



Awareness on Trans fats among selected Food Service Operators

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Abstract

In recent years, there has been a marked increase in the rates of obesity in countries such as India. This is attributed to unhealthy lifestyle practices associated with intake of foods higher in fat and refined carbohydrates. Food options, choices and eating behaviours are influenced by a complex number of factors including the nationality, culture, community, family and the individual's food likes and dislikes and are affected by global attributes. Industrially produced trans fats are formed during partial hydrogenation and could be as high as 50 to 60 per cent of total fat content. Growing concerns are about the potential health effects of trans fatty acids particularly those derived from vanaspathi, margarine, desi ghee and butter. Commercially fried, processed ready to eat bakery foods are potential source of trans fats. Hence the study was undertaken with the objective to assess the awareness on trans fat among the food service operators and to standardise the trans fat free recipes to be used at food service operations. A total of four commercial food service operations were selected at Coimbatore, Tamil Nadu by judgement sampling. The selected food service operations were observed at various functional areas namely purchasing, receiving, storage, preparation and service areas with a checklist to elicit details from the selected food service operations. The ten commonly consumed and most preferred foods by the customers with trans fat like donuts, biscuits, pastries, chocolate, cakes, french fries, fried chicken, cookies and potato chips were chosen and the procedure adopted for production were studied. And these recipes were reformulated to make trans fat free recipes. All the recipes were standardised using olive oil instead of vanaspathi, margarine or butter. These products were standardised and was statistically interpreted using the 't' test to find the difference in the nutritive value for energy and fat of the recipes. The recipes on making trans fat free showed a positive significance revealing the reduced energy and fat content. With mushrooming of food service operations and increased eating out pattern, it will be a mutual benefit to the food service operators and the customers in promotion of quality living.

Keywords: Obesity, customers, quality living.

Introduction

In recent years, there has been a marked increase in the rates of obesity in countries such as India that has been attributed to unhealthy lifestyle practices associated with the introduction of Western-style fast foods that are higher in fat and refined carbohydrates¹.

Indians from a high income neighbourhood were more familiar with fast food as it is defined in the West, and they dined at fast food restaurants more frequently. Furthermore, they were more likely to report that they enjoy eating at Western-style fast food restaurants compared to their low-income neighbourhood counterparts. On the other hand, Indians living in a low-income neighbourhood were more likely to buy and report food sold by street vendors as fast food².

The average American meat eater puts over 50 pounds of fat (cholesterol) into their body per year! This fat clogs the arteries, ultimately causing the heart attacks and strokes that will kill approximately 50 percent of our population³.

India has a wide and rich heritage of foods and recipes. Popular north Indian fast foods include aloo tikki, bhel puri, chaat, pakora, chole bhature, pav bhaji, dhokla, samosa and panipuri. Calorie and fat content in Indian fast food depends on the cooking method. Most of Indian fast foods are prepared by deep frying in fats especially trans fat and saturated fats⁴.

Obesity is a globally faced major health problem, growing as an epidemic in many nations like India, affecting wide spectrum of age groups, from young to elderly⁵⁻⁷

Transfatty acids are unsaturated fatty acids with at least one double bond in the trans configuration and has a straight chain that is similar to those structure of saturated fatty acid. Trans fatty acids are found in two major sources, natural and industrial. In natural source, trans fatty acids originate from milk fat and tissue fat of ruminants such as cows, goat and sheep. Bacteria in their stomach can be producing trans fatty acids by a biological hydrogenation process. Industrial trans fatty acids are mainly generated from vegetable oil polyunsaturated fatty acids, either during partial hydrogenation or during refining process⁸.

Major dietary sources of industrial trans fatty acids include bakery products (e.g., cakes, cookies and pies), deep fried and frozen foods (e.g., French fries, breaded chicken and fish), packaged snacks (e.g., popcorn), margarines and partially hydrogenated fats directly used for cooking and also ruminant derived foods (dairy products and meat)⁹. Industrially produced trans fats are formed during partial hydrogenation, a process used by the vanaspathi industry to harden and stabilize liquid vegetable oils. This process maintains the taste and smell characteristics of oils while enabling a longer shelf life for final food products.

The majority of the trans fats in the food are industrially produced and are typically found in foods made with partially hydrogenated oil, baked and fried foods. Trans fats also occur naturally. They are found at low levels (generally two-five per cent of fat content) in ruminant based foods, such as, dairy products and meat. Trans fatty acids level in vanaspathi depends on multiple factors and could be as high as 50 to 60 per cent of total fat content. There are growing concerns about the potential health effects of trans fatty acids particularly those derived from vanaspathi. In India, vanaspathi, margarine, desi ghee, butter etc. are sources of trans fatty acids. Commercially fried, processed ready to eat bakery foods are potential source¹⁰.

Major contributors to artificial trans fat intake include fried items, savory snacks (like microwave popcorn), frozen pizzas, cake, cookies, pie, margarines and spreads, ready-to-use frosting, and coffee creamers. The amount of trans fat can vary among similar food categories. Trans fat are also found in restaurant and cafeteria foods that contain or are prepared with partially hydrogenated oil¹¹.

Material and Methods

A total of four commercial food service operations comprising a two star Hotel category Hotel Alankar Grande, Hotel Heritage Inn and two bakery units A.K.R Bakers and Donuts were selected at Coimbatore by judgement sampling. The selected food service organisations were observed at various functional areas with a checklist to elicit details on the selected food service operations.

A well designed questionnaire including components such as name, communication details, type of ownership, category of recipes namely starters; main dishes, side dishes, baked items and ice creams were elicited with the cost. The details observed in the functional areas of the selected food service operations are as follows:

Purchasing Area: Purchasing good food is the basis for preparation and serving meal to the customers¹², information on how the procurement of raw materials were noted in person. The method of purchasing, frequency of purchase of the raw materials, the quality check of the ingredients, using perishable foods within two days of purchase. When used fresh, use of

packaged foods should be first in first out so that older packages are used first were observed in purchasing area.

Receiving Area: In receiving area, the delivery note is checked with copy of the order placed, counts, weighs or volume are checked to tally with the amount of various items on the delivery note, the qualities of ingredients perishable, semi perishable, non perishable were checked and hygiene and sanitation of the area and employees were observed.

Storage Area: The types of storage like refrigerator or freezer storage is necessary for meat, dairy products, eggs, and cut fruits and vegetables. Then the storage of food is freeze on the day of purchase, use within a month, defrosts thoroughly before use and once opened use within three days were also elicited. In storage area it was checked whether all the foods and paper supplies are stored six to eight inches off the floor, the foods are labelled with name and received data, the foods are stored in containers with tight fitting lid with a labelled common name, the first in and first out was checked, there is no leakage or bulging in bags and cans, whether the surface is clean, chemicals are clearly labelled and stored away from food and food related supplies, and do they have regular cleaning schedule, sanitation and hygiene was maintained, storage adequacy at dry and cold storage areas, prevention of cross contamination was checked in storage area.

Pre Preparation Area: In pre-preparation area rinsing and sanitising of the utensils before every use, the cleaning and washing of the pre cut food items, the use of clean equipments for cutting and also for placing the cut items were noted. Furthermore the importance on thawing of frozen food to conserve time and energy were seen. On the personnel front, their washing of hands before handling the food, wearing clean clothes and putting on an apron was noted; hygiene and sanitation of both the personnel and work area were closely observed.

Production Area: Foods are prepared in small batches to limit the temperature danger zone, clean reusable towel are used for cleaning the equipment, food is handled using suitable utensil, tasting the prepared food is done using proper procedure.

Usage of trans fat in food products, amount of fat, type of oil used and type of fuel used like wood, and coal, liquid fuel like gasoline, fuel oil were obtained. The prepared food like fresh, washed, and dried fruits and vegetables, packed trays or plastic bags are used or not and if they have used for which purpose were elicited. The foods prepared were given separately as starters like french fries and potato chips, main dish like pastries, baked foods like donuts, biscuits, cookies, and cakes, the tandoor item like fried chicken, and chocolates all these foods are rich sources of trans fat were elicited. The production process for the above selected foods were studied the ingredients and method of production were elicited. The oils used in the establishment and the amount of fat used in the product were elicited.

Food Service Area: In service area, it was observed food is heated to the required safe internal temperature before placing in hot holding, hot holding unit is pre-heated before hot food is placed in unit, food is protected from contamination and type of service of food namely self-service, waiter service, buffet service, or door delivery adopted by the food service organisation were brought out.

Waste Disposal Area: The mode of disposal of waste products like incinerators, bins, and whether it is bio-degradable or non-biodegradable were elicited. In garbage disposal certain activities were observed such as cleanliness of kitchen garbage cans, loading dock and area around dumpster.

Sales Promotion Techniques: The promotion of products and services with marketing and advertising tools like newspaper, media and catalogue were elicited.

Standardisation of Trans Fat Free Recipes: An unhealthy substance also known as trans fatty acids, is made through the chemical process of hydrogenation of oils. Hydrogenation solidifies liquid oils and increases the shelf life and the flavour stability of oils and food that contain those¹³. The ten commonly consumed and most preferred foods by the customers with trans fat like donuts, biscuits, pastries, chocolate, cakes, french fries, fried chicken, cookies and potato chips were chosen the procedure adopted for production were extracted. And these recipes were reformulated without the addition of trans fat to make trans fat free recipes. All the recipes were standardised by using olive oil instead of vanaspathi, margarine or butter. These procedures were adopted and the products were standardised and was statistically interpreted using the student 't' to find the difference the nutritive value for energy and fat of the recipes.

Sensory Evaluation of the Recipes: For the sensory evaluation, a group of 20 women who gave their consent formed the panel. Information on the study, preparation method and scoring procedure were outlined to the panel members. The recipes were evaluated based on the sensory qualities like appearance, colour, flavour, texture and taste. Three trials were conducted and triplicate scores were taken and consolidated to get the mean values. It was found that the recipes prepared from olive oil had the best score and the score for the sensory evaluation for the trans fat free recipes were analyzed for mean and standard deviation.

Promotion of Healthy Food Choices to Employees: The employees and customers were schooled with the help of flash cards, posters, power point presentation which includes awareness on general information on fats and oils, classification of fats and oils, amount and usage of fats and oils, Transfat and Transfat products and ill effects on the usage of trans fat. In each hotel there were two sessions conducted for half an hour with 30 employees depending upon shift works and imparting knowledge mainly focusing on trans fat. In addition, the

reformulated recipes were also demonstrated, which brought the ways of reducing trans fat in foods and this is a healthier option

Results and Discussion

Types of the food service operation: The Alankar Grande and AKR bakers were with sole proprietorship form of organisation "sole proprietorship is a form of business organisation in which an individual introduces his own capital, uses his own skills and intelligence in the management of its affairs and is solely responsible for the results of its operation"¹⁴.

Hotel Heritage Inn and The Donuts were with partnership form of organisation "A partnership is an association of two or more persons to carry on as co-owners a business for profit"¹⁵.

Details of employees in the food service organizations: Employees play a major role in food service operation as the service has to be provided round the clock and customers choice is varied. Hotel Alankar Grande had the maximum employees of male and female in the ratio of 1:15. In Bakery AKR bakers had maximum employees in the ratio of 1:2.

Food workers play a critical role in ensuring food safety, those who do not practice proper personal hygiene, including hand washing at the appropriate times and using appropriate methods, can contaminate food¹² identified risk practices and behaviours that contributed to food borne illnesses: improper holding/time and temperature; poor personal hygiene; and contaminated equipment/prevention of contamination. Studies have found that food safety training is positively associated with self-reported changes in food safety practices¹⁶.

Details of food production carried out: Frequency of purchase of the raw materials: The raw materials which were perishable foods like milk and meat were purchased daily, semi perishable foods like vegetables and fruits are purchased weekly and non perishable foods like cereals and pulses were purchased once in a month by all the four food service operations and none of the food service operation had the option of storing foods for a year. In accordance to McDonald's Corporation, 2008; the raw materials are the ingredients that will go into producing the finished product. For McDonald's, these will include the buns, beef patties, paper cups, salad ingredients and packaging. These are delivered to the restaurants between three and five times a week. The raw materials arrive together on one lorry with three sections so that each product can be stored at a suitable temperature.

Type of storage in food service operation: Food and Drug Administration, 2000 stated that the plan review for storage needs to provide adequate refrigeration facilities for the proper storage, transportation, display, and service of potentially hazardous foods. Specific refrigeration needs will be based upon the menu, number of meals, frequency of delivery, and preparation in advance of service. All refrigerators must be

capable of maintaining Potentially Hazardous Foods (PHF) at 41°F or below.

All the four food service operation had appropriate storage facilities. Hotel Alankar Grande was the only hotel which had dry storage, cold storage, refrigerator and walk in cooler. AKR Bakers and Donuts had only two types of storage facilities.

Duration of storage of the foods in storage area: when the food was purchased Hotel Alankar and AKR bakers freeze on the day of purchase, the Donuts use within a month and Hotel Alankar Grande defrost thoroughly before use. Hotel Alankar Grande and Heritage Inn use prepared foods like fresh, washed and dried fruits and vegetables, packed trays or plastic bags. None of the food service operation kept the food beyond the expiry date (best before date).

A wide variety of fruits and vegetables are sold at Lotus Supermarket, packaged and processed indifferent forms such as canned, bottled, pickled and dehydrated, etc. Fresh produce are packed in nets, PolyStyrene (PS) trays with shrink wrap, cartons and transparent plastic bags. The majority of the fresh produce, however, remains unpacked. These are arranged in 'field' containers as per product type, with price tags attached¹⁷.

The factors influencing the choice of food production: The factors influencing the choice of food production was simple to manage such as system's ease of use is a large influence on which system a company chooses to implement across its production and distribution chain. An easy user interface allows a larger number of employees to use the system with a greater degree of success and this reduces the potential for errors. These were informed by Hotel Heritage Inn and AKR Bakers, the choice of food production for Hotel Alankar Grande was dependent upon the price, and for Donuts it was special offers of sales promotions such as vouchers. Each organisation had their own factors influencing the choice of food production.

Results from a survey of college and university foodservice directors in an agriculture-based Midwestern state show support for purchasing from local sources, primarily to support regional economies, provide fresher and higher quality food, good public relations, availability of safer food and the ability to purchase smaller quantities. Obstacles identified were adequacy, seasonality and reliability of supply, cost, dealing with more vendors, and getting approval for new suppliers¹⁸.

Foods prepared in food service operation: Hotel Alankar Grande prepare french fries, cakes, fried chicken and chocolate, Hotel Heritage inn prepare french fries, pastries, cakes, fried chicken and chocolate, the Donuts prepare pastries, donuts, biscuits, cookies, and cakes and AKR bakes prepare pastries, donuts, biscuits, cookies, cakes and chocolate. In accordance to British Dietetic Association, 2013 trans fats are found in cakes, biscuits, hard margarines, takeaways, pastry, pies and fried foods.

Types of fats used for cooking: The usages of vanaspathi were more in deep fat frying instead of oils and butter also were replaced with vanaspathi. Nine different brands of commercial vanaspathi were analyzed for their various physicochemical characteristics. The fatty acid composition showed that the transfatty acid (elaidic acid) content ranged from 5.9 to 30.0 per cent. In India, about 1.1 million metric tons of vanaspathi is being produced annually, and a large amount is utilized in confectionery, bakery and ready-to-eat foods¹⁹.

Refined oils were not used in Donuts, palm oil was used by AKR bakers for shallow and deep fat frying, butter is used in all the food service operation except AKR bakers, and ghee was used by Hotel Alankar and Donuts.

Reuse of heated oil and ways of reuse of heated oil in cooking: The reused oil was used for deep fat fry and was added in curries by Donuts and Hotel Alankar Grande. The Hotel Heritage inn does not reuse the oils. According to Nurhan, 1996 simplest deep-fat frying was conducted in a kettle of oil heated on a stove or over an open fire. Small batches of food are immersed in hot oil and removed when fried as determined by the experience of the cook. The first real technological advance in frying was the introduction of continuous cookers. The development of continuous fryers provided a boost for the commercial development of frying.

Types of fuels used in food service operation: The gaseous fuel and electricity are the most widely used in all the types of fuels selected food service operation because of the technological development and availability. The recent rebound in US oil and gas production, driven by upstream technologies that are unlocking light tight oil and shale gas resources, is spurring economic activity – with less expensive gas and electricity prices giving industry a competitive edge – and steadily changing the role of North America in global energy trade²⁰. Solar energy has to be used as a renewable source in food service operations.

Information provided on dishes and products: AKR Bakers and Donuts provide the information only on origin in the packaging products while the rest of the information such as certificate, ingredients, nutritional content and organic was not provided. The Hotel Alankar Grande and Heritage Inn gives information on method of preparation in their menu cards.

Types of service in food service operations: Hotel Alankar Grande and Heritage had waiter service as well as buffet service, Donuts and AKR bakers had self service. Silver service usually includes serving food at the table. It is a technique of transferring food from a service dish to the guest's plate from the left. It is performed by a waiter using service forks and spoons from the diner's left. In France, this kind of service is known as service à l'anglaise.

According to Buffet Service Guidelines, 2008 buffet service refers generally to any permanent, regularly occurring, or temporary operation in a permanent retail food facility where unpackaged prepared food is displayed, served and/or prepared for the general public from counters, tables or similar equipment or installations in dining rooms, lobbies, meeting rooms, bar areas, ballrooms, and other areas not traditionally used for food preparation.

Ways of waste disposal: Hotel Alankar and Donuts dispose their waste by bins; pits were used to throw the waste by AKR Bakers and incinerators were used for waste disposal in Hotel Heritage inn. Food waste is immediately placed in the designated bin. Waste handler collects and disposes of food waste immediately after meal times. If offsite disposal is practiced, food waste should only be stored for up to 1 day. If on site disposal, waste handler buries food waste daily. It is highly recommended that food waste should be composted.

Sales Promotion Techniques: Among the sales promotion techniques, Newspaper was the major advertising, marketing and promotion tool for products. And senses at all selected food service operation.

Details of employees in the selected food service operation:
Employee benefits: Recognising the excellence in the performance of employees, the organisation offers competitive pay and benefits programs designed to attract and retain people. Package of benefit choices that meet employees financial security needs at an affordable cost and to promote and maintain good health, to provide for retirement, to help meet the cost of illness and accident, and to help provide financial security for employees and beneficiaries.

According to National Insurance contributions series, 2013 benefits to the employees to allow them to carry out their duties. Medical and life insurance is provided in all the four food service operation Hotel Heritage Inn and AKR Bakers offers

contribution to pension after their retirement. AKR Bakers provide all the facilities to the employees except housing.

Evaluation on trans fat free recipes: The student ‘t’ test was employed to find the difference in the nutritive value such as energy and fat of the recipes prepared with trans fat free alternatives such as butter and olive oil against the standard procedure per ten portions is given in table 1.

There was a five per cent significance found in the fat content and one per cent significant of energy contents of french fries prepared with olive oil respectively. It was also found that the nutritive value such as fat and energy content of the donuts showed one per cent significance whereas in sponge cake there was five percent significance in the energy content.

There was one per cent significance seen in energy content of fried chicken. In potato chips fat content was found to be with five per cent significance and energy content was one percent significant. In biscuits the energy content was one per cent significant. Sweet pastry showed one percent significant in the energy content, in chocolate and cookies the fat and energy content was found to be one percent significant.

The recipes on making trans fatfree showed a positive significance revealing the reduced energy and fat content in all the deep fried foods such as fried chicken, potato chips, and french fries. In all the other baked foods, though the energy content increased, the contribution of fat was from omega 3 unsaturated fat sources, olive oil.

Mean score for the sensory evaluation of trans fat free recipes: This table shows the mean score for the sensory evaluation of trans fat free recipes on a five point scale On a total score of 25, the mean scores and standard deviation of the trans fat free recipes showed a fair acceptability to all the attributes such as appearance, texture, flavour, colour and taste.

Table-1
Nutritive Value for Trans Fat Free Recipes

Name of the recipe	Fat			Energy		
	Before	After	‘t’ value	Before	After	‘t’ value
French fries	50.25	45.8	2.567*	629.5	647.5	6.285**
Sponge cake	145.6	148.8	0.894 ^{ns}	4331.6	4371.4	2.068*
Fried chicken	56.53	52.33	1.537 ^{ns}	621.9	576.9	13.74**
Donuts	167.9	185.9	5.381**	3971.8	4109.7	35.282
Potato chips	50.10	45.9	2.362*	547	502	11.559**
Biscuit	186.1	186.1	0	3345.6	3505.2	22.814**
Sweet pastry	123.8	123.8	0	2422.2	2535.6	8.426**
Chocolate	163.05	180.7	7.215**	2191.7	2327.8	27.831**
Cookies	184.7	208.5	9.359**	3035	3217	18.635**

ns – not significant; * - Significant at 5 per cent level ; ** - Significant at one percent level

Conclusion

Food has always played an extra-ordinarily vital role in the rise and growth or the fall and decline of a nation because of its effect on the health of the population. Consumption of unsafe, contaminated food leads to food-borne diseases which cause considerable morbidity and mortality. There is a wide recognition of the role of foods in spreading diseases and there is a general awareness of the need to set up safety and quality systems in food production²¹. The food service operations must ensure education to employees on ways to reduce trans fats in production and implement alternative strategies. With mushrooming of food service operations and increased eating out pattern, it will be a mutual benefit to the food service operators through promotion of healthy food choices and thereby ensure quality living of the population.

References

1. Yadav K. and Krishnan A., National prevalence of obesity: changing patterns of diet, physical activity and obesity among urban, rural and slum populations in north India, *Obese* 9(5), 400-408 (2008)
2. Christopher R.A., Danijela D., Salim Y., Koon T., Arun Chockalingam., Binod Kumar Patro., Rajesh Kumar and Scott Alexander Lear., Differences in perceptions and fast food eating behaviours between Indians living in high- and low-income neighbourhoods of Chandigarh, India, *nutritionjournal* 12:4. National Insurance contributions on benefits in kind a guide for employers, (2013)
3. Chandran R., A hand book on natural health in today's lifestyle scenario, International E-Publication, 42(1) (2013)
4. Friedrich M.J., Epidemic of obesity expands its spread to developing countries, *JAMA*, 287(11), 1382-86 (2002)
5. Friedman N. and Fanning E.L., Overweight and obesity: an overview of prevalence, clinical impact, and economic impact, *Dis. Manag.*, 7 Suppl. 1, S1-6 (2004)
6. Bellows-Riecken K.H. and Rhodes R.E., A birth of inactivity? A review of physical activity and parenthood, *Prev. Med.*, 46(2), 99–110 (2008)
7. Pfeuffer M., Schrezenmeir J., Impact of trans fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk, *International dairy j*, 16, 1383–88 (2006)
8. Micha R. and Mozaffarian D., Trans fatty acids: effects on cardio metabolic health and implications for policy. prostaglandins, leukotrienes and essential fatty acids, 79, 147-152 (2008)
9. Dhir S., Regulation of trans fatty acids in partially hydrogenated, *Food journal*, 109(2), 182-195(2009).
10. Center for Science in the Public Interest, Trans fat: On the way out Available at: <http://www.cspinet.org/transfat> (2012)
11. Sethi M. and Surjeet M., catering management – an integrated new age international (2007)
12. Mytton O., Gray A., Rayner M. and Rutter H., Could targeted food taxes improvehealth?, *Journal of Epidemiology and Community Health*, 61, 689–694 (2007)
13. Bhushan, Types of organization (2006)
14. Angela Schneeman, The Law of Corporations and Other Business Organizations (2012)
15. The FDA Report on the Occurrence of Food borne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types, (2009)
16. Clayton D.A., Griffith C.J., Price P. and Peters A.C., Food handlers' beliefs and self reported practices, *International journal of environmental health research*, 12 *Journal of economic perspectives*, (1), 25–39 (2002)
17. Alastair Hicks, Multi-Country Study Mission on Minimum-Packaging Technology for Processed Foods, Thailand (2001)
18. Mary B. Gregoire, Ph.D., RD and Catherine H. Strohbehm, Ph.D., RD, CFSP, Local Foods: From Farm to College and University Food service (2005)
19. Jeyarani T. and Yella Reddy S., Physicochemical Evaluation of Vanaspati Marketed In India, Lipid Science and Traditional Foods, Central s Food Technological Research Institute, Mysore (2005)
20. International Energy Agency, World Energy Outlook, *International Journal of Food Science and Technology*, 42, 503-517 (2012)
21. Waghode S.M. and A.M. Garode., *International Research Journal of Biological Sciences*, 2(2), 78-80, February (2013)