CSE/PML/PR-41/2012

Nutritional Analysis of Junk Food

INVESTIGATORS

Dr. Sapna Johnson

Mr. Ramakant Sahu

Ms. Poornima Saxena

CONSULTANTS

Prof. H. B. Mathur

Prof. H. C. Agarwal

DATE

March, 2012

CENTRE FOR SCIENCE AND ENVIRONMENT

41, Tughlakabad Institutional Area, New Delhi 110062

Website: www.cseindia.org; Email: cse@cseindia.org

POLLUTION MONITORING LABORATORY

India Habitat Centre, Core 6A, Fourth Floor

Lodhi Road, New Delhi – 110003

Contents

S. No.	Topic	Page No.
1.	About the CSE Laboratory	3
2.	Introduction & Origin of the Study	3
3.	Major Nutrients in Diet and their Recommendations	4
4.	What is Junk Food?	4
5.	Junk Food Market in India	5
6.	Health Implications of Consumption of Junk Food	5
7.	Regulations on Junk Food	7
8.	Materials and Methods	8
	8.1 Sampling Methodology	
	8.2 Equipments	
	8.3 Standards	
	8.4 Reagents	
	8.5 Salt	
	8.6 Total Carbohydrates	
	8.7 Total Fats	
	8.8 Trans Fats	
	8.9 Chromatography	
9.	Results and Discussion	13
10.	Conclusions	15
Annexures- I-II		17
Chromatograms		21
	A.Standard mixture of trans- Fatty Acid Methyl Esters(FAME)	
	B.Chromatogram of French Fries- KFC	
	C.Chromatogram of Aaloo Bhujia -Haldiram	
References		24

1. About the CSE Laboratory

The Centre for Science and Environment (CSE), a non-governmental organization based in New Delhi, has set up the Pollution Monitoring Laboratory (PML) to monitor environmental pollution and food contamination. PML is an ISO 9001:2008 certified laboratory accredited by SWISO, CH-5610, Wohlen, Switzerland, conducting Pollution Monitoring and Scientific Studies on Environmental and food Samples. The Lab has qualified and experienced staff who exercise Analytical Quality Control (AQC) and follow Good Laboratory Practices. It is equipped with state-of-art equipments for monitoring and analysis of air, water and food contamination, including Gas Chromatograph with Mass Spectrometer (GC-MS), Gas Chromatograph (GC) with ECD, NPD, FID and other detectors, High Performance Liquid Chromatograph (HPLC), Atomic Absorption Spectrometer, UV-VIS Spectrophotometer, Mercury Analyzer, Respirable Dust Sampler etc. Its main aim is to undertake scientific studies to generate public awareness about food contamination and environmental pollution. It provides scientific services at nominal cost to communities that cannot obtain scientific evidence against polluters in their area. The lab and its work is directed to use science to achieve environmentally sound and socially relevant public policy.

2. Introduction & Origin of the Study

Junk food term refers to fast foods which are easy to make and quick to consume. They are zero in nutritional value and often high in fat, salt, sugar, and/or calories. Common junk foods include salted snack foods, fried fast food, and carbonated drinks. Junk Food has become a major problem and many countries are taking action – banning junk food advertising in childrens programmes, removing it from schools and even imposing a fat tax. Many junk foods also have trans fats. Trans fats behave like saturated fats when they get in the body. They clog up the human arteries and cause plaque to build up contributing to heart disease and stroke symptoms.

A 2008 report suggests that mothers who eat junk food while pregnant or breast-feeding have children who are more prone to obesity. The children are also more prone to diabetes, raised cholesterol, and high blood fat.[1]

A recent report suggests that pregnant mothers who eat high sugar and high fat diets have babies who are likely to become junk food junkies themselves, this happens because the high fat and high sugar diet leads to changes in the fetal brain's reward pathway, altering food preferences.[2]

Salt, sugar and fat are items that need to be regulated. Junk Food is not standardized under Indian regulations. It comes under the category of food which is only expected to declare their composition or nature of food and comply with general regulations under the Food Safety and Standards Act in India. As there are no reports of nutritional analysis in Junk Food in India, Pollution Monitoring laboratory undertook a study on Nutritional analysis of Junk Food. A total of 23 junk food samples

from seven different food categories like potato chips (4), snacks (2), instant noodles (2), burgers (6), pizzas (3), french fries (2) and carbonated drinks (2) were collected from Delhi Outlets and analysed for salt content, total carbohydrate, total fat and trans fat with a widely and internationally used methodology of Association of Official Analytical Chemists (AOAC).

3. Major nutrients in diet and recommended dietary guidelines

Carbohydrate, protein, and fat are the main sources of calories in the diet. The nutrient requirements vary with age, gender, physical activity and physiological status. It can be easily achieved through a blend of the basic food groups. Most foods and beverages contain combinations of these macronutrients in varying amounts. According to the Dietary Guidelines for Indians (National Instititute for Nutrition, 2011) a balanced diet should provide 50-60% of calories from carbohydrates, 10-12% from proteins and 20-25% from fats. Though NIN reccommends a maximum of two per cent of total calories to come from trans fats, the reccomendations of WHO is one per cent.

Balanced diet should provide other non nutrients such as dietary fibre, antioxidants and phytochemicals which bestow positive health benefits. Antioxidants such as vtamin C and E, beta carotene, riboflavin and selenium protect the human body from free radical damage other phytochemicals such as polyphenols, flavones etc. also afford protection against oxidant damage.

Table 1. Recommended Dietary Guidelines for Indians

Carbohydrate	Free Sugars	Protein	Salt	Fat
50-60%	<10%	10-15%	6 g per day	20-30%

Source: National Institute for Nutrition (2011)

4. What is Junk Food?

Junk Food is defined as "any food, which is low in essential nutrients and high in everything else—in particular calories and sodium. Junk foods contain little or no proteins, vitamins or minerals but are rich in salt, sugar, fats and are high in energy (calories). Highly salted like chips, high in refined carbohydrates (empty calories) like candy, soft drinks and high in saturated fats like cake and chocolates."[3]

Major Contents in Junk Food

Carbohydrates: The free sugar content has generally been found to be high in carbonated beverages and desserts offered by the fast food chains. The desserts and shakes offered by KFC and McDonalds invariably contain very high sugar content (Official websites' information).

Fats: Junk foods like potato chips, burgers, pizza, fried chicken etc. have high fats content. The link between saturated fat and trans-fat and increased risk of heart disease is well established. There is

also evidence that the risk of type 2 diabetes is directly associated with consumption of saturated fat and trans-fat and inversely associated with polyunsaturated fat from vegetable sources.

Trans Fat: It is the common name for unsaturated fat with *trans*-isomer (E-isomer) fatty acid(s). Trans fatty acids (TFA) are the geometrical isomers of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids having at least one non-conjugated,(interrupted by at least one methylene group), carbon-carbon double bond in the *trans* configuration rather than the more common *cis* configuration. The *trans* configuration has an effect on the functional and physiochemical properties of these fatty acids which in turn effects their metabolism in humans. High levels of TFA are a public health concern due to some evidence associating TFA with coronary heart disease [4]. There is also evidence that the risk of type 2 diabetes is directly associated with consumption of saturated fat and trans-fat and inversely associated with polyunsaturated fat from vegetable sources.

Salt: The amount of dietary salt consumed is an important determinant of blood pressure levels and overall cardiovascular risk. Salt intake should not be more than 6 g per person per day. WHO recommends salt intake of less than 5 grams per person per day for the prevention of cardiovascular disease. WHO estimates that decreasing dietary salt intake from the current 9-12 grams per day to the recommended level of 5 grams per day would have a "major impact on reducing blood pressure and cardiovascular disease."

5. Junk Food Market in India

According to the National Restaurant Association of India (NRAI) 2010 report, the fast food industry in India is currently estimated to be between Rs 6750- Rs 8000 crore, growing at a compound annual growth rate of 35-40 per cent. A major chunk of these markets is ruled by global players like McDonald's, Yum! Brands (Kentucky Fried Chicken, Pizza Hut), Domino's, Subway, Taco Bell, Coca Cola and Barista but domestic players are not lagging behind. Nirula's, Pizza Corner, Coffee Day Group, *Haldiram's*, *Bikanervala* capture a fair share of the fast food segment.

Junk foods are high visibility products: easily available almost everywhere, extensively advertised through every media, these foods find a key target group among children. Their manufacturers and sellers also take recourse to attractive packaging and addition of food additives and colors to enhance flavor, texture, appearance and shelf life.

The Integrated Disease Surveillance Report, which found that rural India was fast catching up with modern diseases, also noted that people were eating less fruits and vegetables and more fast food. It found that across the seven states in which it conducted its study, in urban and rural areas, there was a growing and substantial percentage of people eating fast food.[5]

6. Health Implications of Consumption of Junk Food

Junk food is popular because it is tasty. But it is unhealthy. It is low in fiber, it is high in fat, high in sugar in liquid form. Studies have shown that despite being unhealthy, junk food induces gorging that leads to obesity.

The study published in 2009 in *The Journal of Clinical Investigation*, showed that the fat from fatty acids affected the brain. The study by Deborah Clegg, Assistant Professor of Internal Medicine at UT Southwestern, suggested that when we eat something high in fat, the brain gets 'hit' with the fatty acids, and the fat molecules cause the brain to send messages to the body's cells, warning them to ignore the appetite-suppressing signals from leptin and insulin, hormones that are involved in weight regulation. Since the body does not get the signal that it is satiated it leads to over eating [6].

Another study published in *Neurology* shows that eating too much junk food or food rich in trans fats can shrink the brain similar to that associated with the Alzheimer's disease. The study also showed that if the diet was rich in vitamin B, C, D and E and high in omega-3 fatty acids, the brain benefitted from it. Though this study was done on a set of people above 65 years, the impact of trans fatty acids begins to take place at the fetal stage[7].

Adverse fatty acid supplies during fetal and child development can alter fatty acid composition of membrane phospholipids (these are the lipid/ fatty cells in the membrane- and has an impact on the fattiness of the child) and storage triglycerides (they store unused calories in humans, they are an important measure for the health of the heart, the lower the better). This can lead to disruption in cellular environments, structure and function.

Avoiding hydrogenated fats is also important for the growing brains of children as it can lead to attention deficit hyperactive disorder (this is a psychiatric disorder mainly in children where in there is a co existence of both attention problem and hyperactivity).

Studies also show that too much of junk food alters the chemistry of the brain and are addictive like cocaine. High-fructose corn syrup (HFCS), monosodium glutamate (MSG), hydrogenated oils, refined salt, and various other chemical preservatives found in processed junk food do the same thing to a person's brain as cocaine does[8].

Another study conducted by researchers at both the University of Texas in Austin (UT) and the Oregon Research Institute found that prolonged consumption of junk foods results in reduced activity in the striatum, a section of the forebrain that registers reward. In other words, just like with drugs, those addicted to junk food require ever-increasing amounts of it to get the same "high." In a correlative study, researchers identified a similarity in dopamine production levels between drug addicts and junk food addicts. Addiction to either one essentially causes the brain receptors that receive dopamine signals to lose their responsiveness. As a result, addicts require increasing amounts of the addictive substance to receive the same level of satisfaction.

Studies have also shown that as early as the age of 30, arteries could begin clogging and lay the groundwork for future heart attacks. What children eat from puberty affects their risks of prostate and breast cancer. Osteoporosis and hypertension are other diseases that appear to have their earliest roots in childhood when lifelong eating habits are being formed[9].

Children are especially vulnerable. Poor diets can slow growth, decay new teeth, promote obesity and sow the seeds of infirmity and debilitating disease that ultimately lead to incurable disease. Food containing low nutrition value tends to reduce the IQ level of children.

Doctors at Harvard University and the University of Murcia, Spain, have found that junk food can make young men infertile, even if they're physically fit and in good health. Those who ate high amounts of junk food, such as fried foods, processed red meats, potato chips were found to have sperm of poorer quality than their counterparts who ate a nutritious diet high in whole grains, vegetables and fish. In particular, participants who consumed high levels of trans fats, naturally found in fried foods, had the worst quality sperm – that is, sperm that was least likely to survive the journey to fertilize an egg. This was the case, even for men who were a healthy weight and exercised[10.]

7. Regulations on junk food

WHO: On January 21, 2011, WHO formally issued a recommendation asking for a ban on junk food in schools and playgrounds in order to promote healthy diet and tackle child obesity"[11]. Settings where children gather should be free from all forms of marketing of foods high in saturated fats, trans-fatty acids, free sugars or salt," said WHO. "Such settings include, but are not limited to, nurseries, schools, school grounds and pre-school centers, playgrounds, family and child clinics and pediatric services and during any sporting and cultural activities that are held on these premises," it added.

Britain: has the biggest obesity problem in Europe with nearly 26 per cent of the population being obese[12.]

The country banned junk food in schools in 2005[13]. A ban on junk food ads during television programs aimed at children below 16 years came into force in August 2008[14.]

UK consumer advocates are now calling for their government to explore provisions allowed under the new EU regulation on food information to make sure that the 'traffic light labelling' is used on all food products[15].

Scotland: In March 2012 proposed to ban advertisements of junk foods in television shows aired before 9 pm[16].

US: In 2010, the US government proposed a law to ban junk food in schools; a decision that still remains to be taken[17]. Meanwhile elementary schools in Arizona, Georgia, Kentucky, Louisiana, Maryland, Mississippi, Nebraska, New Jersey, New York, and West Virginia have already banned the sale of junk food in schools until at least after lunch[18].

Mexico: Has banned junk food in all of its public, private and elementary schools in May 2010. This was a part of their nation wide anti-obesity campaign[19].

United Arab Emirates: Banned junk food and soft drinks in all its schools in Abu Dhabi in 2010[20].

Canada: The Ontario state government banned candy, chocolate, fries, pop and energy drinks in school premises in September 2010[21].

Denmark: Imposed a fat tax on junk food in October 2011. The surcharge will be levied on food items like butter, milk, cheese, pizza, meat, bacon, ice cream and processed food if they contain more than

2.3 per cent saturated fat. According to the new taxes imposed, the consumers will have to shell out about 20 per cent more for a packet of butter and a little more than half a Krone (1 Krone = 01.7 US $) for a packet of chips[22]. The government hopes that by imposing such taxes on junk food they will be able to fund the increased health care costs of treating the obese population. Also, high prices will dissuade people from eating food high on saturated fat[23].

Hungary: In September 2011 had imposed tax on food that was high in sugar, fat, carbohydrates and salt. A tax was also imposed on carbonated beverages, alcohol and drinks with high caffeine levels such as energy drinks[24].

Other countries in Europe too have experimented with similar taxes. Switzerland and Austria, along with Denmark have already banned trans-fats, while Finland and Romania are considering fat taxes[25]. Brazil, Mexico and Taiwan too are working towards getting a fat tax in place.

India: According to FSSAI, junk food is not defined, but instead calls within the category of proprietary food – which is food not standardized under regulations. This category of food is only expected to declare their composition or nature of food and comply with general regulations under the food act.

8. Materials and Methods

8.1. Sampling methodology

A total of 23 junk food samples from seven different food categories were collected from Delhi Outlets and analysed for Salt, Total carbohydrates, Total Fat and Trans Fat content. One sample of potato chips was tested just for Total Fat and Trans fat content. The range of food categories tested in the survey included potato chips (4), Indian snacks (2), Instant noodles (2), Burgers (6), Pizzas (3), French Fries (2) and carbonated drinks (2) samples with a widely and internationally used methodology of Association of Official Analytical Chemists (AOAC). Details of the samples are given in Annexure I. Each sample was analysed in duplicate.

8.2. Equipments

- Gas Chromatograph: Thermoquest-Trace GC equipped with Flame Ionization Detector with advanced software (Chromcard-32 bit Ver 1.06 October 98).
- Capillary column: HP - 88 (100 m x 0.25 mm i.d x 0.20 μm).
- GC conditions: Oven temperature programme - Initial temperature 140 ^{0}C, hold time of 1.0 minutes; ramp of 10 ^{0}C/min upto 180 ^{0}C, hold time of 1.0 minutes; ramp of 1.0 ^{0}C/min upto final temperature 230 ^{0}C; hold time 4.0 minutes. Total run time 60 minutes. Injector port 250 ^{0}C; Detector 285 ^{0}C; The gas flow rates used were 0.5 mL/min carrier gas (Nitrogen), 15 mL/min makeup gas (Nitrogen) and 15 and 350 mL/min of Hydrogen and Air respectively.
- Analytical balance: Weighing to $\pm$ 0.0001g

- Vortex mixer
- Sonicator
- Centrifuge of Remi equipments
- Oven, Hot plate, Ultracentrifuge, Rotary evaporator (Buchi type) etc.
- Nitrogen evaporator with 40 $\pm$ 5 ^{0}C water bath
- Vials
- Burette - Class A
- Pippetes
- A 10-µl syringe from Hamilton Co. was employed for injection

8.3. Standards

The *trans* and *cis* fatty acid methyl ester standards of known composition; and triglyceride internal standard solution - C11:0 – triundecanoin available from Supelco, Inc were used to prepare mixed *trans* - Fatty Acid Methyl Ester (*t* - FAME) standards solution Table 2.

8.4. Reagents

All the reagents and solvents used were of AR grade.

- Boron trifluoride reagent (7% BF_3 in methanol, made from commercially available 14% BF_3 solution)
- Hydrochloric acid – 12 M and 8.3 M
- Pyrogallic acid
- Diethyl ether
- Ethanol
- Petroleum ether
- Chloroform
- n-Hexane
- Toluene
- Sodium sulfate
- Ferric Alum indicator
- $KMnO_4$
- Silver Nitrate
- Nitric acid
- Ammonium Thiocyanate
- Anthrone Reagent
- Glucose standard

8.5. Salt (Chlorine as NaCl) - The Association of Official Analytical Chemists International (AOAC) method (937.09) was used for determination of Salt (Chlorine as Sodium Chloride). The sample was treated with $AgNO_3$ then wet washed and then excess $AgNO_3$ is back titrated with NH_4SCN solution employing ferric ammonium sulfate solution as indicator. The completion of reaction is indicated by light brown colored, ferric isocyanate FeSCN ++, indicating the end point

Approximately 2.0 g of finely comminuted and thoroughly mixed sample was taken into an Erlenmeyer flask. 25.0 mL of 0.1 N $AgNO_3$ solution was added and flask was swirled until the sample and solution were in intimate contact, and then 20.0 mL of conc. HNO_3 was added. Sufficient boiling chips were added and sample boiled until digested. Approximately 50 mL of water was added and boiled for 5 minutes, cooled to room temperature in the fume hood. 5 mL of the ferric alum indicator were added and swirled. The excess $AgNO_3$ was titrated with NH_4SCN solution to a permanent, light brown, end point.

8.6. Total Carbohydrate

Total Carbohydrates in food were estimated by the Anthrone method which is a simple colorimetric method. Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone reagent (9,10-dihydro-9-ozoanthracene) a green coloured product with an absorption maximum at 630 nm.

100 mg of finely comminuted and thoroughly mixed sample was taken into a boiling tube and was hydrolysed by keeping it in a boiling water bath for three hours with 5 mL of 2.5N HCl. The hydrolysed sample was then cooled to room temperature and then neutralised with solid sodium carbonate until the effervescence ceased. The volume was made to 100 mL and centrifuged and the supernatant liquid was collected. From this appropriate aliquots were taken for analysis. The calibration standards were prepared using the working standard (100 ppm glucose). '0' served as blank. The volume was made to 2.0 mL in all the tubes including the sample tubes by adding distilled water. Then 8.0 mL of anthrone reagent was added and heated for eight minutes in a boiling water bath, then cooled rapidly and finally the absorbance was recorded at 630 nm (Clegg K.M. 1956).

8.7. Total Fat: The Association of Official Analytical Chemists International (AOAC) method (922.06) was used for the total fat analysis. Acid hydrolysis of food followed by the liquid - liquid extraction method with combination of ethyl ether and petroleum ether, finally the total fat content was determined by gravimetric method.

2.0 g sample was taken in a conical flask and 2.0 mL of ethanol was added and stirred well. 10.0 mL HCl (25 + 11) was added and mixed well and then the flask was heated in water bath maintained at 70-80 ^{0}C for 40 minutes. During this period the contents of the flask were stirred 3 times. 10.0 mL ethanol was added and cooled to room temperature. The mixture was transferred to separatory funnel. Conical flask was rinsed into separatory funnel with 25 mL ether added in three portions. The separatory funnel was shaken vigorously for 1.0 minute. 25.0 mL petroleum ether was added and

again shaken vigorously for 1.0 minute. The two layers were allowed to separate. The upper layer of ether-fat solution was collected in a flask. The separatory funnel was rinsed into flask with 15 mL 1:1 diethyl ether-petroleum ether mixture. Volume was reduced to about 5 mL by slowly evaporating the ethers in water bath and was transferred to a dried and pre-weighed beaker. The flask was rinsed twice with 1:1 mixture of diethyl ether and petroleum ether. The ethers were slowly evaporated in water bath. Then the fat was dried in an oven at 100 ^{0}C and allowed to stand in air till weight was found to be constant. Finally the beaker with fat was weighed.

8.8. Trans Fats

The Association of Official Analytical Chemists International (AOAC) method (996.06) was used for the trans fat analysis. Acid hydrolysis for most foods, acid and alkaline hydrolysis for food containing cheese, followed by the liquid - liquid extraction method with combination of ethyl ether and petroleum ether. Fat is extracted into ether, then methylated to Fatty Acid Methyl Esters (FAMEs) using BF_3 in methanol. FAMEs are quantitatively measured by capillary gas Chromatography against C11:0-triundecanoin; internal standard. Trans fats were expressed as sum of individual fatty acids expressed as triglyceride equivalents.

a. Preparation of Fatty Acid Methyl Ester

(i) Foods excluding dairy products and cheese: Finely ground and homogenized sample was accurately weighed (containing approximately 100 – 200 mg fat) in to labelled flask. Approximately 100 mg pyrogallic acid, 2.0 mL of triundecanion internal standard, 2.0 mL of ethanol and few boiling chips were added and mixed well until entire product was dispersed. Then 10.0 mL of 8.3 M HCl was added and mixed well. Flask was placed in water bath maintained at 70 – 80 ^{0}C temperature for 40 minutes. During this period the flask was agitated 8 – 10 times.

(ii) Foods containing cheese: Finely ground and homogenized sample was accurately weighed (containing approximately 100 – 200 mg fat) in to labelled flask. Approximately 100 mg pyrogallic acid, 2.0 mL of triundecanion internal standard, 2.0 mL of ethanol and few boiling chips were added and mixed well until entire product was dispersed. 4.0 mL of H_2O was added and mixed well, then 2.0 mL of 58% NH_4OH was added and mixed well. Flask was placed in water bath maintained at 70 – 80 ^{0}C temperature for 20 minutes. During this period the flask was agitated 2 – 3 times. Then 10.0 mL of 12M HCL was added into the flask and was placed into steam bath and maintained for 20 minutes. During this period the flask was agitated 2 – 3 times.

b. Extraction of Fat: After digestion flask was removed from water bath and allowed to cool to room temperature. Approximately 25 mL of ethanol was added to the flask and mixed gently. Then 25 mL of diethyl ether was added into the flask and stoppered and shaked for 5 minutes. Stopper was rinsed into flask with 1:1 diethyl ether - petroleum ether mixture. The contents of the flask were transferred into separatory funnel and 25 mL of petroleum ether was added to it and shaken for 5 minutes and the contents were allowed to separate. The upper layer of ether was collected in a beaker and was slowly

evaporated on water bath using nitrogen stream to aid the evaporation. The residue remaining in beaker contains extracted fat.

Table 2. Mixed *Trans* Fatty Acid Methyl Ester (*t*- FAME) Standard Solution

S. No	Trivial Name	IUPAC Name	Carbon Number	Retention time (minutes)
1	Internal Standard - Triundecanoin methyl ester			17.75
2	Myristelaidic acid methyl ester	*trans*-methyl tetradec-9-enoate	C14: 1 9*t*	22.92
3	Petroselaidic acid methyl ester	*trans*-methyl octadec-6-enoate	C18: 1 6*t*	33.04
4	Elaidic acid methyl ester	*trans*-methyl octadec-9-enoate	C18: 1 9*t*	33.17
5	Vaccenic Acid methyl ester	*trans*-methyl octadec-11-enoate	C18: 1 11*t*	33.31
6	Linoleic acid methyl ester	*trans*, *trans*-methyl octadeca-9,12-dienoate (50%)	C18: 2 9*t*, 12*t*	35.32
7		*cis, trans*-methyl octadeca-9,12-dienoate (20%)	C18: 2 9c, 12*t*	36.12
8		*trans, cis*-methyl octadeca-9,12-dienoate (20%)	C18: 2 9*t*, *12c*	36.42
9		*cis, cis*-methyl octadeca-9,12-dienoate (10%)	C18: 2 9*c*,12*c*	36.86
10	Linolenic acid methyl ester	*trans, trans, trans*-methyl octadeca-9,12,15-trienoate (30%)	C18:3 9*t*,12*t*,15*t*	37.86
11		*trans, trans, cis*-methyl octadeca-9,12,15-trienoate (15%)	C18:3 9*t*,12*t*,15c	38.78
12		*trans, cis, trans*-methyl octadeca-9,12,15-trienoate (15%)	C18:3 9*t*, 12c,15*t*	38.92
13		*cis, trans, trans*-methyl octadeca-9,12,15-trienoate (15%)	C18:3 *9*c, 12*t*, 15*t*	39.21
14		*cis, cis, trans*-methyl octadeca-9,12,15-trienoate (7%)	C18:3 *9c, 12c,* 15*t*	39.37
15		*cis, trans, cis*-methyl octadeca-9,12,15-trienoate (7%)	C18:3 *9c,12t,15c*	40.02
16		*trans, cis, cis*-methyl octadeca-9,12,15-trienoate (7%)	C18:3 *9t, 12c, 15c*	40.15
17		*cis, cis, cis*-methyl octadeca-9,12,15-trienoate (4%)	C18:3 9*c*, 12*c*,15c	40.64
18	Eicosenoic acid methyl ester	*trans*-methyl eicos-11-enoate	C20:1 11*t*	40.20

c. Methylation: Extracted fat was dissolved in 2-3 mL chloroform and 2-3 mL diethyl ether. The mixture was transferred into glass vial and evaporated to dryness in water bath maintained at 40 ^{0}C under nitrogen stream then 2.0 mL of 7% BF_3 reagent and 1.0 mL of toluene were added to it. The glass vial was sealed with screw cap top containing teflon and then heated in oven for 45 minutes at 100 ^{0}C. During this period the vial was shaken gently 4 - 5 times. Vial was cooled to room temperature and then 5.0 mL H_2O, 1.0 mL hexane and approximately 1.0 g Na_2SO_4 were added. Vial was capped and shaken for 1.0 minute then layers were allowed to separate. Top layer was transferred to another vial containing 1.0 g Na_2SO_4. Top layer contains fatty acid methyl ester (FAME) including FAME of triglyceride internal standard.

8.9. Chromatography

Obtained relative retention times (vs FAME of triglyceride internal standard solution) and response factors of individual FAMEs by GC analysis of individual FAME standard solutions and mixed FAME standard solutions.

Injected 2µl each of individual FAME standard solutions and 2µl each of mixed FAMEs standard solution. Used mixed *trans* FAME standard solutions to optimize chromatographic response before

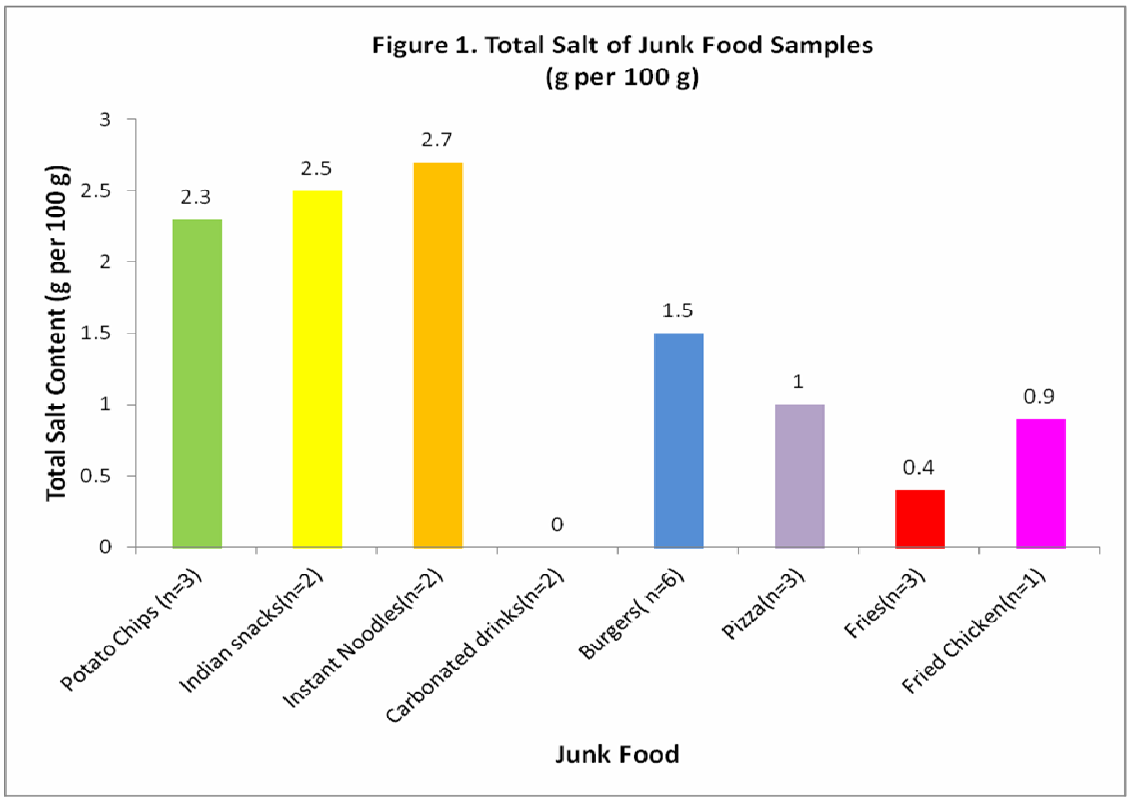
injecting the test solution. Injected 2 µl of test solution into GC column. Trans fats were expressed as sum of individual fatty acids expressed as triglyceride equivalents.

9. Results and Discussion

Twenty three (n=23) junk food samples comprising – Potato Chips (4), Indian Snacks (2) , Instant noodles (2), Carbonated drinks (2), Burgers (6), Pizza (3), Fries (3) and fried Chicken (1) were purchased from different outlets and analyzed in duplicate. Twenty three (n=23) samples were tested for Salt, Total Carbohydrate, Total fats and Trans fats. One sample (Lays, American Style Cream & Onion Flavour) was only tested for fats and trans fats. AOAC methodology was used and results are provided in Annexure II.

Salt: The National Institute of Nutrition(NIN) 2010 dietary guidelines recommend restricted salt consumption and reducing salt intake to the minimum. The NIN recommends that the daily intake of salt be reduced to 6 g per person per day. WHO reccommends 5 g per person per day.

Salt Content in the 22 junk food samples in g per 100 g of sample was in the range 0.2 to 4.2. The highest salt content was found in Instant noodles; Maggi Masala 4.2 g and Top ramen Noodles 3.2 g. Salt content in Potato Chips was 1.2-3.5 g per 100 g. Average salt content in vegetarian burgers (3) was 1.7 g and in non-vegetarian burgers (3) 1.5 g per 100 g. Average salt content in Pizza (3) was 1.0 g; in fries (3) 0.4 g and and fried chicken (1) was 0.9 g per 100 g (see Figure 1).



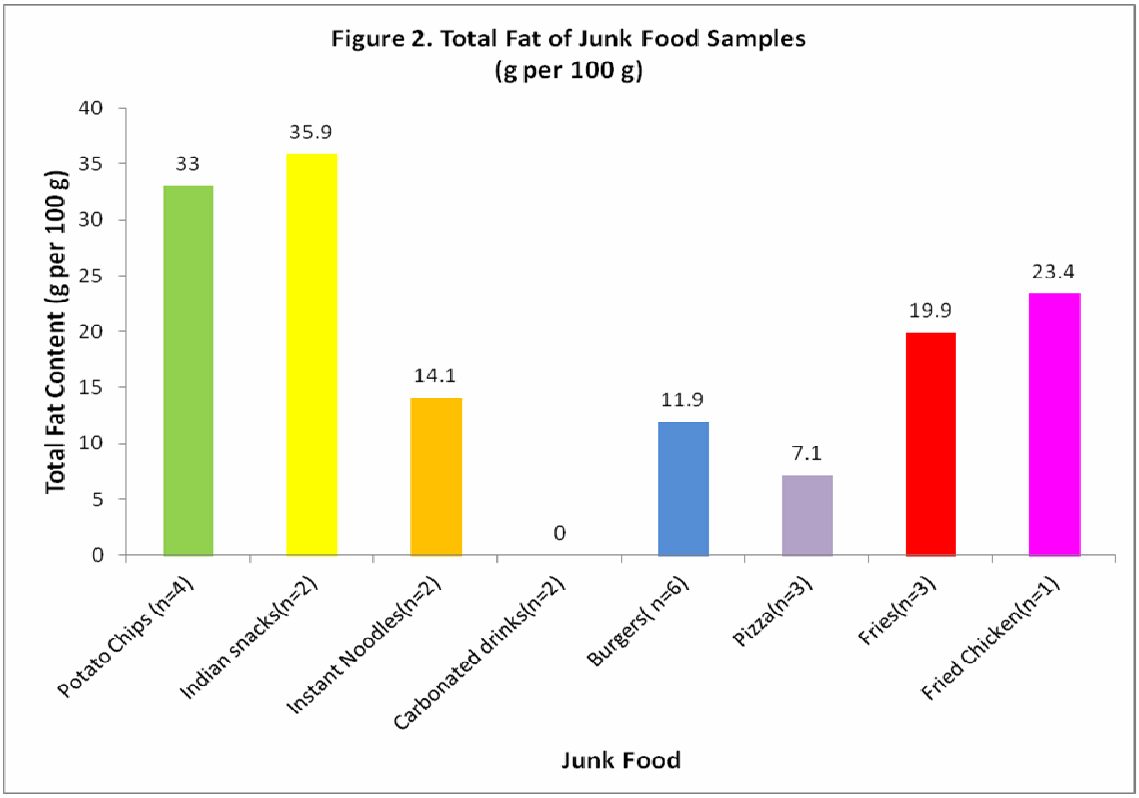
Carbohydrate: NIN reccommends 50-60% of the total energy intake from carbohydrates. Total carbohydrate content in g per 100 g of sample in Potato chips (3) was 57.5 g; in Indian snacks (1 sample each of Aaloo bhujia and Kurkure masala munch) 49.9 g; in instant noodles (2) 71.6 g and in carbonated drinks (2) 14.4 g. Carbohydrate content in Vegetarian Burgers was 43.4 g and in Non-vegetarian Burgers was 32.9 g per 100 g of sample. Pizza (3) had 50.3 g of carbohydrates and Fries (3) and fried chicken (1) had 56.5 g and 14 g per 100 g, respectively.

Highest level of carbohydrate was detected in Top Ramen Noodles at 73.3 g per 100 g of sample

Fats: The Indian dietary guidelines (NIN, 2010) recommends that 15-30 percent of calories should be from total fat with not more than 10 percent from saturated fats; trans fats should contribute one or 2 per cent.

Total fat: Levels of total fat in 23 junk food samples ranged from a low of 7.1 g per 100 g in Pizzas (3) to a high of 35.9 g per 100 g in Indian Snacks (Aaloo bhujia and Kurkure masala munch). Total fat content in g per 100 g of sample was: Potato chips (4) 33.0 g, Indian snacks (2) 35.9 g, Instant noodles (2) 14.1 g, Burgers (6) 11.9 g, Pizza (3) 7.1g, Fries (3) 19.9 g and Fried chicken (1) 23.4 g. (see Figure 2)

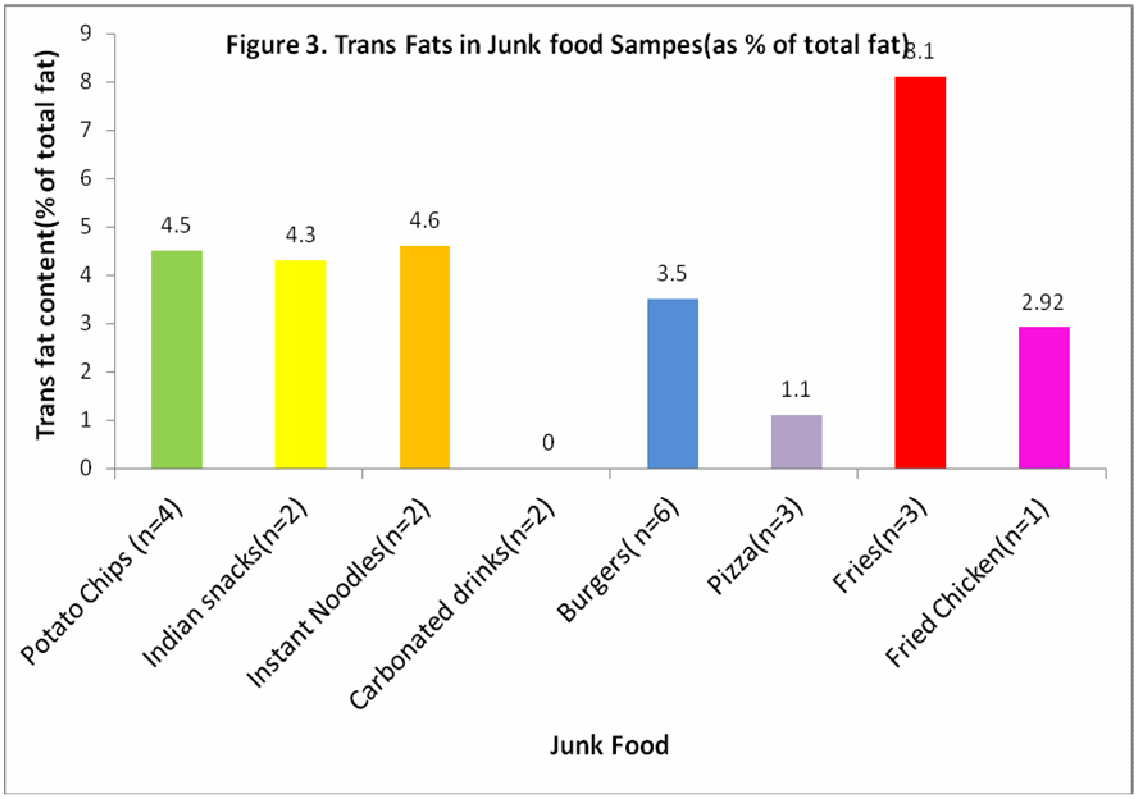
Highest level of total fat was found in Indian Snack (Haldiram's Aaloo bhujia) 37.8g per 100 g of the sample.



Trans fats: The WHO current recommendation on intake of trans fats is that it should be less than 1% of daily energy intake.

Maximum amount of trans fats was found in Fries (3) at 1.6 g per 100 g sample (~8.1% trans fats as percent of total fat). Trans fats in Instant noodles (2) was 0.6 g per 100 g sample (~4.6% trans fats as percent of total fat); in Indian snacks 1.6 g per 100 g sample (~4.3% Trans fats as percent of total fat); in Burgers (6) 0.4 g per 100 g sample (~3.5% trans fats as percent of total fat); in Fried chicken 0.7 g per 100 g sample (~2.9% Trans fats as percent of total fat); in Potato chips it was 1.5 g per 100 g sample (~4.5 % trans fats as percent of total fat). Least amount of trans fats were found in Pizzas at 0.1 g per 100 g sample (~1.1 % trans fats as percent of total fat) (see Figure 3).

Highest levels of trans fats were found in KFC's French fries 1.7 g per 100 g (~9.2% trans fats as percent of total fat).



10. Conclusions

The results of the study indicate that junk food contain high levels of sugars, salt and trans fats. High levels of trans fats are a public health concern due to its assiciation with Chronic heart diseases. The trans fat content was highest in French fries (8.1% of total fat) followed by instant noodles (4.6% of total fat) and potato chips (4.5% of total fat). There should be regulations to reduce trans fats in junk foods and its should be properly labeled.

Similarly, high intake of salt is associated with hypertension. The salt content was highest in Instant noodles (3.7 g per 100 g of sample). Consumption of a packet of instant noodles, therefore, will cover about half of the daily salt quota. Salt content was not given by companies on the label. There should an all India action plan to reduce salt in diets and junk food companies should be asked to label salt quantity of their package.

The provision of nutritional information is currently not a legal requirement unless a claim is made. None of the takeaway foods like pizzas burgers, fries,and potato chips provide nutritional information on the product packs. The absence of nutritional data per/100g makes comparisons between products difficult. There should be mandatory labeling, at least for serving size , trans fats, saturated fats, sugars and salt along with already mandatory labeling nutritional information for all processed foods including takeaway foods.

As the the junk food industry targets children, it is important to ban junk food from schools and places where children have easy access to these foods. Government should also start awareness campaign to increase awareness of consmers about the ill health effects of regularly consuming junk foods.

Annexure I-Details of samples of junk foods

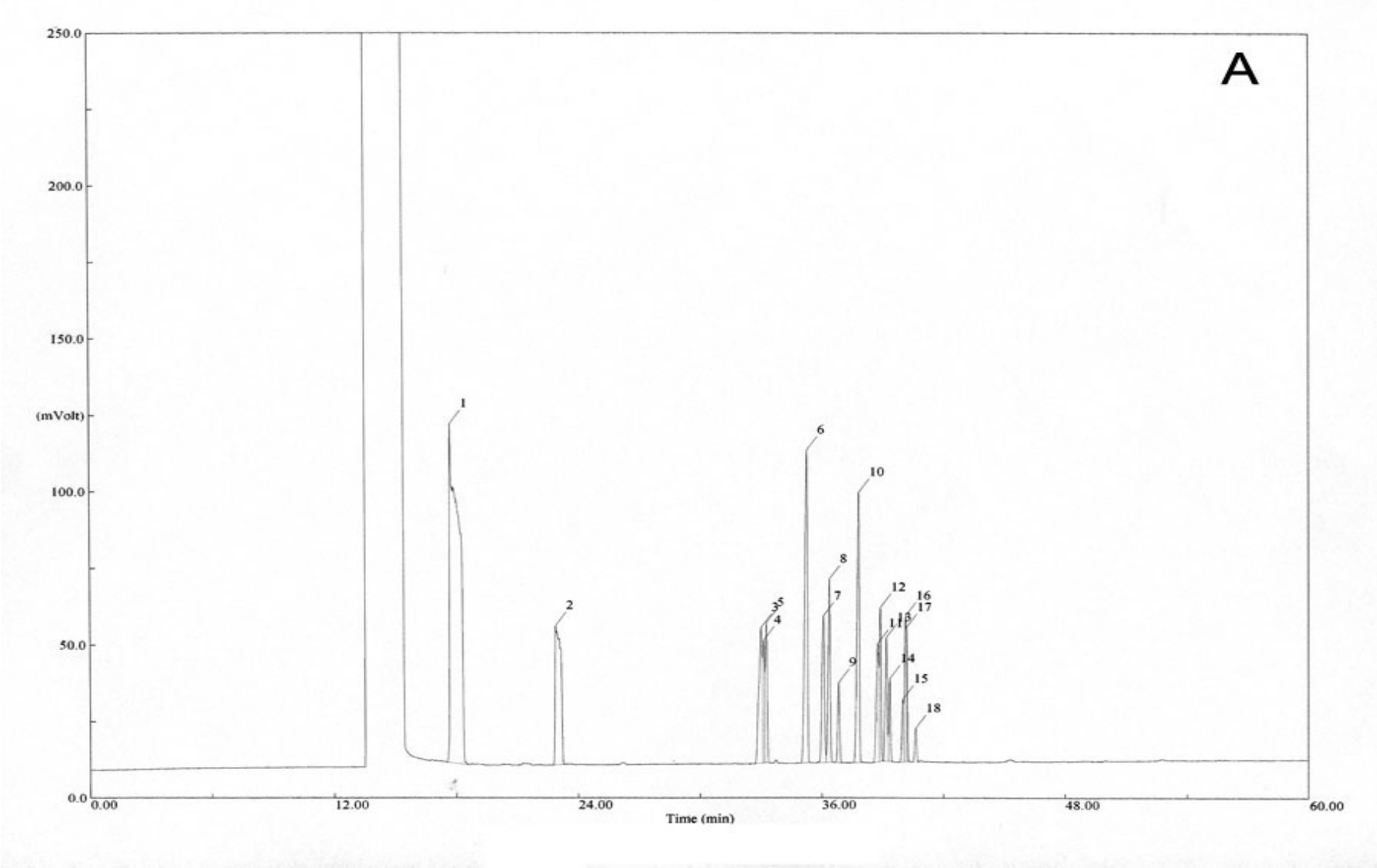
S. No.	Brand	Manufacturer	Date of Manufacture	Expiry	Reported Nutritional Information (Per 100 g/100 mL)	Remarks
A	**Potato Chips**					
1.	Uncle Chips, Spicy Treat	Pepsico India Holding Pvt. Ltd. (Frito-Lay Division), Manufacturing unit – Atop Food Products, Morbi Rajkot Highway, P.O. Box No. 007, Morbi – 363641, Gujarat	09 Jan 12	Best before four months from manufacture	Energy – 554 kcal Protein – 7.0 g Carbohydrate – 52.7 g Sugars – 1.3 g Fat – 35 g Trans Fats – Not mentioned	
2.	Lays, American Style Cream & Onion Flavour	Pepsico India Holding Pvt. Ltd. (Frito-Lay Division), Manufacturing unit – JL No. 2 & 4 (Kendua Panchayat), Mouja Jaldhulagari via Andul Maouri, P.O. Dhulagarh, P.S. Sankrail, Distt. Howrah, Pin – 711302, West Bengal	28 Dec 11	Best before four months from manufacture	Energy – 549 kcal Protein – 8.0 g Carbohydrate – 52.7 g Sugars – 3.7 g Fat – 34 g Trans Fats – 0 g Cholesterol – 0 mg	Snack Smart
3.	Bingo, Oye Pudina	ITC Ltd, Manufacturing unit – ITC Ltd., Foods division, snacks unit, plot no. 1, sector 11, IIE, BHEL, Haridwar – 249403.	18 Aug 11	Best before six months from manufacture	Energy – 527 kcal Protein – 8.1 g Carbohydrate – 54.1 g Sugars – 4.5 g Total Fat – 30.9 g Trans Fats – 0 g Cholesterol – 0 mg	Cholesterol & trans fats free, no artificial flavor, no added MSG
4.	Lays, American Style Cream & Onion Flavour	Pepsico India Holding Pvt. Ltd. (Frito-Lay Division), Manufacturing unit – JL No. 2 & 4 (Kendua Panchayat), Mouja Jaldhulagari via Andul Maouri, P.O. Dhulagarh, P.S. Sankrail, Distt. Howrah, Pin – 711302, West Bengal	21 Feb 2012	Best before four months from manufacture	Energy – 549 kcal Protein – 8.0 g Carbohydrate – 52.7 g Sugars – 3.7 g Fat – 34 g Cholesterol – 0 mg	
B.	**Indian snacks**					
5.	Aloo Bhujia	Haldiram Manufacturing Co. Pvt. Ltd., B – 1/F – 12, Mohan Co-operative, Industrial Estate, Mathura Road, New Delhi - 110044	Sep 11	15 March 2012	Energy – 630 kcal Protein – 5 g Total Carbohydrate – 40 g Total Fat – 50 g 6Trans Fats – 0 g Cholesterol – 0 mg Sodium – 670 mg	
6.	Kurkure Masal Munch	Pepsico India Holding Pvt. Ltd. (Frito-Lay Division), Manufacturing unit – Village Channo, Patiala, - Sangrur Road, P.O. Bhawanigarh, Distt. Sangrur – 148026, Punjab	31 Jan 12	Best before four months from manufacture	Energy – 561 kcal Protein – 6.4 g Carbohydrate – 53.6 g Sugars – 3.0 Fat – 35.7 g Trans Fats – Not mentioned	
7.	Masala Maggi	Nestle India Ltd.	Jan 12	Best before nine months from	Energy – 402 kcal	

		Plot No. 1A, Sector 1, Integrated Industrial Pantnagar – 263145, Uttrakhand		manufacture	Protein – 9.2 g Carbohydrate – 58.9 g Sugars – 1.2 Fat – 14.4 g Trans Fats – Not mentioned Calcium – 150 mg Potassium – 365 mg	
8.	Top Ramen, Super Noodles, Masala	Indo Nissin Foods Limited Manufacturing unit – 91 kms, Delhi Jaipur Road, (NH – 8), Village – Deodhai, Tehsil – Bawal, Distt. – Rewari, Haryana – 123401	Dec 2011	Best before ten months from manufacture	Energy – 467 kcal Protein – 7.6 g Carbohydrate – 73.3 g Sugars – 1.3 Fat – 15.9 g Trans Fats – 0 g Cholesterol – 0 mg Vitamin B1 – 0.6 mg Vitamin B2 – 0.7 mg	No added MSG
C.	**Carbonated drinks**					
9.	Pepsi	Pearl Drinks Ltd., Plot No 2E, Udyog Kendra, Ecotech III, Greater Noida, - 201304 (U.P.)	08-02-12	Best before three months from manufacture	Energy – 44 kcal Protein – 0 g Carbohydrate – 11 g Sugars – 11 g Fat – 0 g	Contains Caffeine
10.	Coca Cola	Hindustan Coca-Cola Beverages Pvt. Ltd., Tehsil Hapur, Distt. Ghaziabad – 201009, U.P.	07-02-12	Best before two & half months from manufacture	Energy – 44 kcal Protein – 0 g Carbohydrate – 11 g Sugars – 11 g Fat – 0 g	Contains Caffeine
E	**Burgers**					
11	McAloo Cheese	Mc Donald's	06-03-12			
12	Veg Zinger with cheese	KFC	06-03-12			
13	Subz Burger with cheese	Nirula's	06-03-12			
14	McChicken	Mc Donald's	25-03-12			
15	Chicken Zinger	KFC	25-03-12			
16	Chicken burger	Nirulas's	25-03-12			
F.	**Pizza**					
17	Margherita PAN -PER	Pizza Hut	06-03-12			
18	Margherita	Dominos	06-03-12			
19	Margherita Classic	Slice of Italy	05-03-12			
G.	**Fries**		06-03-12			
20	Crispy, golden fries	McDonald's	06-03-12			
21	French fries	KFC	05-03-12			
22	Fries	Nirula's	06-03-12			
H.	**Fried Chicken**					
23	Hot and Crispy	KFC	20-03-12			

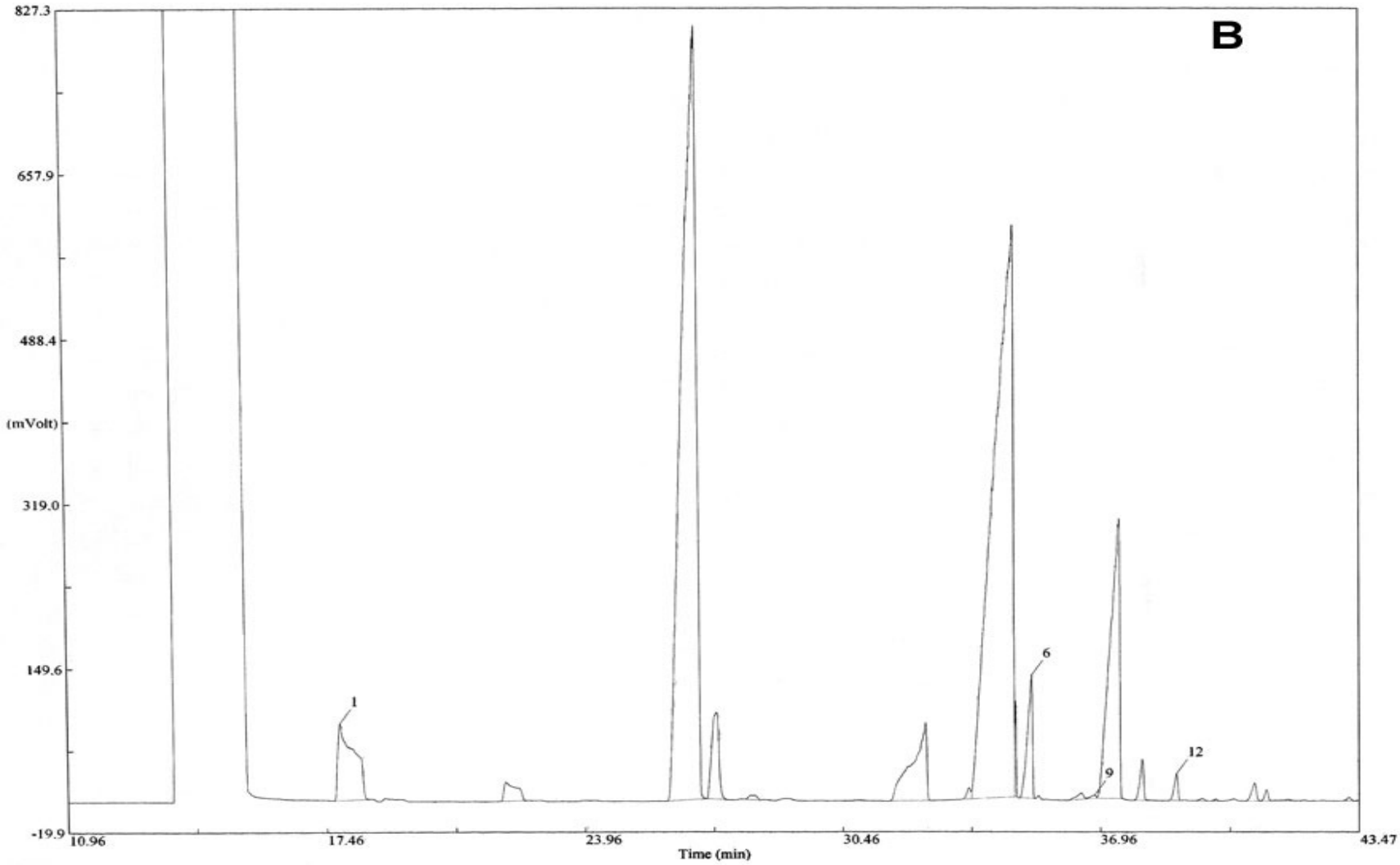
Annexure II. Salt, Total Carbohydrate, Total Fat and Trans fat in Junk Food samples.

Nutrition Guidelines (NIN, 2010)	Person	Kilo calories allowed /day	Salt (g/day)	Carbohydrate (g/day)	Total Fat (g/day)	Trans Fat (g/day)	Trans Fat (% of total Fat)
	Adults Male	2320	6	290-348	39-78	2.6	
	Adult Female	1900	6	263-315	35-70	2.1	
	Children (10-12 yr)	2100	6	238-285	32-64	2.3	
S.No	**Sample name**	**Manufacturer**	**Salt (g per 100 g)**	**Total Carbohydrate (g per 100 g)**	**Total Fat (g per 100 g)**	**Trans Fats (g/100 g of sample)**	**Trans Fat (% of total Fat)**
A.	**Potato chips**						
1.	Uncle chips spicy treat	Pepsico India Holding Pvt. Ltd	3.5	52.2	34.3	0.8	2.32
2.	Lays American Style Cream & Onion Flavour	Pepsico India Holding Pvt. Ltd	1.2	56.9	33.1	0.9	2.80
3.	Bingo, oye pudina	ITC Ltd	2.3	63.4	31.0	0.6	1.79
4.	Lays American Style Cream & Onion Flavour	Pepsico India Holding Pvt. Ltd			33.5	3.7	10.93
		Average(Potato Chips)	**2.3**	**57.5**	**33.0**	**1.5**	**4.5**
B.	**Indian snacks**						
5.	Aloo bhujia	Haldiram Manufacturing Co. Pvt. Ltd.,	3.3	45.6	37.8	2.5	6.48
6.	Kurkure masala munch	Pepsico India Holding Pvt. Ltd.	1.6	54.2	34.0	0.7	2.13
		Average(Indian Snacks)	**2.5**	**49.9**	**35.9**	**1.6**	**4.3**
C.	**Instant Noodles**						
7.	Masala Maggi	Nestle India Ltd.	4.2	69.8	14.0	0.6	4.07
8.	Top Ramen noodles	Indo Nissin Foods Limited.	3.2	73.3	14.3	0.7	5.09
		Average(Instant Noodles)	**3.7**	**71.6**	**14.1**	**0.65**	**4.6**
D	**Carbonated drinks**						
9.	Pepsi	Nestle India Ltd.	0.0	14.0	0.0	0.0	0.00
10.	Coca cola	Indo Nissin Foods Limited.	0.0	14.8	0.0	0.0	0.00
		Average (carbonated drinks)	**0.0**	**14.4**	**0.0**	**0.0**	**0.0**

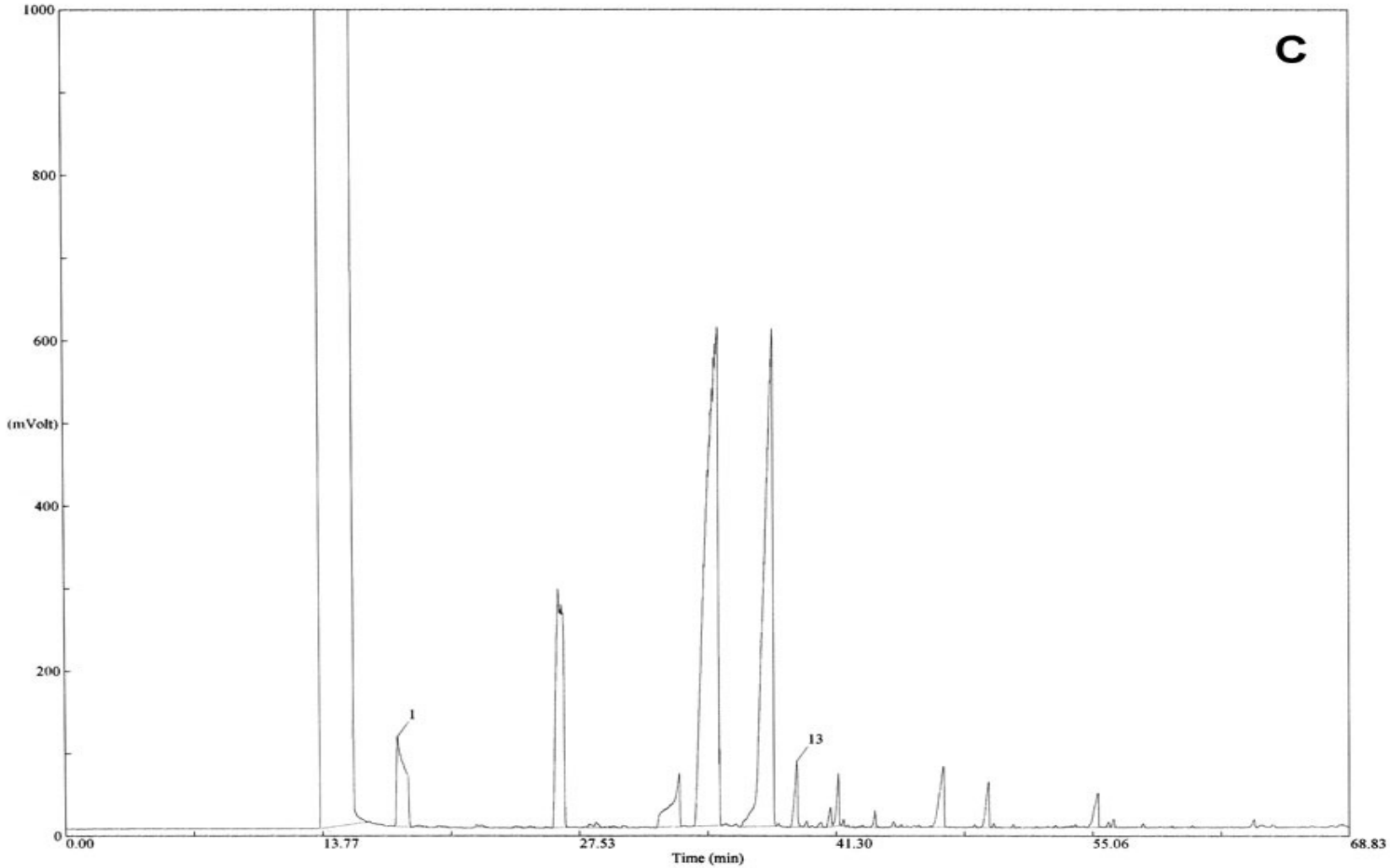
E	**Burgers**						
11	McAloo Cheese	Mc Donald's	2.0	48.1	8.3	0.3	3.39
12	Veg Zinger with cheese	KFC	1.7	45.4	13.7	0.7	4.86
13	Subz Burger with cheese	Nirula's	1.7	36.8	9.6	0.3	3.15
		Average (Veg Burgers)	**1.8**	**43.4**	**10.5**	**0.4**	**3.8**
14	McChicken	Mc Donald's	1.1	37.6	10.7	0.4	3.80
15	Chicken Zinger	KFC	1.2	30.2	16.9	0.5	3.10
16	Chicken burger	Nirulas's	1.2	31.0	12.2	0.3	2.40
		Average (Non Veg Burgers)	**1.2**	**32.9**	**13.3**	**0.4**	**3.1**
F.	**Pizza**						
17	Margherita Pan	Pizza Hut	1.4	51.6	7.9	0.1	0.70
18	Margherita	Dominos	0.6	43.7	6.3	0.1	1.62
19	Margherita Classic	Slice of Italy	1.0	55.6	7.0	0.1	1.10
		Average((Pizza's)	**1.0**	**50.3**	**7.1**	**0.1**	**1.1**
G.	**Fries**						
20	Crispy, golden fries	McDonald's	0.3	55.8	16.6	1.3	7.65
21	French fries	KFC	0.8	59.0	19.0	1.7	9.20
22	Fries	Nirula's	0.2	54.8	24.1	1.8	7.33
		Average(Fries)	**0.4**	**56.5**	**19.9**	**1.6**	**8.1**
H	**Fried Chicken**						
23	Hot and Crispy	KFC	0.9	14.0	23.4	0.7	2.92



A. Standard mixture of trans- Fatty Acid Methyl Esters



B. Chromatogram of French Fries- KFC



C. Chromatogram of Aaloo Bhujia -Haldiram

References

[1] Bayol SA, Simbi BH, Bertrand JA & Stickland NC. 2008. Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced infemales. *Journal of Physiology* ***586*** *3219–3230.*

[2] Z. Y. Ong, B. S. Muhlhausler. Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. *The FASEB Journal*, 2011; DOI: 10.1096/fj.10-178392

[3] http://medical-dictionary.thefreedictionary.com/Junk+Food

[4] Crupkin M, Zambelli A. 2008. Detrimental impact of *trans* fat on human health: Stearic acid-rich fats as possible substitutes. *Comp Rev Food Sci Food Saf* **7**:273–9

[5] NIMS 2009, Integrated Disease Surveillance Project, Non-Communicable Disease Risk Fact Survey, National Institute of Medical Statistics, New Delhi)

[6] http://www.jci.org/articles/view/36714/version/2

[7] http://www.downtoearth.org.in/content/food-brain

[8] http://www.naturalnews.com/034478_junk_foods_addictive_brain_chemistry.html

[9] www.cseindia.org/userfiles/Factsheet-Junk_food.pdf

[10] http://naturalsociety.com/fast-food-makes-men-infertile

[11] 2010 study conducted by scientists at Scripps Research Institute (SRI)

[12] Statistics on obesity, physical activity and diet: England, 2012

[13] http://www.abs-cbnnews.com/lifestyle/01/22/11/who-calls-junk-food-ban-schools-playgrounds

[14] http://news.bbc.co.uk/2/hi/health/7166510.stm

[15] http://www.guardian.co.uk/politics/2010/jul/11/food-standards-agency-abolished-_health-secretary

[16] http://www.bbc.co.uk/news/uk-scotland-17414707

[17] http://www.reuters.com/article/2010/03/17/food-usa-schools-_idUSN1715006520100317

[18] http://www.huffingtonpost.com/2011/07/14/massachusetts-bans-junk-__f_n_899325.html

[19] http://www.guardian.co.uk/world/2010/may/27/mexico-bans-junk-food-schools

[20] http://www.thenational.ae/news/uae-news/junk-food-banned-from-every-dubai-school-_canteen

[21] http://www.canada.com/life/Ontario+schools+junk+food+free+2011/2467809/story.html

[22] http://www.cbsnews.com/8301-504763_162-20114811-10391704.html)

[23] OECD Health data 2011

[24] http://www.spiegel.de/international/europe/0,1518,783862,00.html

[25] http://www.dw.de/dw/article/0,,15445105,00.html