Analysis of salt, total fat, trans fat and carbohydrate in junk food

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Contents

1. Introduction ........................................................................................................................................ 3

2. Materials and methods ....................................................................................................................... 4

3. Results ................................................................................................................................................ 8

Annexures 1-4 ....................................................................................................................................... 11-18

References ............................................................................................................................................. 19
1. Introduction

India has been going through an epidemiological transition i.e. the proportion of disease burden attributable to non-communicable diseases (NCDs) is increasing. A 2016 analysis by the India State-level Disease Burden Initiative found that NCDs accounted for 61.8 per cent of deaths compared to communicable, maternal, neonatal diseases, and undernutrition, which together accounted for 27.5 per cent of deaths. National Capital Territory of Delhi is amongst the few states with the highest-middle epidemiological transition. In 2012, the Centre for Science and Environment (CSE) had tested 23 samples of junk food across seven different categories such as chips, snacks, instant noodles, burger, pizza, fries, fried chicken, and carbonated beverage for salt, carbohydrate, fat and trans fat. The samples were collected from various outlets in Delhi. The tests were done using methods listed by the Association of Official Analytical Chemists (AOAC), which are accepted internationally and by the apex food regulator of the country – the Food Safety and Standards Authority of India (FSSAI). This study found high levels of sugar, salt and fat in junk foods tested which could be unhealthy to the consumers and it also highlighted misleading labeling on certain food products.

Junk food refers to foods which are often ultra-processed, low in nutritional value and high in fat, salt, sugar (HFSS) and calories. They are usually popular across all age groups and aggressively promoted particularly towards children. Unlike junk foods, a balanced diet is one which provides nutrients in required quantities and proportions. It is recommended that around 50-60 per cent of total calories should be derived from carbohydrates, preferably from complex carbohydrates, about 10-15 per cent from proteins and 20-30 per cent from both visible and invisible fat, according to the Dietary Guidelines for Indians put forth by the National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad. Consumption of junk foods have both short-term and long-term ill effects on the body as high fat, salt and sugar intake are linked to obesity and NCDs such as diabetes, hypertension and heart disease. Reducing unhealthy diet is one of the four modifiable risk factors to prevent NCDs in addition to physical inactivity, alcohol and tobacco, as per the World Health Organization (WHO).

High intake of salt (sodium chloride) which is 40 per cent sodium predisposes a risk for high blood pressure. According to the WHO, salt intake of less than 5 grams per day for adults helps to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart attack. It has been identified as one of the most cost-effective measures countries can take to improve population health outcomes.

Fats are a combination of saturated fatty acids, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The energy yield from a gram of fat is approximately 9 Kcal (37.7 kj)/g, which is higher compared to 4 Kcal (16.8 kj)/g from a molecule of protein or a carbohydrate. While saturated fatty acids have no double bond in their chemical structure, MUFA have one and PUFA have two or more. The double bonds of naturally occurring unsaturated fatty acids are often of the cis orientation, which means that the hydrogen atoms
attached to the double bonds are on the same side. If the hydrogen atoms are on opposite sides, the configuration is termed trans. Trans fats are unsaturated fatty acids that contain at least one double bond in the trans configuration. They are formed during industrial partial hydrogenation of vegetable oil, a process widely commercialized to produce solid fats or vanaspati. Trans fat content of such fats can account for up to 60 per cent of the fatty acid content and depends on parameters like time, catalyst, temperature, and hydrogen pressure during hydrogenation process as well as the composition of MUFA and PUFA in the oil used. To minimize their health impact, trans fats should not exceed 1 per cent of energy intake. High levels of trans fats intake are known to affect blood lipid profile, including elevation of LDL (low density lipoprotein) cholesterol, which is a well-accepted biomarker for risk of cardiovascular disease.

Carbohydrates are compounds that contain carbon, hydrogen, and oxygen in the ratio of 1:2:1. They are directly transformed into glucose after digestion, or undergo oxidation into pyruvate, including some sugar alcohols in the body. Therefore, carbohydrate intake raises blood glucose and generates a positive energy balance, which is one of the reasons of obesity.

Junk foods are not adequately regulated in India. Nutritional analysis of popular junk foods is important to improve consumer awareness and help policy makers develop suitable interventions for regulating such foods.

2. Materials and methods

2.1 Sample collection

A total of 33 junk food samples comprising 14 packaged foods and 19 fast foods were collected from retail outlets and fast food chain restaurants from different locations in Delhi between July and September of 2019. Packaged food belonged to four categories including chips (6), namkeen (4), instant noodles (3), soup (1), and fast food were from five categories namely burger (8), fries (3), fried chicken (1), pizza (4), and sandwich and wrap (3). Details of samples are provided in Annexure 1 (see Annexure 1: Details of food samples tested). All packaged food samples were tested before expiry date and fast food samples were tested within 30 days of purchase. Samples were tested for salt, total fat, trans fat and carbohydrate except instant noodles and soup, which were not tested for trans fat. Test for salt and total fat were run in triplicate and trans fat and carbohydrate were conducted in duplicate.

2.2 Sample preparation

Packaged food samples were weighed and stored in air-tight desiccator maintained at room temperature (20-25°C) for a maximum of up to 60 days until tests were complete. Fast food samples were transported in air-tight zip lock pouches in dry ice, weighed and stored at -20°C for a maximum of 30 days until tests were complete. Samples were ground to fine powder/paste
using a mixer grinder (Sujata) set at high speed for 2-3 minutes. All samples were cooled to room temperature before tests were conducted.

2.3 Sample testing

2.3.1 Salt

AOAC 937.09 (volumetric analysis for salt/Chlorine as Sodium Chloride) was used for testing salt in food samples. To summarize the method, 2g finely ground sample was mixed with 25mL AgNO3 (0.1M, Merck) in an Erlenmeyer flask. 20mL HNO3 (concentrated, Merck) was added with boiling chips, followed by boiling until the test sample was completely digested. 50mL deionised water was added to the digested sample, boiled for 5 minutes and cooled to room temperature in a fume hood. Ferric alum indicator (5mL, Fischer Scientific) was added and gently mixed. Digestion and titration steps were performed in fume hood. Test sample was titrated with NH4SCN (0.1M, Merck) until a permanent, light brown endpoint was achieved. Calculation of salt was done as follows.

\[ C = A - B \]

A= Added volume of 0.1M AgNO3  
B= Titer volume of 0.1M NH4SCN  
C= Consumed volume of AgNO3 for NaCl (calculate this difference as NaCl). 
With 10g test sample each ml AgNO3 = 0.058 per cent NaCl. 
Values of salt were converted to sodium content using the following formula: 
Salt (g)/2.54= Sodium (g)

2.3.2 Carbohydrate

Anthrone method (colorimetric analysis) was used (Clegg KM, 1956).\textsuperscript{66} 100mg finely ground sample was mixed with 5ml HCl (2.5N, Merck) and kept in water bath maintained at 100°C for 3 hours for sample hydrolysis. Hydrolyzed samples were cooled to room temperature and neutralized by adding solid Na2CO3 (Qualigens), until the effervescence ceased. After adjusting the volume to 100ml by adding deionised water and centrifuged at 4000rpm for 10 minutes at room temperature, supernatant was collected in separate vials. Anthrone reagent (4ml, Loba Chem) was added to test vials with 1ml of supernatant, after which the test sample was boiled in a water bath kept at 100°C for eight minutes. After rapid cooling of the samples to room temperature at ice (4°C), absorbance was measured at 630nm (UV/VIS Spectrophotometer, Agilent). A blank sample (no test product), and quality control standards (30ppm and 140ppm) and six standards of glucose solution (20ppm, 40ppm, 60ppm, 80ppm, 100ppm and 200ppm) (Merck), each diluted in distil water, served as calibration controls.

2.3.3 Total fat

AOAC 922.06 (acid hydrolysis and gravimetric analysis) method was used. To summarize the
method, 2g finely ground test food sample was mixed with 2ml ethanol (Fischer Scientific) in a conical flask. 10ml HCl (concentrated, Merck) was added, followed by heating the sample in a water bath at 70-80°C for 40 minutes with regular agitation. 10ml ethanol (Fischer Scientific) was added and sample flask was cooled to room temperature before the contents were transferred to a separatory funnel. The conical flask with test contents was rinsed into the separatory funnel using 25 ml ether (Merck) in three portions. After shaking the contents in the separatory funnel for 1 minute, 25 ml petroleum ether (40-60°C, Qualigens) was added and the contents were thoroughly mixed for 1 minute. The test mixture was allowed to separate into layers by centrifuging at 600 rpm for 20 minutes. Of the two layers obtained, upper layer (of ether-fat solution) was collected in a new flask. The separatory funnel was rinsed into the new conical flask with 15ml 1:1mixture of diethyl ether and petroleum. The total test volume was reduced to about 5ml by slow evaporation in water bath at 70°C for 40 minutes. The fat obtained was dried in an oven at 100°C until a constant weight of contents was obtained. The weight of the fat (adjusted with the weight of the pre-weighed beaker) was recorded.

2.3.4 Trans fat

AOAC 996.06 (Hydrolysis, Gas Chromatography based) method was used. The protocol involves hydrolysis, extraction of fat into ether, methylation of extracted fat to FAME (fatty acid methyl esters), and finally, quantitative estimation of FAME using gas chromatography based method against a C11:0-triundecanoin internal standard. Details of FAME standard solution is provided in annexure 2 (see Annexure 2: Details of FAME standard solution). The method is outlined below.

2.3.4.1 Preparation of Fatty Acid Methyl Ester (FAME)

Foods excluding dairy products and cheese: Sample containing 30-50mg fat was mixed with 100mg pyrogallic acid (Merck), 596μg glyceryl triundecanoate internal standard (59.6μl of 10,000ppm working standard, Sigma), 2ml ethanol (Merck) and few boiling chips until these were completely dispersed. This was followed by addition of 10ml HCl (8.3M, Merck). Contents were placed in a water bath maintained at 70-80°C for 40 minutes with regular agitation.

Foods containing cheese: Sample containing 30-50mg fat was mixed with 100mg pyrogallic acid (Merck), 596μg glyceryl triundecanoate internal standard (59.6μl of 10,000ppm working standard, Sigma), 2ml ethanol (Merck) and few boiling chips until these were completely dispersed. This was followed by addition of 4ml distil water and 2ml NH4OH (58% w/v, Merck). Contents were placed in a water bath maintained at 70-80°C for 20 minutes with regular agitation. 10ml HCL(12M, Merck) was added followed by a steam bath for 20 minutes with regular agitation.

2.3.4.2 Fat extraction

After cooling the above-said contents to room temperature, 25mL ethanol (Merck) was added to
digested test sample and mixed gently. This was followed by adding 25ml diethyl ether (Merck), sealing the flask using a cork stopper and vigorous shaking for 5 minutes. The stopper was rinsed into the flask using a 1:1 mixture of diethyl ether and petroleum ether (40-60°C, HPLC grade, Spectrochem). Contents were transferred to a separatory funnel with 25 ml of petroleum ether. This was followed by rigorous shaking for 5 minutes to allow layer separation. The upper layer (containing extracted fat) was collected in a beaker, and was kept at 40°C in a water bath to allow slow evaporation under a Nitrogen stream. The residue in the beaker contains extracted fat.

2.3.4.3 Methylation of fat

Extracted fat was dissolved in 2-3 mL1:1 mixture of chloroform (LiChrosolv, HPLC grade, Merck) and diethyl ether (Merck). The mixture was transferred to a glass vial and evaporated in water bath maintained at 40°C under a Nitrogen stream until the mixture was completely dried. 2ml BF3 reagent (10% w/v, Sigma) and 1ml toluene (LiChrosolv, HPLC grade, Merck) were added to the dried mass obtained after evaporation in glass vial, which was sealed followed by heating in an oven at 100°C for 45 minutes with regular agitation. After cooling the vial contents to room temperature, 5ml distil water, 1ml hexane (LiChrosolv, HPLC grade, Merck) and 1g Na2SO4 (Merck) were added, followed by re-sealing the vial and rigorous shaking for 1 minute to allow layer separation. The upper layer (which contains FAME including that of internal standard) was transferred to a fresh vial containing 1g Na2SO4.

2.3.4.4 Detection of trans fat

Samples were analyzed i.e. trans fats were detected using Gas Chromatography (Thermoquest-Trace GC, Thermo Fischer Scientific) with Flame Ionization Detector. 2μl of test sample were injected in GC (see Table 1: Gas chromatography conditions). A five point calibration curve (range 25 to 200ppm) was employed for analysis. Internal controls (calibration check standard, matrix spiked samples, etc) were run in all batches. GC conditions are provided in the table below. Chromatograms of FAME with internal standard of few samples are provided in annexure 3 (see Annexure 3: Chromatograms of standard and few study samples). Trans fat was calculated as sum of individual eighteen fatty acids (as triglyceride equivalents).

### Table 1: Gas chromatography conditions

<table>
<thead>
<tr>
<th>Step</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oven temperature programme (45 minutes)</td>
<td>• Initial Temperature 140°C&lt;br&gt;• Hold time 1 minute; ramp 10°C/min up to 180°C&lt;br&gt;• Hold time 1 minute; ramp 1.0°C/min up to 214°C&lt;br&gt;• No hold time; ramp 4°C/min up to final temperature 230°C&lt;br&gt;• Hold time 1 minute</td>
</tr>
<tr>
<td>SSL injector (10μL syringe, Hamilton)</td>
<td>• Capillary column HP - 88 (100 m x 0.25 mm i.d. x 0.20 im)Temperature 250°C with Split less time 0.8 minutes</td>
</tr>
</tbody>
</table>
3. Results

Tests for salt, total fat, trans fat and carbohydrate in junk food samples were done. Results except for trans fat in instant noodles and soup which were not tested, are reported as mean values in Table 2 (see Table 2: Quantitative analysis of salt, total fat, trans fat and carbohydrate in junk food). Replicate test values are provided in annexure 4 (see Annexure 4: Test results-replicate values).

In chips, salt content was ranging from 1.38–3.23g per 100g. Too Yumm Multigrain Chips Chinese Hot and Sour by RP-Sanjiv Goenka Group had the highest salt. Total fat ranged from 17.17–36.70g per 100g with highest value in Pudina Treat Chips by Haldiram’s. Trans fat varied from 0.08–0.33g per 100g with highest levels in Classic Salted Chips by Haldiram’s. Carbohydrate levels were of the order 48.96–64.85 g per 100 g.

Namkeen samples had salt levels from 1.69–4.99g per 100g, total fat levels ranging from 34.03–44.79g per 100g and trans fat content ranging from 0.20–0.56g per 100g. Classic Nut Cracker by Haldiram’s had the highest levels of salt, total fat and trans fat amongst all namkeens tested. Carbohydrate levels in namkeens ranged from 17.66–54.90g per 100g.

Instant noodles had salt ranging from 3.69–4.25g per 100g. Ching’s Secret Schezwan by Capital Foods had the highest salt in this category. Total fat in instant noodles ranged from 14.93–22.06g per 100g and was highest in Atta Noodles Chatpata by Patanjali Ayurved. Carbohydrate was present in the range 51.23–62.18g per 100g in instant noodles tested.

Knorr Classic Thick Tomato Soup by Hindustan Unilever had 10.44g per 100g salt, 8.43g per 100g fat and 64.61g per 100g carbohydrate.

Burgers had salt ranging from 1.02–1.54g per 100g. Veg Zinger (with cheese) by KFC had the highest salt amongst burgers tested. Total fat content varied from 9.11–19.47g per 100g and trans fat content ranged from 0.05–0.20 g per 100g. Chicken Classic Zinger (with cheese) by KFC had the highest fat and trans fat in this category. Carbohydrate levels were of the order 12.51–33.16g per 100g.

Fries had salt content ranging between 0.47–0.85g per 100g. Highest salt was present in Fries (regular) by Burger King. Total fat in fries was from 13.60–14.90g per 100g and trans fat was in the range 0.12–0.15 g per 100g. Fries (medium) by KFC had the highest fat. Fries (medium) by KFC and Fries (medium) by McDonald’s both had highest level of trans fat. Carbohydrate content
varied from 25.94–44.77g per 100g.

Hot Wings (4 pieces) by KFC fried chicken sample had 1.44g per 100g salt, 21.42g per 100g fat, 0.14g per 100g trans fat and 10.51g per 100g carbohydrate.

In pizzas, salt content ranged from 1.18–1.70g per 100g. Chicken Supreme (personal) by Pizza Hut had the highest salt. Total fat ranged from 7.40–12.31g per 100g with highest fat in Non-veg Supreme (regular) by Domino’s. Trans fat values in pizzas ranged from 0.21–0.23g per 100g. Trans fat values were highest in Classic Tomato Margherita (personal) and Chicken Supreme (personal) by Pizza Hut. Carbohydrate levels in pizzas varied from 20.40–35.81g per 100g.

Sandwiches and wraps had salt ranging from 1.38–1.88g per 100g with highest content in Chicken Seekh Kabab (6 inch) by Subway. Total fat values ranged from 13.90–18.27g per 100g, with highest levels in Paneer Tikka (6 inch) by Subway. Trans fat levels varied from 0.22–0.24g per 100g and carbohydrate values ranged from 16.83–21.12g per 100g. Big Spicy Paneer Wrap by McDonald’s had the highest levels of trans fat.
Table 2: Quantitative analysis of salt, total fat, trans fat and carbohydrate in junk food

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured weight (g)</th>
<th>Salt (g/100g)</th>
<th>Total fat (g/100g)</th>
<th>Trans fat (g/100g)</th>
<th>Carbohydrate (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chips</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lay’s India’s Magic Masala by PepsiCo</td>
<td>51.75</td>
<td>1.94</td>
<td>32.5</td>
<td>0.21</td>
<td>51.53</td>
</tr>
<tr>
<td>Lay’s American Style Cream and Onion Flavour by PepsiCo</td>
<td>52.75</td>
<td>1.55</td>
<td>29.19</td>
<td>0.27</td>
<td>48.96</td>
</tr>
<tr>
<td>Uncle Chipps Spicy Treat by PepsiCo</td>
<td>55.25</td>
<td>2.81</td>
<td>35.04</td>
<td>0.17</td>
<td>51.81</td>
</tr>
<tr>
<td>Classic Salted Chips by Haldiram’s</td>
<td>61.2</td>
<td>1.38</td>
<td>36.52</td>
<td>0.33</td>
<td>52.19</td>
</tr>
<tr>
<td>Pudina Treat Chips by Haldiram’s</td>
<td>16.38</td>
<td>2.28</td>
<td>36.70</td>
<td>0.23</td>
<td>52.93</td>
</tr>
<tr>
<td>Too Yumm Multigrain Chips Chinese Hot and Sour by RP-Sanjiv Goenka Group</td>
<td>89</td>
<td>3.23</td>
<td>17.17</td>
<td>0.08</td>
<td>64.85</td>
</tr>
<tr>
<td><strong>Namkeen</strong></td>
<td></td>
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<tr>
<td>Classic Nut Cracker by Haldiram’s</td>
<td>231.65</td>
<td>4.99</td>
<td>44.79</td>
<td>0.56</td>
<td>17.66</td>
</tr>
<tr>
<td>Aloo Bhujia by Haldiram’s</td>
<td>231.14</td>
<td>3.05</td>
<td>43.48</td>
<td>0.33</td>
<td>34.69</td>
</tr>
<tr>
<td>Bingo! Mad Angles Delight Achaari Masti by ITC</td>
<td>80.7</td>
<td>1.69</td>
<td>42.94</td>
<td>0.22</td>
<td>53.71</td>
</tr>
<tr>
<td>Kurkure Masala Munch by PepsiCo</td>
<td>49.97</td>
<td>2.49</td>
<td>34.03</td>
<td>0.20</td>
<td>54.90</td>
</tr>
<tr>
<td><strong>Instant noodles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maggi Masala by Nestle</td>
<td>71.2</td>
<td>3.69</td>
<td>14.93</td>
<td>NT</td>
<td>62.18</td>
</tr>
<tr>
<td>Atta Noodles Chatpata by Patanjali Ayurved</td>
<td>60.40</td>
<td>3.95</td>
<td>22.06</td>
<td>NT</td>
<td>51.23</td>
</tr>
<tr>
<td>Ching’s Secret Schetzwan by Capital Foods</td>
<td>60.35</td>
<td>4.25</td>
<td>17.82</td>
<td>NT</td>
<td>53.80</td>
</tr>
<tr>
<td><strong>Soup</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Knorr Classic Thick Tomato Soup by Hindustan Unilever</td>
<td>51.90</td>
<td>10.44</td>
<td>8.43</td>
<td>NT</td>
<td>64.61</td>
</tr>
<tr>
<td><strong>Burger</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Cheese Whopper Veg by Burger King</td>
<td>259.47</td>
<td>1.36</td>
<td>13.88</td>
<td>0.19</td>
<td>20.22</td>
</tr>
<tr>
<td>Cheese Whopper Chicken by Burger King</td>
<td>258.08</td>
<td>1.35</td>
<td>10.65</td>
<td>0.16</td>
<td>12.51</td>
</tr>
<tr>
<td>Veg Zinger (with cheese) by KFC</td>
<td>250.31</td>
<td>1.54</td>
<td>10.89</td>
<td>0.10</td>
<td>19.69</td>
</tr>
<tr>
<td>Chicken Classic Zinger (with cheese) by KFC</td>
<td>252.84</td>
<td>1.22</td>
<td>19.47</td>
<td>0.20</td>
<td>16.41</td>
</tr>
<tr>
<td>McVeeggie Burger by McDonald’s</td>
<td>153.95</td>
<td>1.22</td>
<td>9.95</td>
<td>0.08</td>
<td>33.16</td>
</tr>
<tr>
<td>McAlloo Tikki Burger by McDonald’s</td>
<td>141.25</td>
<td>1.02</td>
<td>9.11</td>
<td>0.08</td>
<td>29.16</td>
</tr>
<tr>
<td>McChicken Burger by McDonald’s</td>
<td>145.31</td>
<td>1.11</td>
<td>9.51</td>
<td>0.08</td>
<td>27.68</td>
</tr>
<tr>
<td>Chicken Maharaja Mac by McDonald’s</td>
<td>317.40</td>
<td>1.45</td>
<td>10.05</td>
<td>0.05</td>
<td>20.25</td>
</tr>
<tr>
<td><strong>Fries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fries (regular) by Burger King</td>
<td>92.26</td>
<td>0.85</td>
<td>13.6</td>
<td>0.12</td>
<td>25.98</td>
</tr>
<tr>
<td>Fries (medium) by KFC</td>
<td>91.76</td>
<td>0.47</td>
<td>14.9</td>
<td>0.15</td>
<td>25.94</td>
</tr>
<tr>
<td>Fries (medium) by McDonald’s</td>
<td>79.25</td>
<td>0.71</td>
<td>14.48</td>
<td>0.15</td>
<td>44.77</td>
</tr>
<tr>
<td><strong>Fried chicken</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot Wings (4 pieces) by KFC</td>
<td>88.91 (without bone)</td>
<td>1.44</td>
<td>21.42</td>
<td>0.14</td>
<td>10.51</td>
</tr>
<tr>
<td><strong>Pizza</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Peppy Paneer cheese burst (regular) by Domino’s</td>
<td>342.57</td>
<td>1.34</td>
<td>9.55</td>
<td>0.21</td>
<td>20.40</td>
</tr>
<tr>
<td>Non-veg Supreme (regular) by Domino’s</td>
<td>355.01</td>
<td>1.41</td>
<td>12.31</td>
<td>0.22</td>
<td>26.53</td>
</tr>
<tr>
<td>Classic Tomato Margheritra (personal) by Pizza Hut</td>
<td>221.78</td>
<td>1.18</td>
<td>7.4</td>
<td>0.23</td>
<td>35.81</td>
</tr>
<tr>
<td>Chicken Supreme (personal) by Pizza Hut</td>
<td>306.74</td>
<td>1.70</td>
<td>10.32</td>
<td>0.23</td>
<td>26</td>
</tr>
<tr>
<td><strong>Sandwich and wrap</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Big Spicy Paneer Wrap by McDonald’s</td>
<td>255.14</td>
<td>1.58</td>
<td>17.71</td>
<td>0.24</td>
<td>21.12</td>
</tr>
<tr>
<td>Paneer Tikka (6 inch) by Subway</td>
<td>263.61</td>
<td>1.38</td>
<td>18.27</td>
<td>0.23</td>
<td>16.83</td>
</tr>
<tr>
<td>Chicken Seekh Kabab (6 inch) by Subway</td>
<td>280.48</td>
<td>1.88</td>
<td>13.9</td>
<td>0.22</td>
<td>16.95</td>
</tr>
</tbody>
</table>

NT: Not tested; pizza samples were tested after sprinkling one sachet of oregano provided with the pizza.
## Annexure-1 Details of food samples tested

### Table 1: Packaged foods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Manufacturer</th>
<th>Date of manufacture</th>
<th>Best before/ date of expiry</th>
<th>Source of collection</th>
<th>Batch Number</th>
<th>Pack size/ serving quantity</th>
<th>Declared value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lay’s India’s Magic Masala by PepsiCo</td>
<td>PepsiCo India Holdings Pvt Ltd., Village Channo, Patiala - Sangrur road, PO Bhawarigarth, Dist Sangrur, 148026, Punjab</td>
<td>May 22, 2019</td>
<td>4 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>N122051974</td>
<td>52g/30g</td>
<td>815</td>
</tr>
<tr>
<td>Lay’s American Style Cream and Onion Flavour by PepsiCo</td>
<td>PepsiCo India Holdings Pvt Ltd. Village Channo, Patiala - Sangrur road, PO Bhawarigarth, dist Sangrur, 148026, Punjab</td>
<td>June 10, 2019</td>
<td>4 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>N110061951</td>
<td>52g/30g</td>
<td>742</td>
</tr>
<tr>
<td>Uncle Chipps Spicy Treat by PepsiCo</td>
<td>MD Printing and packaging private limited, Plot Number 49&amp;50, Sector-5, SIDCUL, Haridwar, Pin 249403, Uttarakhund</td>
<td>June 16, 2019</td>
<td>4 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>MD160619</td>
<td>55g/30g</td>
<td>1080</td>
</tr>
<tr>
<td>Classic Salted Chips by Haldiram’s</td>
<td>Haldiram Snack Pvt. Ltd., A-11, Sector-68, Noida-201307, Gautam Budh Nagar, UP</td>
<td>July 2, 2019</td>
<td>November 1, 2019</td>
<td>Haldiram's Lajpat Nagar</td>
<td>RAG02814</td>
<td>60.5g/ND</td>
<td>629</td>
</tr>
<tr>
<td>Too Yumm Multigrain Chips Chinese Hot and Sour by RP-Sanjiv Goenka Group</td>
<td>Adiguru Food Processing and Packaging, Plot A/11, Phase-1, New Industrial Estate, Jagatpur, Cuttack-754021, Odisha</td>
<td>April 11, 2019</td>
<td>6 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>M3101</td>
<td>90g/ND</td>
<td>760</td>
</tr>
<tr>
<td>Classic Nut Cracker by Haldiram’s</td>
<td>Haldiram Snack Pvt. Ltd., A-11, Sector 68, Noida-201307, Gautam Budh Nagar, UP</td>
<td>June 27, 2019</td>
<td>5 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>RAF27A</td>
<td>230g/ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aloo Bhuja by Haldiram’s</td>
<td>Haldiram Snack Pvt. Ltd., C-3, Sector 67, Noida-201307, Gautam Budh Nagar, UP</td>
<td>June 21, 2019</td>
<td>6 months from manufacture</td>
<td>Sidhi Vinayak Departmental Store, Jangpura Extension</td>
<td>DAF21B</td>
<td>230g/ND</td>
<td>41.5</td>
</tr>
<tr>
<td>Bingo! Mad Angles Delight Achaari Masti by ITC</td>
<td>Kanchan Metals Pvt. Ltd. Everest house, 19G, 19th Floor, 46C, Jawaharlal Nehru Road, Kolkata - 700071, at plot no.69, 70A, Sector-Ecotech Extension, Greater Noida, Dist-Gautam Buddh Nagar, UP 201306</td>
<td>June 20, 2019</td>
<td>6 months from manufacture</td>
<td>Sidhi Vinayak Departmental Store, Jangpura Extension</td>
<td>DR06</td>
<td>80g/ND</td>
<td>789</td>
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<tr>
<td>Kurkure Masala Munch by PepsiCo</td>
<td>Daawat Foods Ltd., 45 Km stone, GT road, Kamaspur, Distt Sonepat-131021, Haryana</td>
<td>June 21, 2019</td>
<td>4 months from manufacture</td>
<td>Reliance Fresh, Lajpat Nagar IV</td>
<td>DF21061908</td>
<td>50g/30g</td>
<td>892</td>
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<tr>
<td>Sample</td>
<td>Manufacturer</td>
<td>Date of manufacture</td>
<td>Best before/ date of expiry</td>
<td>Source of collection</td>
<td>Batch Number</td>
<td>Pack size/ serving quantity</td>
<td>Declared value</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
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<tr>
<td>Maggi Masala by Nestle</td>
<td>VPO- Nangal Kalan, Industrial Area Tahiwal, Tehsil-Haroli, Uda-174507, Himachal Pradesh</td>
<td>June, 2019</td>
<td>9 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>91586640DA</td>
<td>70g/70g</td>
<td>1172.3</td>
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<tr>
<td>Atta Noodles Chatpata by Patanjali Ayurved</td>
<td>SwasthaAahar Pvt. Ltd., 15km milestone, Delhi-Rohtak National Highway-10, Kharawar, Dist- Rohtak-124021, Haryana</td>
<td>June, 2019</td>
<td>9 months from manufacture</td>
<td>Shri Siddhi Retail, Kalkaji</td>
<td>APCH240619A9</td>
<td>60g/ND</td>
<td>1364</td>
</tr>
<tr>
<td>Ching’s Secret Schewan by Capital Foods</td>
<td>Indo Nissin Foods Pvt Ltd. 91kms, NH-8, Delhi-Jaipur road, Village-Deodhai, Tehsil-Bawal, District-Rewari, Haryana 123401</td>
<td>May 27, 2019</td>
<td>9 months from manufacture</td>
<td>Big Bazaar, Kalkaji</td>
<td>I14795CN06B</td>
<td>60g/60g</td>
<td>452.8</td>
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<tr>
<td>Knorr Classic Thick Tomato Soup by Hindustan Unilever</td>
<td>Hindustan Unilever Limited, At plot A-8/9 MIDC industrial estate, Malegaon, Sinnar, Nashik-422103, Maharashtra</td>
<td>November 30, 2018</td>
<td>12 months from packaging</td>
<td>Reliance Fresh, Lajpat Nagar IV</td>
<td>A5770103</td>
<td>53g/13.25g</td>
<td>4029</td>
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</table>

S: Sodium, TF: Total fat, tf: Trans fat, C: Carbohydrate, ND: Not Declared

### Table 2: Fast foods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Company/manufacturer</th>
<th>Source of collection</th>
<th>Weight (g)</th>
<th>S (mg)</th>
<th>TF (g)</th>
<th>tf (g)</th>
<th>C (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese Whopper Veg by Burger King</td>
<td>Burger King India Pvt. Ltd.</td>
<td>GF &amp; Mezz Flr, E 8, Connaught Place</td>
<td>296</td>
<td>540.5</td>
<td>10.61</td>
<td>0</td>
<td>33.99</td>
</tr>
<tr>
<td>Cheese Whopper Chicken by Burger King</td>
<td>Burger King India Pvt. Ltd.</td>
<td>GF &amp; Mezz Flr, E 8, Connaught Place</td>
<td>287</td>
<td>487.80</td>
<td>10.38</td>
<td>0</td>
<td>21.01</td>
</tr>
<tr>
<td>Veg Zinger (with cheese) by KFC</td>
<td>KFC (Sapphire Foods India Pvt. Ltd.)</td>
<td>6 &amp; 7, Scindia House, Connaught Place</td>
<td>200</td>
<td>600</td>
<td>4</td>
<td>0</td>
<td>38.5</td>
</tr>
<tr>
<td>Chicken Classic Zinger (with cheese) by KFC</td>
<td>KFC (Sapphire Foods India Pvt. Ltd.)</td>
<td>6 &amp; 7, Scindia House, Connaught Place</td>
<td>196</td>
<td>510</td>
<td>10.71</td>
<td>0</td>
<td>21.94</td>
</tr>
<tr>
<td>McVeggie Burger by McDonald’s</td>
<td>McDonald’s (Connaught Plaza Restaurants Pvt. Ltd.)</td>
<td>42, Janpath</td>
<td>169</td>
<td>432.5</td>
<td>8.28</td>
<td>0.06</td>
<td>33.14</td>
</tr>
<tr>
<td>McAloo Tikki Burger by McDonald’s</td>
<td>McDonald’s (Connaught Plaza Restaurants Pvt. Ltd.).</td>
<td>42, Janpath</td>
<td>146</td>
<td>371.9</td>
<td>8.22</td>
<td>0.07</td>
<td>34.25</td>
</tr>
<tr>
<td>McChicken Burger by McDonald’s</td>
<td>McDonald’s (Connaught Plaza Restaurants Pvt. Ltd.)</td>
<td>42, Janpath</td>
<td>173</td>
<td>454.9</td>
<td>8.67</td>
<td>0.12</td>
<td>27.75</td>
</tr>
<tr>
<td>Chicken Maharaja Mac by McDonald’s</td>
<td>McDonald’s (Connaught Plaza Restaurants Pvt. Ltd.)</td>
<td>42, Janpath</td>
<td>290</td>
<td>555.2</td>
<td>11.72</td>
<td>0.07</td>
<td>20.69</td>
</tr>
<tr>
<td>Fries (regular) by Burger King</td>
<td>Burger King India Pvt. Ltd.</td>
<td>GF &amp; Mezz Flr, E 8, Connaught Place</td>
<td>74</td>
<td>270.3</td>
<td>14.32</td>
<td>0</td>
<td>50.95</td>
</tr>
<tr>
<td>Fries (medium) by KFC</td>
<td>KFC (Sapphire Foods India Pvt. Ltd.)</td>
<td>6 &amp; 7, Scindia House, Connaught Place</td>
<td>100</td>
<td>200</td>
<td>14</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Fries (medium) by McDonald’s</td>
<td>McDonald’s (Connaught Plaza Restaurants Pvt. Ltd.)</td>
<td>42, Janpath</td>
<td>109</td>
<td>234.9</td>
<td>15.6</td>
<td>0.09</td>
<td>37.61</td>
</tr>
<tr>
<td>Hot Wings (4 pieces) by KFC</td>
<td>KFC (Sapphire Foods India Pvt. Ltd.)</td>
<td>6 &amp; 7, Scindia House, Connaught Place</td>
<td>155</td>
<td>903.2</td>
<td>20</td>
<td>0</td>
<td>7.10</td>
</tr>
<tr>
<td>Sample</td>
<td>Company/manufacturer</td>
<td>Source of collection</td>
<td>Weight (g)</td>
<td>S (mg)</td>
<td>TF (g)</td>
<td>tf (g)</td>
<td>C (g)</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------</td>
<td>---------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Peppy Paneer cheese burst (regular) by Domino’s</td>
<td>Domino’s Pizza (Jubilant FoodWorks Limited)</td>
<td>M 40, Connaught Circus</td>
<td>272</td>
<td>660.9</td>
<td>6.62</td>
<td>ND</td>
<td>36.47</td>
</tr>
<tr>
<td>Non-veg Supreme (regular) by Domino’s</td>
<td>Domino’s Pizza (Jubilant FoodWorks Limited)</td>
<td>M 40, Connaught Circus</td>
<td>320</td>
<td>560.7</td>
<td>4.75</td>
<td>ND</td>
<td>34.75</td>
</tr>
<tr>
<td>Classic Tomato Margherita (personal) by Pizza Hut</td>
<td>Pizza Hut (Devyani International Ltd.)</td>
<td>Shop No. 58, Janpath, Connaught Place</td>
<td>256</td>
<td>390.6</td>
<td>7.88</td>
<td>&lt;0.1</td>
<td>38.61</td>
</tr>
<tr>
<td>Chicken Supreme (personal) by Pizza Hut</td>
<td>Pizza Hut (Devyani International Ltd.)</td>
<td>Shop No. 58, Janpath, Connaught Place</td>
<td>346.8</td>
<td>495.7</td>
<td>9.69</td>
<td>ND</td>
<td>25.72</td>
</tr>
<tr>
<td>Big Spicy Paneer Wrap by McDonald’s</td>
<td>McDonald’s (Connaught Plaza Restaurants Pvt. Ltd.)</td>
<td>42, Janpath</td>
<td>272</td>
<td>644.8</td>
<td>16.54</td>
<td>0.22</td>
<td>23.16</td>
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<tr>
<td>Paneer Tikka (6 inch) by Subway</td>
<td>Subway (A Unit of Aarbee International LLP)</td>
<td>M-08, Barakhamba road, Connaught place</td>
<td>238</td>
<td>410.1</td>
<td>11.76</td>
<td>ND</td>
<td>16.81</td>
</tr>
<tr>
<td>Chicken Seekh Kabab (6 inch) by Subway</td>
<td>Subway (A Unit of Aarbee International LLP)</td>
<td>M-08, Barakhamba road, Connaught place</td>
<td>238</td>
<td>259.2</td>
<td>6.3</td>
<td>ND</td>
<td>16.81</td>
</tr>
</tbody>
</table>

S: Sodium, TF: Total fat, tf: Trans fat, C: Carbohydrate, ND: Not Declared
### Annexure 2 Details of FAME standard solution

<table>
<thead>
<tr>
<th>Name</th>
<th>Peak</th>
<th>IUPAC Name</th>
<th>Carbon</th>
<th>Retention time (minutes)</th>
</tr>
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<tbody>
<tr>
<td>Internal Standard— glyceryl triundecanoate</td>
<td>1</td>
<td>trans-methyl tetradec-9-enoate</td>
<td>C14:19t</td>
<td>14.94</td>
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<tr>
<td>Myristelaidic acid methyl ester</td>
<td>2</td>
<td>trans-methyl octadec-6-enoate</td>
<td>C18:16t</td>
<td>28.88</td>
</tr>
<tr>
<td>Petroselaidic acid methyl ester</td>
<td>3</td>
<td>trans-methylundec-9-enoate</td>
<td>C18:19t</td>
<td>28.99</td>
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<tr>
<td>Elaidic acid methyl ester</td>
<td>4</td>
<td>trans-methyloctadec-9-enoate</td>
<td>C18:19t</td>
<td>28.99</td>
</tr>
<tr>
<td>Vaccenic acid methyl ester</td>
<td>5</td>
<td>trans-methyloctadec-11-enoate</td>
<td>C18:111t</td>
<td>29.15</td>
</tr>
<tr>
<td>Linoleic acid methyl ester</td>
<td>6</td>
<td>trans,trans-methylundec-9,12-dienoate(50%)</td>
<td>C18:29c,12t</td>
<td>30.94</td>
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<tr>
<td></td>
<td>7</td>
<td>cis,trans-methyl octadeca-9,12-dienoate (20%)</td>
<td>C18:29c, 12t</td>
<td>31.71</td>
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<tr>
<td></td>
<td>8</td>
<td>trans,cis-methylundec-9,12-dienoate (20%)</td>
<td>C18:29c, 12c</td>
<td>31.98</td>
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<tr>
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<td>9</td>
<td>cis,cis-methylundec-9,12-dienoate (10%)</td>
<td>C18:29c,12c</td>
<td>32.42</td>
</tr>
<tr>
<td>Linolenic acid methyl ester</td>
<td>10</td>
<td>trans,trans,trans-methyl octadeca-9,12,15-trienoate(30%)</td>
<td>C18:39t,12t, 15t</td>
<td>33.36</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>trans,trans,cis-methylundeca-9,12,15-trienoate(15%)</td>
<td>C18:39t,12t, 15c</td>
<td>34.23</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>trans,cis,trans-methylundec-9,12,15-trienoate(15%)</td>
<td>C18:39t,12c, 15t</td>
<td>34.35</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>cis,trans,trans-methylundec-9,12,15-trienoate(15%)</td>
<td>C18:39c,12t, 15t</td>
<td>34.65</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>cis,cis,trans-methylundec-9,12,15-trienoate(7%)</td>
<td>C18:39c,12c, 15t</td>
<td>34.77</td>
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<tr>
<td></td>
<td>15</td>
<td>cis,trans,cis-methylundec-9,12,15-trienoate(7%)</td>
<td>C18:39c,12t, 15c</td>
<td>35.43</td>
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<td>16</td>
<td>trans,cis,cis-methylundec-9,12,15-trienoate(7%)</td>
<td>C18:39t,12c, 15c</td>
<td>35.55</td>
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<td>17</td>
<td>cis,cis,cis-methylundec-9,12,15-trienoate(4%)</td>
<td>C18:39c,12c, 15c</td>
<td>36.03</td>
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<tr>
<td>Eicosenoic acid methyl ester</td>
<td>18</td>
<td>trans-methyleicos-11-enoate</td>
<td>C20:111t</td>
<td>35.78</td>
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</tbody>
</table>
**Annexure 3** Chromatograms of standard and few study samples

FAME profile of standard

FAME profile of Peppy Paneer cheese burst (regular) by Dominos’s

FAME profile of Big Spicy Paneer Wrap by McDonald’s

FAME profile of Paneer Tikka (6 inch) by Subway
Annexure 4: Test results -replicate values

<table>
<thead>
<tr>
<th>Sample</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mean</th>
<th>STD</th>
<th>COV</th>
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<th>R3</th>
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<th>STD</th>
<th>COV</th>
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<th>R3</th>
<th>Mean</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mean</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lay’s India’s Magic Masala by PepsiCo</td>
<td>1.989</td>
<td>1.953</td>
<td>1.871</td>
<td>1.94</td>
<td>0.06</td>
<td>3.12</td>
<td>31.74</td>
<td>31.83</td>
<td>33.94</td>
<td>32.50</td>
<td>1.25</td>
<td>3.83</td>
<td>0.212</td>
<td>0.2112</td>
<td>0.2112</td>
<td>0.2112</td>
<td>54.3</td>
<td>48.76</td>
<td>51.53</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lay’s American Style Cream and Onion Flavour by PepsiCo</td>
<td>1.543</td>
<td>1.493</td>
<td>1.627</td>
<td>1.55</td>
<td>0.07</td>
<td>4.36</td>
<td>28.39</td>
<td>28.23</td>
<td>30.96</td>
<td>29.19</td>
<td>1.53</td>
<td>5.25</td>
<td>0.3005</td>
<td>0.237</td>
<td>0.27</td>
<td>0.27</td>
<td>48.29</td>
<td>49.62</td>
<td>48.96</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncle Chipps Spicy Treat by PepsiCo</td>
<td>2.856</td>
<td>2.85</td>
<td>2.718</td>
<td>2.81</td>
<td>0.08</td>
<td>2.78</td>
<td>34.67</td>
<td>35.05</td>
<td>35.39</td>
<td>35.04</td>
<td>0.36</td>
<td>1.03</td>
<td>0.1627</td>
<td>0.1763</td>
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<td>0.17</td>
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R: Replicate, STD: standard deviation, COV: coefficient of variation, NT: Not tested
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