stearic acid, Eicosanoic acid, Archidic acid and Behenic acid 9.844+2.926+0.965+0.969+0.771) and rest 84.525% is the unsaturated fatty acids. Major unsaturated fatty acid of the peanut oil was Oleic acid (60.341%) and unsaturated fatty acid is Linoleic acid. According to figure 5, saturated fat content of palm oil is 44.732% (sum of the Palmitic acid and Stearic acid; 40.008+4.724) and the rest 55.268 % is the unsaturated fatty acids. Major unsaturated fatty acids of the palm oil are Oleic acid and unsaturated fatty acid is Linoleic acid.

According to the results, saturated fat content of all tested oils is less than that of palm oil which is the control and unsaturated fat content of the tested oils are higher than the palm oil.

According to the results unsaturated fat content of the tested oils were as follows.

Palm Oil < Soybean Oil < Peanut Oil < Corn Oil < Sunflower Oil

55.268 % < 83.882 % < 84.525 % < 86.611% < 89.114%

Free fatty acid level of the selected oils

The quality of corn, sunflower, soybean, peanut and palm oils were analyzed by evaluating physicochemical properties such as specific gravity, iodine value, free fatty acid values. Results are presented in Table 1.

<table>
<thead>
<tr>
<th>Type of Oil</th>
<th>Free fatty acid level (%)</th>
<th>Iodine Value g/100g</th>
<th>Specific Gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm Oil</td>
<td>0.03</td>
<td>55</td>
<td>0.934</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>0.41</td>
<td>127</td>
<td>0.918</td>
</tr>
<tr>
<td>Sunflower Oil</td>
<td>0.31</td>
<td>130</td>
<td>0.920</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>0.20</td>
<td>130</td>
<td>0.925</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>0.36</td>
<td>95</td>
<td>0.920</td>
</tr>
</tbody>
</table>

The results given in the table 1 clearly indicate that the iodine value and free fatty acids content of the all selected oils were within the accepted range. Free fatty acids (FFA) are produced by the hydrolysis of oils and fats. The level of FFA depends on time, temperature and moisture content, storage, processing, heating or frying.

Iodine value (IV) measures the degree of unsaturation in a fat or oil. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively. Moreover, the table 1 shows that the Specific gravity of the all selected oils was within the accepted range.

Evaluation of Characteristics properties of Soft Dough Biscuits

Moisture content of the prepared biscuits (using different types of the oils) were determined and results are given in the table 2.
Table 2: Analysis Results of Moisture content and pH of the biscuits

<table>
<thead>
<tr>
<th>Types of Oil Incorporated Biscuits</th>
<th>Moisture Content (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm Oil</td>
<td>2.35</td>
<td>6.89</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>2.25</td>
<td>6.81</td>
</tr>
<tr>
<td>Sunflower Oil</td>
<td>2.27</td>
<td>6.88</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>2.67</td>
<td>7.04</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>2.60</td>
<td>6.84</td>
</tr>
</tbody>
</table>

According to the results shown table 2, moisture content of all biscuits samples, prepared from sunflower, corn, soya bean, peanut and palm oils is within the accepted range (According to the sri lankan, SLS 251:2010 biscuits standard, Maximum moisture content of biscuits is 4.5%). Moisture content of the biscuits is depend on the quality of the raw materials, composition of the product, baking time and baking temperature of the biscuits. pH also in all tested biscuits samples (Table 2) was within the range (SLS 251:2010 biscuits standard, pH value of the biscuits is between 6.8-7.2).

Nutritional Composition of Soft Dough Biscuits

Table 3: Analysis Results of Nutritional composition of the biscuits

<table>
<thead>
<tr>
<th>Types of Oil Incorporated Biscuits</th>
<th>Protein Content %</th>
<th>Fat  %</th>
<th>Sugar %</th>
<th>Total Carbohydrate %</th>
<th>Energy (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm Oil</td>
<td>8.07</td>
<td>13.67</td>
<td>22.69</td>
<td>74.96</td>
<td>455.15</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>8.58</td>
<td>13.70</td>
<td>23.36</td>
<td>74.50</td>
<td>455.62</td>
</tr>
<tr>
<td>Sunflower Oil</td>
<td>8.02</td>
<td>13.78</td>
<td>24.05</td>
<td>74.98</td>
<td>456.02</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>8.47</td>
<td>11.75</td>
<td>24.34</td>
<td>75.81</td>
<td>442.87</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>9.04</td>
<td>14.85</td>
<td>23.87</td>
<td>72.64</td>
<td>460.37</td>
</tr>
</tbody>
</table>

Protein content of the biscuits was determined according to the standard protocol kjeldhal method and results were shown in table 3. Results revealed that the protein content of all biscuits were within the acceptable range and there was no significant difference between the treatments and the reference sample of palm oil incorporated biscuits. The total fat content of all treatments are also within the acceptable range and there was no significant difference between the treatment and the reference sample.

In soft dough biscuits, generally fat content is high and it is range from 18-25%. One of the objectives in this study is to reduce the fat content of the biscuits as it is important as a health food. Results of this study are showing that total fat content of biscuits is ranging from 11.75-14.85%. Reducing of total fat content in biscuits beyond that limit is not possible due to some of practical limitations such as declining of sensory properties, sticking the dough to the rotary mould and contracting size and shape of the biscuits. The results pertaining to the Sugar content, revealed that the sugar content was within the acceptable range and there is no significant difference between the reference sample (palm oil incorporated biscuits) and the treatments. In soft dough biscuits, generally sugar content is high and it is above 25- 30%. Total carbohydrate content given in the table 3 also indicate, it is within the acceptable range and there is also no significant difference between the treatments and control. Energy values of the biscuits were determined by calculation method and results revealed that there was no significant difference between the treatment and control.

Saturated fat content of biscuits

Saturated fat content of the biscuits were determined the using GCMS spectroscopic method and results pertaining to the sunflower oil, soybean oil, corn oil, peanut oil and palm oil incorporated biscuits are given figure 6, 7, 8, 9 and 10.

Figure 6: Sunflower oil
Figure 7: Soybean oil
Figure 8: Corn oil
Figure 9: Peanut oil
Figure 10: Palm oil

Figure 6, 7, 8, 9 and 10: Gas Chromatogram of the fatty acid composition of the biscuits produced from sunflower oil, soybean oil, corn oil, peanut oil and palm oil
The figure 6 shows that the chromatogram indicating the fatty acid composition of the biscuit produced from the Sunflower oil. Saturated fat content of the biscuits produced from sunflower oil was only 16.998% (sum of Palmitic acid and Stearic acid). Unsaturated fat content of the biscuits produced from sunflower oil was 83.002%. Whereas, the figure 7 shows the fatty acid composition of the biscuits produced from Soybean oil. Saturated and unsaturated fatty acid contents of these biscuits were around 20.429% and 79.871% respectively. In the case of corn oil (Fig. 8) incorporated biscuits, Saturated and unsaturated fatty acids contents were 21.395% and 78.606 % respectively. Saturated fatty acids content in peanut oil incorporated biscuits was only 18.957% (Fig. 9).

According to sri lankan regulatory requirement, if unsaturated fatty acid content exceeds the value of 70% it can be categorized as a health food. Hence, biscuits produced from sunflower oil, soybean oil, corn oil and peanut oil were fulfilled this requirement. As far as control treatment is concerned, which saturated and unsaturated fatty acid composition is 43.592 & 56.388% respectively. Therefore, biscuits prepared from palm oil are not accepted as a health food.

Normally commercial scale biscuits manufacture used palm oil as the fat source. However, results of this study indicate that palm oil was not suitable for biscuits because, it increases the unsaturated fat content. Finally, the quantitative results pertaining to the saturated fatty acid levels in biscuits produced from different oils are presented in the Table 5.

Table 5: Analysis Results of fatty acids levels in biscuits produced from different oils

<table>
<thead>
<tr>
<th>Types of Oil Incorporate in Biscuits</th>
<th>Saturated Fat Content (% of the extracted Fat (From biscuits))</th>
<th>Unsaturated Fat Content (% of the extracted Fat (From biscuits))</th>
<th>Total Energy (Kcal)</th>
<th>Energy % from Un saturated Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm Oil</td>
<td>43.592</td>
<td>56.388</td>
<td>455.15</td>
<td>15.24</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>21.395</td>
<td>78.606</td>
<td>456.02</td>
<td>22.81</td>
</tr>
<tr>
<td>Sunflower Oil</td>
<td>16.998</td>
<td>83.002</td>
<td>442.87</td>
<td>19.33</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>20.429</td>
<td>79.871</td>
<td>455.62</td>
<td>21.57</td>
</tr>
<tr>
<td>Peanut Oil</td>
<td>18.957</td>
<td>81.033</td>
<td>460.37</td>
<td>23.83</td>
</tr>
</tbody>
</table>

According to the results in table 5, there is a clear indication that the unsaturated fatty acid levels in biscuits produced from all tested oils were comparatively higher than that of the control (Palm oil incorporated biscuits) treatment. Amongst the tested oils, sunflower oil gave the better results in terms of the unsaturated fatty acid level in biscuits. Further, results revealed that sunflower oil is superior in terms of high in unsaturated fat content comparatively other treatments. Oleic acid and linoleic acid were common in the unsaturated fatty acids of all sunflower oil, soybean oil, corn oil, peanut oil and palm oil incorporated biscuits. Palmitic acid and stearic acid were common in the saturated fatty acids of all sunflower oil, soybean oil, corn oil, peanut oil and palm oil incorporated biscuits. Linolenic acid was only found in soybean oil incorporated biscuits as fatty acids. These results were depicting in figure 6, 7, 8, 9 and 10.

Evaluation of Organoleptic properties of biscuits

Five types of biscuits prepared from different oil types were evaluated sensoraly and results were ranked according to the method describe by friedman test. Rank sum pertaining to the five sensory stimuli are given bellow Table 6.

Table 6: Rank sums for five sensory stimuli for five types of biscuits

<table>
<thead>
<tr>
<th>Sample</th>
<th>Texture</th>
<th>Aroma</th>
<th>Taste</th>
<th>After Taste</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>456</td>
<td>71.5</td>
<td>81.0</td>
<td>76.5</td>
<td>75.5</td>
<td>75.0</td>
</tr>
<tr>
<td>584</td>
<td>93.0</td>
<td>101.5</td>
<td>103.0</td>
<td>101.5</td>
<td>98.5</td>
</tr>
<tr>
<td>632</td>
<td>107.0</td>
<td>97.5</td>
<td>92.5</td>
<td>91.5</td>
<td>101.5</td>
</tr>
<tr>
<td>796</td>
<td>96.0</td>
<td>93.0</td>
<td>95.0</td>
<td>93.5</td>
<td>99.5</td>
</tr>
<tr>
<td>829</td>
<td>102.5</td>
<td>77.0</td>
<td>83.0</td>
<td>88.0</td>
<td>85.5</td>
</tr>
</tbody>
</table>

456 – Biscuits sample prepared with Soybean oil
584 – Biscuits sample prepared with Corn oil
632– Biscuits sample prepared with Palm oil
796– Biscuits sample prepared with Sunflower oil
829– Biscuits sample prepared with Peanut oil

The rank sum of sensory stimuli such as texture, aroma, taste, after taste and overall acceptability were compared with difference between rank sum in p=0.05. Results revealed (Table 3) that there is no significant difference between the all treatments except the sensory stimulus the texture. However, there is a significant difference between the control treatment (632) and all other treatments pertaining to the sensory stimulus texture. The reason may be due to the panelists who may have been exposed to the biscuits produced from palm oil (632).

To further validate the outcome of the study, sensory profiles pertaining to the 5 treatments were drawn using mean values of the sensory stimuli texture, aroma, taste, after taste and overall acceptability, which are depicted by Fig. 11.
Figure 11: Mean ranks of sensory stimuli of biscuits

Sensory profiles also clearly indicate that there is no significant difference between treatments, because sensory profile of each treatment, are nearly overlapping to each other except the sensory stimulus “texture”. Color and flavor of the product was not considered as parameters since no any color and flavor additives were used in this study.

According to the draft Food labeling and Advertising regulation in Sri lanka, USE OF NUTRITION AND HEALTH CLAIMS, Regulation 12(5) (ii) SCHEDULE VII described that high in unsaturated fat in food item, at least 70% of the fatty acids derived from unsaturated fat under the condition that unsaturated fat shall provides more than 20% of the energy. One of the important objectives of this study is to achieve above requirements from the unsaturated fat and to accomplish the state energy level. The results given in table 5 clearly indicate that unsaturated fat content in sunflower oil, soybean oil, corn oil and peanut oil is higher than 70%.

Even unsaturated fat provide more than 20% of the energy requirement fulfill the sunflower oil, corn oil and peanut oil. Amongst, Sunflower oil seems superior in terms of lowest saturated fat content and 70% of the unsaturated fat and more than 20% energy provided from unsaturated fat. (Blue area is problematic, therefore omit it)

Therefore, the biscuits prepared from sunflower oil is already qualified to claim the ‘nutrition and health claims’, as this product contains High percentage of unsaturated fatty acids.

CONCLUSIONS

This study was conducted to select high unsaturated edible grade fat type for development of soft dough biscuits. Four types of different oils namely Sunflower oil, soybean oil, corn oil and peanut oil were used for the study as treatments and palm oil was used as the standard/ control.

Chemical and physical parameters of oils were determined for its initial characterzation. The all the parameters tested were within the acceptable range for regulatory requirement. Only deviation was found in saturated fatty acid content in tested oils and control which were 10.866%, 16.118%, 13.389%, 15.475% and 44.732% for sunflower oil, soybean oil, corn oil, peanut oil and palm oil respectively. Tested oils were also contained higher percentage of unsaturated fatty acids, except the palm oil.

Soft Dough biscuits developed by using sunflower oil, soybean oil, corn oil and peanut oil were remained within the stipulated physical and chemical requirements. According to the SLS 251:2010 for biscuits standard, regulatory requirements for moisture content and pH value shall be 4.5%(Max) and in between 6.8-7.2 respectively. Biscuits prepared from all of these treatments were in compliance with those regulatory requirements. Nutritional analysis of the prepared soft dough biscuits revealed that there were no differences between the treatments.

Unsaturated fat contents of the soft dough biscuits prepared from sunflower oil, soybean oil, corn oil, peanut oil and palm oil were remained higher than that of the biscuits prepared from palm oil which were for Palm Oil 56.408% , Sunflower Oil 83.002%, Soybean Oil 79.571%, Corn Oil 78.605% and Peanut Oil 81.043%. Results of the sensory evaluation revealed that the acceptance of the all the parameters except the texture. Hence, Sunflower oil, Soybean oil, Corn oil and Peanut oil can be used for the soft dough biscuit production. Amongst the tested treatments, Sunflower oil identified as the best, in terms of unsaturated fatty acid content. Therefore, with this product can go for ‘nutrition and health claim’, which describes High in unsaturated fat in food item.

REFERENCES