S. FILIP *et al.*: *Trans* Fatty Acids in Food, *Food Technol. Biotechnol.* **48** (2) 135–142 (2010)

135

Trans Fatty Acids in Food and Their Influence on Human Health

Sebastjan Filip[1], Rok Fink[2], Janez Hribar[1] and Rajko Vidrih[1]*

[1]Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

[2]Department of Sanitary Engineering, Faculty of Health Sciences, University of Ljubljana, Zdravstvena pot 5, SI-1000 Ljubljana, Slovenia

Summary

Hydrogenated oils tend to have a higher *trans* fatty acid (TFA) content than oils that do not contain hydrogenated fats. Prospective epidemiological and case-control studies support a major role of TFAs in the risk of cardiovascular disease. In the partially hydrogenated soybean oil, which is the major source of TFAs worldwide, the main isomer is *trans*-10 C18:1. In the European countries with the highest TFA intake (the Netherlands and Norway), consumption of partially hydrogenated fish oils was common until the mid-1990s, after which they were omitted from the dietary fat intake. These partially hydrogenated fish oils included a variety of very long-chain TFAs. Recent findings from Asian countries (India and Iran) have indicated a very high intake of TFAs from partially hydrogenated soybean oil (4 % of energy). Thus TFAs appear to be a particular problem in developing countries, where soybean oil is used. In 2003, the United States Food and Drug Administration issued a final ruling that required food manufacturers to list the TFAs in their foods on the nutritional facts label. One way to produce 'zero' levels of TFAs is the *trans*-esterification reaction between vegetable oils and solid fatty acids, like C8:0, C12:0, C14:0 and C16:0.

Key words: *trans* fatty acids, human nutrition, human health, hydrogenated fats

Introduction

According to various studies, fats of animal and vegetable origins satisfy 22 to 42 % of the daily energy demands of human beings (*1–3*). Some fats, and especially those that are hydrogenated, contain *trans* fatty acids (TFAs), *i.e.* unsaturated fatty acids with at least one double bond in a *trans* configuration (*4*). This *trans* double-bond configuration results in a greater bond angle than for the *cis* configuration, thus producing a more extended fatty acid carbon chain that is more similar to that of the saturated fatty acids (SFAs), rather than to that of the *cis*-unsaturated double-bond-containing fatty acids (*5,6*).

Formation of these double bonds thus impacts the physical properties of a fatty acid. Fatty acids that contain a *trans* double bond have the potential for closer packing and alignment of their acyl chains, which results in decreased molecular mobility (*2*). Therefore, the oil fluidity will be reduced when compared to that of fatty acids that contain a *cis* double bond. Partial hydrogenation of unsaturated oils results in the isomerisation of some of the remaining double bonds and the migration of others, producing an increase in the TFA content and hardening of the fat. It has been shown that foods that contain hydrogenated oils tend to have a higher TFA content than those that do not contain hydrogenated oils (*4–6*). Nevertheless, the hydrogenation of oils,

*Corresponding author; Phone: ++386 1 423 1161; Fax: ++386 1 256 5782; E-mail: rajko.vidrih@bf.uni-lj.si

such as corn oil, can result in both *cis* and *trans* double bonds, which are generally located anywhere between carbon 4 and carbon 16 of the fatty acids.

Elaidic acid (*trans*-9 C18:1) is one of the major TFAs, although during hydrogenation of polyunsaturated fatty acids (PUFAs), small amounts of several other TFAs are produced, including: *trans*-9,*cis*-12 C18:2; *cis*-9,*trans*-12 C18:2; *cis*-9,*cis*-12,*trans*-15 C18:3; and *cis*-5,*cis*-8,*cis*-11, *cis*-14,*trans*-17 C20:5 (*1*,*4*,*6*).

Correlations between high intake of industrially produced TFAs (IP-TFAs) and the increased risk of coronary heart disease (CHD) have been reported (*7*,*8*). Lowering the intake of TFAs can also reduce the incidence of CHD (*2*). Estimates based on changes in plasma concentrations of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) indicate around 4 % reduction in CHD incidence, while based on epidemiological associations, when TFA intake is lowered by 2 % (5 g/day), the estimates indicate >20 % reduction (*2*,*5*,*9*). In the Netherlands, a major reduction in the TFA content of retail foods was achieved in the 1990s, through the efforts of the industry and with minimal government intervention (*9*). Society pressure is also now helping to reduce the TFA content of 'fast food'. This illustrates the feasibility of reducing TFAs in fast food without increasing the saturated fats, with the daily intake being kept as low as possible to minimise health risks (*7*).

Prospective epidemiologic studies and case-control studies using adipose tissue analyses have confirmed a major role of TFAs in the risk of CHD. The magnitude of the association with CHD is considerably stronger than for SFAs, and it is stronger than that predicted for the effects of TFAs on LDL and HDL cholesterol (*2*,*5*, *6*,*9*). In this context, it needs to be considered that data for the Russian Federation show that every year 1005 people per 100 000 of the population between 25 and 64 years of age die because of circulatory system diseases (*10*). Consequently, in 2003, the United States Food and Drug Administration issued a final ruling that required food manufacturers to list the TFAs in the nutritional facts labels of all packaged food products (*11*), with the food industry being given until 1 January, 2006 to comply. Along with these growing health concerns about TFAs, this mandate has led to marked changes in the fat and oil industries, with newer technologies developed to reduce the TFA content of fats and oils used in the manufacture of food products. Given the labelling mandate and these technological advances, it is possible that food products traditionally considered to be sources of TFAs are now much lower in, or indeed do not contain, TFAs (*12*). In late 2006, New York City became the first major city in the United States of America to pass a regulation limiting IP-TFAs in restaurants. This has served as a model for others to follow, with these regulations including: a maximum level per serving size of 0.5 g of TFAs; a distinction between frying and baking, with a phased-in implementation; a help centre to assist restaurants in making the switch to more healthy options; and plans to evaluate the regulation and its impact on CHD (*12*).

Accurate quantification of C18:1 TFAs in food products is thus an important issue, with policies recently implemented in different countries to limit their consumption and their occurrence in food products because of their relationship with CHD (*11*,*13*,*14*).

History

Margarine was invented in 1869 by Hippolyte Mège Mouriès, a French food research chemist, in response to a request by Napoleon III for a wholesome butter alternative. It is not entirely clear whether the primary aim was the betterment of the working classes or the economics of the food supply to the French army. In the laboratory, Mège Mouriès solidified purified fat, after which the resulting substance was pressed in a thin cloth, which formed stearine and discharged oil. This oil formed the basis of the butter substitute. For the new product, Mège Mouriès used margaric acid, a fatty acid component isolated in 1813 by the Frenchman Michel Eugène Chevreuil. While analysing the fatty acids that are the building blocks of fats, he singled out this one and named it margaric acid because of the lustrous pearly drops that reminded him of the Greek word for pearls, *i.e.* margarites (*4*,*14*,*15*).

In 1871, Mège Mouriès sold this know-how to the Dutch firm Jurgens, which is now part of Unilever. In the early days, margarine contained two types of fat: a large proportion of animal fat and a small proportion of vegetable fat. As time passed, the small vegetable element increased, through two specific stages in the process. First, by improving the process of refining vegetable oils, the use could be made of a greater variety of liquid oils and a higher proportion of solid vegetable fats. Second, through the development of processes for turning liquid oils into solid fats on a commercial scale, the use could be made of larger quantities of liquid vegetable oils (*15*).

During the early years of that period, in the late 1800s, TFA intake from partially hydrogenated vegetable oils was minimal. Indeed, it was not until the late 1800s that the process of partial hydrogenation of oils was invented in Europe. These partially hydrogenated oils apparently entered the United States food supply by 1920. Although the rate of increase before 1950 is not completely clear, by 1950 the amount of IP-TFAs in the food supply was quite substantial. Partly because of economic effects during World War II, margarine production rose rapidly as a replacement for butter (*14*,*15*). Then during the 1960s, margarine became viewed as a healthy alternative to butter because of its absence of cholesterol and its low content of SFAs. Thus, consumption increased further, and so margarine, which was heavily hydrogenated at that time, became widespread in the food supply and was the major source of IP-TFAs. This phenomenon is illustrated in Fig. 1 (*16*). Total consumption was approx. 2 to 3 % of the food energy. Since then, the sources of TFAs have changed from mainly margarine to mainly deep-fried fast foods and commercially baked products, but *per capita*, the intake has remained roughly the same (*2*,*14*,*15*).

After World War II, the process of making hydrogenated and hardened fats from cheaper sources of vegetable oils was widely adopted. Margarines were developed and marketed as alternatives to butter, and vegetable shortening increasingly replaced animal fats
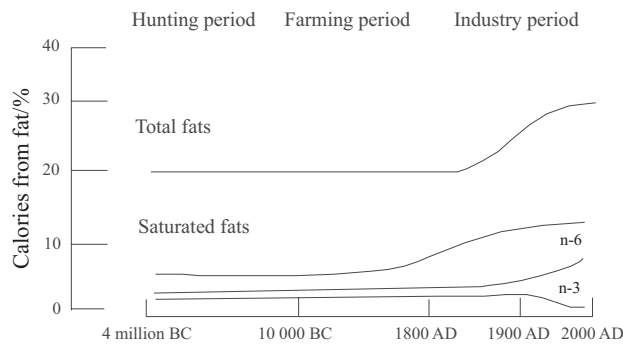
Fig. 1. Relative food energy supplied by the different fatty acids, and the predicted changes for the food industry, including breeding and fat hydrogenation (*16*)

in cooking (*17*). As early as 1975, a group of scientists led by Leo Thomas, at what is now the University of Glamorgan in South Wales, suspected that deaths from CHD were connected with eating partially hydrogenated fats. It is now generally accepted that TFAs are actually worse for health than the SFAs that they were designed to replace (*18*).

Studies on Animals

In milk fat, TFAs are produced by anaerobic fermentation of PUFAs in the rumen of lactating cows (*19,20*). This fermentation process is called biohydrogenation, and it results in TFAs that can be further metabolised in the mammary gland. Accurate estimations of fatty acid composition are vital not only for the definition of the nutrient composition of food, but also to accurately determine treatment effects that can alter the fatty acid composition of the food (*21–25*).

There is a considerable overlapping of TFA isomers in fats of ruminant origin and partially hydrogenated vegetable oils, as they have many isomers in common. However, there are considerable differences in the amounts of individual TFAs in both sources. While there is evidence of unfavourable effects of TFAs from hydrogenated vegetable oils on LDL and other risk factors for atherosclerosis, at present it is uncertain which of the component(s) of the TFAs created by chemical hydrogenation are responsible for such a negative metabolic effect (*21*). Prospective studies addressing the effects of TFA intake on CHD risk, where estimates of TFA intake were based on dietary protocols, have mostly been carried out in populations with a relatively low intake of dairy or ruminant TFAs (*26*). Nevertheless, the biggest effects of fatty acid composition and the nutritive quality of foods of animal origin, like meat and milk products, depend on the feed quality and the health of the animals.

Studies on Humans

These TFA-containing fats can be incorporated into both foetal and adult tissues, although the transfer rate through the placenta continues to be a contradictory subject. In preterm infants and healthy term babies, the *trans* isomers have been inversely correlated with infant

birth mass. Maternal milk reflects precisely the daily dietary intake of TFAs, with 2 to 5 % of total TFAs in human milk. The levels of linoleic acid in human milk are increased by a high *trans* diet, although long-chain polyunsaturated TFAs remain mostly unaffected (*27–29*). Alterations in the maternal dietary intake of PUFAs cause similar changes in the PUFA content of the milk. Several investigations have shown that supplementation of maternal milk with fish oils increases the amounts of C20:5n-3 and C22:6n-3 in the milk and in maternal and infant erythrocyte lipids. Likewise, infant tissues incorporate TFAs from maternal milk, increasing the levels of linoleic acid and decreasing arachidonic and docosahexaenoic acids. This suggests an inhibitory effect of TFAs on liver n-6 fatty acid desaturase activity (*30,31*). As opposed to blood and liver, the brain appears to be protected from TFA accumulation in experimental animals, although no data have yet been reported for human newborns (*32*). A significant interaction between diet and pregnancy was detected in the activities of Δ6-desaturase and glucose-6-phosphatase in liver microsomes; dietary TFAs decreased the activities of both enzymes, although only in pregnant rats (*33–35*). In Spain TFAs in human milk were investigated by Boatella *et al.* (*36*), who showed that the average content of TFAs in 38 samples was 0.98 % of the fatty acids. This value was lower than that for human milk from other developed countries, where consumption of hydrogenated fat was higher. In a study by Chen *et al.* (*37*) on TFAs in human milk in Canada, the mean total TFA content was (7.19± 3.03) % of the total milk fatty acids, with a range from 0.10 to 17.15 %.

Compelling data linking dietary TFAs to increased risk of CHD have originated from large, prospective, population-based studies, which have included from 667 to 80 082 men and women across different age groups who were monitored from 6 to 20 years; this link has also been seen in controlled feeding trials (*6*). Among these studies, there are the United States health professional's follow-up study, the Finnish α-tocopherol, β-carotene cancer prevention study, the United States nurse's health study (with 14-year and 20-year follow-up) (*2*), and the Dutch Zutphen elderly study (*6*). These studies are consistent in the finding of a strong positive association between TFA intake and the risk of CHD. Interestingly, a weaker correlation between SFA intake and the risk of CHD has also been reported (*2*).

The Zutphen elderly study studied 667 men from 64 to 84 years of age and free of CHD at baseline (*6*). Dietary surveys were used to establish the food consumption patterns of the participants. Information on risk factors and diet were obtained in 1985, 1990 and 1995. After a 10-year follow-up, from 1985–1995, there were 98 cases of fatal or non-fatal coronary heart disease. The findings showed that in this period the mean TFA intake decreased from 4.3 to 1.9 % of the food energy. After adjustment for age, body mass index, smoking and dietary covariates, TFA intake at baseline was positively associated with the 10-year risk of CHD. Thus a high intake of TFAs, which included all types of isomers, contributed to the risk of CHD. A substantial decrease in TFA intake, which was mainly due to the lowering of the TFA con-

tent in edible fats in the Dutch industry, would therefore have a large impact on public health (*4,6,32*).

In multiple and rigorous randomised trials, the intake of TFAs has been consistently shown to have adverse effects on blood lipids, and most notably on the LDL/HDL cholesterol ratio, which is a strong marker of cardiovascular risk. When a mixture of TFA isomers obtained by partial hydrogenation of vegetable oils is used to replace oleic acid, there is a dose-dependent increase in the LDL/HDL ratio. The relationship between the levels of TFAs as the percentage of energy and the increase in the LDL/HDL ratio appears to be approximately linear, with no evidence of a threshold at low levels of TFA intake, and with a slope that is twice as steep as that observed by replacing oleic with an SFA (*2,4,12,38*). Studies comparing animal and vegetable TFAs have shown similar effects on the total/HDL cholesterol ratio. Effects of TFA on lipoproteins from both sources appeared at doses exceeding 2 % of energy (*38*). The average impact of TFA-induced changes in the LDL/HDL ratio corresponds to tens of thousands of premature deaths in the United States alone. Although dramatic, this effect is substantially smaller than the increase in cardiovascular mortality associated with TFA intake in epidemiological studies, suggesting that other mechanisms are likely to contribute to the toxicity of TFAs (*39*).

Although there is accumulating evidence linking inflammatory proteins and other biomarkers to CHD, lipid concentrations in the blood remain one of the strongest and most consistent predictors of risk. Therefore, the LDL/HDL cholesterol ratio is probably the best marker to date for estimating the effects of TFAs on plasma lipids, which are most likely relevant to CHD incidence and mortality (*32,39*).

Further rigorous randomised trials to establish the effects of hydrogenated fats and TFA intake on individual lipoprotein classes started in 1990, when a report from the Netherlands suggested that a diet enriched with elaidic acid (*trans*-9 C18:1) increased the total and LDL cholesterol concentrations and decreased HDL cholesterol concentrations, compared to a diet enriched with oleic acid. In contrast, enrichment of the diet with SFAs increased LDL cholesterol, but had no effect on HDL cholesterol, thus resulting in a less adverse change than in the case of elaidic acid (*38,40*).

Trans Fatty Acids and Legislation

Governments are increasingly recognizing that the risks to consumers from the increased consumption of TFAs cannot be ignored. In 2003, Denmark became the first country to introduce laws to control the sale of foods containing TFAs. In January 2006, it became law in the United States of America that the contents of TFAs have to be specifically listed on food labels. There is a complication to this, however, because foods that contain less than 0.5 g of TFA per serving can be labelled as being free of TFAs. Furthermore, the regulations only apply to food that is labelled; food sold in restaurants and canteens is not covered by this law (*5,7,11*).

However, in December 2006, the Board of Health of New York City banned many TFAs from restaurants in the city, prompting similar moves in Philadelphia, Montgomery County in Maryland, and the Boston suburb of Brookline. The first phase of the regulation applies to oils, shortening and margarine used in cooking and as spreads, for recipes that contain more than 0.5 g of TFA per serving. Since 1 July 2007, New York City officials have also called for restaurants to clearly display calorie counts next to their menu items in a bid to increase consumer awareness of the nutritional content of their food. By 1 July 2008, the ban had been extended to include TFAs used in baked goods, including bread and cakes, in prepared foods, salad dressings and oils used for deep frying, and in dough and cake batter. Similar bans are being proposed in Chicago and in the state of Illinois; other cities may follow suit, most likely in California (*17,18*).

The American Heart Association recommends a healthy dietary pattern and lifestyle to combat heart disease, limiting TFA consumption to less than 1 % (or approx. 2 g on a 2000-calorie diet) and saturated fat to less than 7 % of total daily calories (*12*). This is consistent with the TFA recommendations made by the American Dietetic Association and the Dietitians of Canada (*41*).

The Danish story started with the publication of a study in the Lancet by Willett in 1993, and ended when IP-TFAs were reduced in the Danish market following a ban in 2003 (*2*). The Danish Nutrition Council was established in 1992, and it was the driving force behind the campaign that convinced Danish politicians that IP--TFAs can be removed from foods without any effects on taste, price or availability of foods. The Nutrition Council argued that as no positive health effects of IP-TFAs had ever been reported, then just the suspicion that a high intake has harmful effects on health justified the ban (*42*). The Danish success story might be interesting for other countries where this unnecessary health hazard could be eliminated from the foods.

Analytical Methods for *Trans* Fatty Acid Determination

The fatty acid composition of food is usually determined by gas-liquid chromatography of the corresponding methyl esters (*43–50*). Usually, fatty acid methyl esters (FAMEs) can be conveniently prepared by heating lipids with a large excess of either acid- or base-catalysed reagents. Most of the analytical methods are time consuming and impractical for processing large numbers of samples, because lipids have to be extracted prior to FAME preparation. For these reasons, some procedures have been developed that can be used to prepare FAMEs directly from fresh tissue (*46,47*).

Distribution of *Trans* Fatty Acids

Vaccenic acid (*trans*-11 C18:1) accounts for over 60 % of the natural TFAs, whereas among IP-TFAs, a broad mixture of TFAs is produced, with elaidic acid (*trans*-9 C18:1) as the main source (*6*). In recent years, newer

technologies have been developed to reduce the TFA content in fats and oils used in the manufacture of food products. The content of TFAs in Danish food has been monitored for the last 30 years. In margarine and shortening, the content of TFAs has steadily declined, from about 10 g per 100 g of margarine in the 1970s, to practically no TFAs in margarine in 1999. To efficiently reduce the health risk related to TFAs, Denmark decided to impose a maximum level of IP-TFAs of 2 g per 100 g of fat, as labelling was deemed insufficient to protect consumers, and especially for risk groups like children and people with a high intake of fast foods (*3,51*).

In North America, the daily TFA intake was estimated using food frequency questionnaires, and it was found to be 3–4 g per person, and by extrapolation of human milk data, to be greater than 10 g per person. The data show that the levels of TFAs can vary considerably among foods within any specific category, reflecting the differences in the fats and oils used in the manufacturing or preparation processes. For example, the range of TFAs in 17 brands of crackers was 23 to 51 % of total fatty acids, which represents differences of 1 to 13 g of TFAs per 100 g of crackers. This study shows that the wide variability in the TFA content of different foods can result in large errors in the estimation of TFA intake of individuals and, potentially, of groups (*52*).

TFA consumption in European countries varies considerably. The diet in northern European countries traditionally contains more TFAs than that in the Mediterranean countries, where olive oil is commonly used. The diet in France has always been relatively low in TFAs because France has traditionally used predominantly ruminant fats, as compared to hydrogenated vegetable oils. Recent decrease in dietary TFAs has been seen due to the modification of commercial fats and changes in consumer choice (*2,4,32*). In the TRANSFAIR study (*53*), which was based on a market basket analysis of diets in 14 European countries, the mean daily intake of TFAs ranged from minimal intake in Greece (1.4 g of TFA per day) to the highest intake in Iceland (5.4 g of TFA per day) (Fig. 2, *2–4,51,53*).

Milk fat is also the most abundant source of conjugated linoleic acids (CLAs), which refers to a group of geometrical and positional isomers of linoleic acid (LA *cis*-9,*cis*-12 C18:2). The major isomer of the CLAs in milk fat is *cis*-9, *trans*-11, and it represents 80 to 90 g per 100 g of total CLAs (*54–56*). Some of these fatty acids have biological, physiological and nutritional properties that are very interesting for consumer health, as especially seen for butyric acid and CLAs (*17,26,57*). CLAs are synthesised in ruminants both from dietary linoleic acid (*cis*-9, *cis*-12 C18:2) in the rumen by the microbial flora, and from vaccenic acid (*trans*-11 C18:1) in the mammary glands during *de novo* synthesis (*58*).

About half of the convenience products on the Austrian market that were tested contained less than 1 % of TFAs, and one third less than 5 % (*3*). However, almost 5 % of the products tested contained more than 20 % TFAs. A similar level was seen for fast food products, with the highest TFA levels of 8.9 %. Total TFAs of household fats were lower ((1.45±1.99) %) than fats for industrial use ((7.83±10.0) %; p<0.001). Compared to the investigations in Austria and Germany, around 10 years ago the TFA content of the tested foods decreased significantly. About half of the investigated products contained less than 1 % of TFAs or total fatty acids, although very high levels of TFAs (>15 %) are still detected, and an intake of more than 5 g of TFA per portion is possible, which has been shown to significantly increase the risk of CHD (*2,3,6*).

Brát and Pokorný (*59*) investigated a series of 20 margarines, nine cooking fats, and butter that were available on the Czech market. They used the American Oil Chemistry Society (AOCS) standard analysis methods, applying capillary gas chromatography. The margarines contained 15.2 to 54.1 % cooking fats, and 16.5 to 59.1 % SFAs, which was less than the butter. The content of linoleic acid varied between 3.7 and 52.4 % in the margarines, small amounts of linolenic acid were present in most samples, while oleic acid prevailed in cooking fats. Monoenoic TFAs were present only in trace amounts in 10 samples, and *trans*-polyenoic acids were present only in small amounts. Most cooking fats had a high content of TFAs. They summarised these data by indicating that the number of *trans*-free margarines had rapidly increased over a few years.
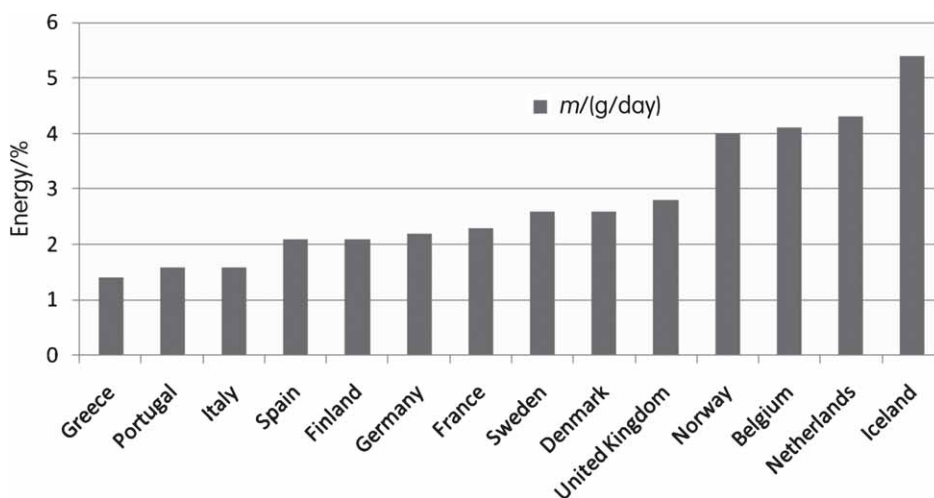

Fig. 2. Daily intake of TFAs in European countries (*2–4,51,53*)

More recently, Cenčič-Kodba (*60*) examined 13 margarines and fatty food samples in Slovenia, which were selected according to the frequency of use within different population groups in the community. All of the fried food and bakery food samples included in this study contained TFAs. The levels varied from less than 0.5 to 6.8 %. The highest TFA content in the margarines was 5.2 %, with 0.3 % as the lowest, and a mean TFA content of 2.3 %. The main TFAs were the *trans* isomers of mononounsaturated octadecenoic acid (C18:1).

The findings of Larqué *et al.* (*61*) suggest that Spanish margarines have moved to becoming products with a potentially healthier distribution of fatty acids. Even so, the great variability shown in fatty acid composition of margarines and the poor labelling highlight the importance of greater consumer information to avoid upsetting the traditional Mediterranean diet in Spain.

Conclusions and Future Trends

It can be concluded at present that the reduction of TFAs in the food supply is a complex issue that has involved interdependent and interrelated stakeholders. Actions to reduce TFAs need to be carefully considered regarding both the intended and unintended consequences related to nutrition and public health. World Health Organization (*62*) has already included the amount of TFAs in daily intake recommendations (Table 1). Many different options of alternative oils and fats that can be used to replace TFAs are available or are being developed. However, decisions on which alternatives to use are complicated and often time-consuming, and they involve considerations of health effects, availability, research and development investments, food quality and

taste, supply chain management, operational modifications, consumer acceptance, and cost (*11,12*).

As industry responses are now well underway following the policy actions of the past few years, it is possible to take a snapshot of industry activities that provides preliminary answers to these considerations. The first results of most anti-*trans* fat campaigns can be seen as modifications of the fatty acid compositions of industrial fats. In those fats, there are significantly higher levels of SFAs and possibly a higher index of atherogenicity. Several major food companies have announced efforts to remove TFAs from their leading brands over the past decade, starting with Unilever in the 1990s, and then more recently with Nestlé in 2002, Kraft in 2003, Campbell's in 2004 (for Goldfish crackers), Kellogg's in 2005, and Frito-Lay in 2006 (for chips). It is of note that the earliest announcements came from European firms, where the use of partially hydrogenated soy was not as common as in the United States, and thus reformulation was not as onerous. The announcements over the last three years have reflected the attention brought to this issue through lawsuits and debates about the nutritional label regulations. Many companies chose to implement the disclosure of these *trans* fat contents earlier than January 1, 2006 deadline, particularly when they were able to advertise zero *trans* fats on their products (*63*).

One aspect for producing 'zero' TFA fats lies in the transesterification reactions between vegetable oils and the SFAs of C8:0, C12:0, C14:0 and C16:0. These reactions are catalysed by an immobilised sn-1,3 specific *Rhizomucor miehei* lipase. One aspect relating to a TFA--free or low TFA fat that is suitable for use in confectionery fats is a non-hydrogenated vegetable fat composed of an inter-esterified fat: this can be obtained by subjecting a blend of at least one fat rich in lauric acid and at least one fat without lauric acid to inter-esterification (*64*).

For all of the products introduced in 2005 and 2006 that claimed to contain 'no *trans* fats', the most commonly used oil ingredients were canola, sunflower and soybean oils. Palm oil, which is high in saturated fat, also appears among commonly used ingredients, but is not as commonly used as an alternative in reducing TFAs. Some products (11 %) still use partially hydrogenated oils, because the regulation allows 0.5 g per serving of *trans* fats in products that can claim to contain 'no *trans* fat', while the use of small amounts of partially hydrogenated oils has facilitated the reformulation of some products (*65*).

Between 2006 and 2007, consumer awareness of *trans* fats increased and attained levels similar to those for saturated fats. This increased awareness is associated with improved self-reporting behaviour in consumer shopping for groceries (*66*). However, food labels and food claims that accompany packed foods are still largely incomprehensible for consumers and therefore they remain almost useless. Moreover, in Europe, consumers still cannot identify the content of TFAs in the labelling of food products, particularly as the only legislation that restricts the content of TFAs in Europe is in Denmark.

At the same time, we have to be aware that some indicators are showing that the world population is still

Table 1. Goals for population dietary intake

Dietary factor	Goal (Energy range/%)[a]
Total fat[b]	15–30
Saturated fatty acids	<10
Polyunsaturated fatty acids	6–10
n-6 polyunsaturated fatty acids	5–8
n-3 polyunsaturated fatty acids	1–2
Trans fatty acids	<1
Total carbohydrate[c]	55–75
Free sugars[d]	<10
Protein	10–15
Cholesterol	<300 mg/day
Sodium chloride (sodium)	<5 g/day (<2 g/day)
Fruits and vegetables	>400 g/day
Total dietary fibre	from foods>25 g/day

[a]except where specified otherwise

[b]as 'total fat (saturated fatty acids+polyunsaturated fatty acids+*trans* fatty acids)'

[c]percentage of total energy available after taking into account what is consumed as protein and fat, hence the wide range

[d]'free sugars' refers to all monosaccharides and disaccharides added to foods by manufacturers, cooks or consumers, plus sugars naturally present in honey syrups and fruit juices

increasing and is expected to reach nearly 8.9 thousand million (8 900 000 000) by the year 2050 (*67*). In the future, it will become increasingly difficult to assure food security as well as food safety, and also the nutritional quality of food. Indeed, it is the nutritional quality of food and its distribution all over the world that are the main factors which will have a huge impact on human health. In this way, human health is more than just of personal value, as it is also part of the welfare of the whole of our society.

References

1. S.C. Srinivasan, X. Irz, B. Shankar, An assessment of the potential consumption impact of WHO dietary norms in OECD countries, *Food Policy, 31* (2006) 53–77.

2. W.C. Willett, *Trans* fatty acids and cardiovascular disease – Epidemiological data, *Atherosclerosis* (Suppl.), *7* (2006) 5–8.

3. K.H. Wagner, E. Plasse, C. Proell, S. Kanzler, Comprehensive studies on the *trans* fatty acid content of Austrian foods: Convenience products, fast food and fats, *Food Chem. 108* (2008) 1054–1060.

4. M.C. Craig-Schmidt, World-wide consumption of *trans* fatty acids, *Atherosclerosis* (Suppl.), *7* (2006) 1–4.

5. J. Moss, Labelling of *trans* fatty acid content in food, regulations and limits – The FDA view, *Atherosclerosis* (Suppl.), *7* (2006) 57–95.

6. C.M. Oomen, M.C. Ocké, E.J.M. Feskens, M.A.J. van Erp-Baart, F.J. Kok, D. Kromhout, Association between *trans* fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: A prospective population-based study, *Lancet, 357* (2001) 746–751.

7. S. Stender, J. Dyerberg, A. Bysted, T. Leth, A. Astrup, A *trans* world journey, *Atherosclerosis* (Suppl.), *7* (2006) 47–52.

8. M.T. Tarrago-Trani, K.M. Phillips, L.E. Lemar, J.M. Holden, New and existing oils and fats used in products with reduced *trans* fatty acid content, *J. Am. Diet. Assoc. 106* (2006) 867–880.

9. M.B. Katan, Regulation of *trans* fats: The gap, the Polder and McDonald's French fries, *Atherosclerosis* (Suppl.), *7* (2006) 63–66.

10. Atlas of Health in Europe, World Health Organisation, WHO (2008) Copenhagen, Denmark.

11. Food Labeling: *Trans* fatty acids in nutrition labeling; Consumer research to consider nutrient content and health claims and possible footnote or disclosure statements, final rule and proposed rule, *Federal Register, Vol. 58*, Food and Drug Administration, USA (2003) 41433–41506.

12. S. Borra, P.M. Kris-Etherton, J.G. Dausch, S. Yin-Piazza, An update of *trans* fat reduction in the American diet, *J. Am. Diet. Assoc. 107* (2007) 2048–2050.

13. M. Carriquiry, W.J. Weber, L.H. Baumgard, B.A. Crooker, *In vitro* biohydrogenation of four dietary fats, *Animal Feed Sci. Technol. 141* (2008) 339–355.

14. J. Chen, Y. Cao, H. Gao, L. Yang, Z.Y. Chen, Isomerization of conjugated linolenic acids during methylation, *Chem. Phys. Lipids, 150* (2007) 136–142.

15. Margarine and spreads, IMACE – International Margarine Association of the Countries of Europe (2009) (*http://www.imace.org/margarine/history.htm*).

16. K. Salobir: Nutritional Functionality of Fats. In: *21st Food Technology Days 2001: Functional Foods*, B. Žlender, L. Gašperlin (Eds.), Biotechnical Faculty, Ljubljana, Slovenia (2001) pp. 121–136.

17. M.J. Albers, L.J. Harnack, L.M. Steffen, D.R. Jacobs, 2006 marketplace survey of *trans*-fatty acid content of marga-rines and butters, cookies and snack cakes, and savoury snacks, *J. Am. Diet. Assoc. 108* (2008) 367–370.

18. T. Blake: *Trans*-Fats, BBC, UK (2009) (*http://www.bbc.co.uk/food/food_matters/transfats.shtml*).

19. F. Destaillats, P.A. Golay, F. Joffre, M. de Wispelaere, B. Hug, F. Giuffrida, L. Fauconnot, F. Dionisi, Comparison of available analytical methods to measure *trans*-octadecenoic acid isomeric profile and content by gas-liquid chromatography in milk fat, *J. Chromatogr. A, 1145* (2007) 222–228.

20. V. Fournier, P. Juanéda, F. Destaillats, F. Dionisi, P. Lambelet, L.L. Sébédio, O. Berdeaux, Analysis of eicosapentaenoic and docosahexaenoic acid geometrical isomers formed during fish-oil deodorization, *J. Chromatogr. A, 1129* (2006) 21–28.

21. A. Ascherio, Epidemiological studies on dietary fats and coronary heart disease, *Am. J. Med. 113* (2002) 9–12.

22. C.M. Murrieta, B.W. Hess, D.C. Rule, Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid, *Meat Sci. 65* (2003) 523–529.

23. D. Triantafillou, V. Zografos, H. Katsikas, Fatty acid content of margarines in the Greek market (including *trans*-fatty acids): A contribution to improving consumers' information, *Int. J. Food Sci. Nutr. 54* (2003) 135–141.

24. F.A. Kummerow, Q. Zhou, M.M. Mahfouz, M.R. Smiricky, C.M. Grieshop, D.J. Schaeffer, *Trans* fatty acids in hydrogenated fat inhibited the synthesis of the polyunsaturated fatty acids in the phospholipid of arterial cells, *Life Sci. 74* (2004) 2707–2723.

25. G.C. Burdge, P.R. Derrick, J.J. Russell, S. Tricon, S. Kew, T. Banerjee, R.F. Grimble, C.M. Williams, P. Yaqoob, P.C. Calder, Incorporation of *cis-9, trans-11* or *trans-10, cis-12* conjugated linoleic acid in human erythrocytes *in vivo*, *Nutr. Res. 25* (2005) 13–19.

26. M. Pfeuffer, J. Schrezenmeir, Impact of *trans* fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk, *Int. Dairy J. 16* (2006) 1383–1388.

27. B. Koletzko, J. Müller, *Cis-* and *trans*-isomeric fatty acids in plasma lipids of newborn infants and their mothers, *Biol. Neonate, 57* (1990) 172–178.

28. B. Koletzko, *Trans* fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man, *Acta Paediatr. 81* (1992) 302–306.

29. B. Koletzko, T. Decsi, Fatty acid composition of plasma lipid classes in healthy subjects from birth to young adulthood, *Eur. J. Pediatr. 153* (1994) 520–525.

30. R.G. Jensen, C.J. Lammi-Keefe, R.A. Henderson, V.J. Bush, A.M. Ferris, Effect of dietary intake of n-6 and n-3 fatty acids on the fatty acid composition of human milk in North America, *J. Pediatr.* (Suppl.), *120* (1992) 87–92.

31. R.G. Jensen, C.J. Lammi-Keefe: Current Status of Research on the Composition of Bovine and Human Milk Lipids. In: *Lipids in Infant Nutrition*, Y.S. Huang, A.J. Sinclair (Eds.), AOCS Press, Champaign, IL, USA (1998) pp. 168–191.

32. E. Larqué, S. Zamora, A. Gil, Dietary *trans* fatty acids in early life: A review, *Early Hum. Dev.* (Suppl.), *65* (2001) 31–41.

33. E. Larqué, F. Pérez-Llamas, V. Puerta, M.D. Girón, M.D. Suárez, S. Zamora, A. Gil, Dietary *trans* fatty acids affect docosahexaenoic acid concentrations in plasma and liver but not brain of pregnant and fetal rats, *Pediatr. Res. 47* (2000) 278–283.

34. E. Larqué, S. Zamora, A. Gil, Dietary *trans* fatty acids affect the essential fatty-acid concentration of rat milk, *J. Nutr. 130* (2000) 847–851.

35. E. Larqué, P.A. García-Ruiz, F. Perez-Llamas, S. Zamora, A. Gil, Dietary *trans* fatty acids alter the compositions of

microsomes and mitochondria and the activities of micro-some Δ6-fatty acid desaturase and glucose-6-phosphatase in livers of pregnant rats, *J. Nutr. 133* (2003) 2526–2531.

36. J. Boatella, M. Rafecas, R. Codony, A. Gibert, M. Rivero, R. Tormo, D. Infante, F. Sánchez-Valverde, *Trans* fatty acid content of human milk in Spain, *J. Pediatr. Gastr. Nutr. 16* (1993) 432–434.

37. Z.Y. Chen, G. Pelletier, R. Hollywood, W.M.N. Ratnayake, *Trans* fatty acid isomers in Canadian human milk, *Lipids, 30* (1995) 15–21.

38. R.P. Mensink, P. Nestel, *Trans* fatty acids and cardiovascular risk markers: Does the source matter?, *Curr. Opin. Lipidol. 20* (2009) 1–2.

39. A. Ascherio, *Trans* fatty acids and blood lipids, *Atherosclerosis* (Suppl.), *7* (2006) 25–27.

40. P.R. Mensink, B.M. Katan, *Trans* monounsaturated fatty acids in nutrition and their impact on serum lipoprotein levels in man, *Prog. Lipid Res. 32* (1993) 111–122.

41. Position of the American Dietetic Association and Dietitians of Canada: Dietary fatty acids, *J. Am. Diet. Assoc. 107* (2007) 1599–1611.

42. A. Astrup, The *trans* fatty acid story in Denmark, *Atherosclerosis* (Suppl.), *7* (2006) 43–46.

43. S.H. Chen, K.C. Chen, H.M. Lien, Determination of fatty acids in vegetable oil by reversed-phase liquid chromatography with fluorescence detection, *J. Chromatogr. A, 849* (1999) 357–369.

44. S.A. Mjøs, Identification of fatty acids in gas chromatography by application of different temperature and pressure programmes on a single capillary column, *J. Chromatogr. A, 1015* (2003) 151–161.

45. I. Bondia-Pons, A.I. Castellote, M.C. López-Sabater, Comparison of conventional and fast gas chromatography in human-plasma fatty-acid determination, *J. Chromatogr. B, 809* (2004) 339–344.

46. W.M.N. Ratnayake, Determination of *trans* unsaturation by infrared spectrophotometry and determination of fatty-acid composition of partially hydrogenated vegetable oils and animal fats by gas chromatography/infrared spectrophotometry: Collaborative study, *J. AOAC Int. 78* (1995) 783–802.

47. F. Ulberth, M. Henninger, Simplified method for the determination of *trans* monoenes in edible fats by TLC-GLC, *J. Am. Oil Chem. Soc. 69* (1992) 829–831.

48. S.R. Baggio, A.M.R. Miguel, N. Bragagnolo, Simultaneous determination of cholesterol oxides, cholesterol and fatty acids in processed turkey meat products, *Food Chem. 89* (2005) 475–484.

49. W.P. Park, E.R. Goins, *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods, *J. Food Sci. 59* (1994) 1262–1266.

50. R. Garces, M. Mancha, One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues, *Anal. Biochem. 211* (1993) 139–143.

51. T. Leth, G.H. Jensen, Æ.A. Mikkelsen, A. Bysted, The effect of the regulation on *trans* fatty acid content in Danish food, *Atherosclerossis, 7* (2006) 53–56.

52. S.M. Innis, T.J. Green, T.K. Halsey, Variability in the *trans* fatty acid content of foods within a food category: Impli-cations for estimation of dietary *trans* fatty acid intakes, *J. Am. Coll. Nutr. 18* (1999) 255–260.

53. G. van Poppel, M.A. van Erp-Baart, T. Leth, E. Gevers, J. van Amelsvoort, D. Lanzmann-Petithory, A. Kafatos, A. Aro, *Trans* fatty acids in foods in Europe: The TRANSFAIR study, *J. Food Compos. Anal. 11* (1998) 112–136.

54. A.K. Seçkim, O. Gursoy, O. Kinik, N. Akbulut, Conjugated linoleic acid (CLA) concentration, fatty acid composition and cholesterol content of some Turkish dairy products, *LWT-Food Sci. Technol. 38* (2005) 909–915.

55. M. Ledoux, J.M. Chardigny, M. Darbois, Y. Soustre, J.L. Sébédio, L. Laloux, Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers, *J. Food Compos. Anal. 18* (2005) 409–425.

56. J.M. Chardigny, R.L. Wolff, E. Mager, J.L. Sébédio, L. Martine, P. Juanéda, *Trans* mono- and polyunsaturated fatty acids in human milk, *Eur. J. Clin. Nutr. 49* (1995) 523–531.

57. A.J. Pandya, K.M. Ghodke, Goat and sheep milk products other than cheeses and yoghurt, *Small Ruminant Res. 68* (2007) 193–206.

58. D.E. Bauman, J.M. Griinari, Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome, *Livest. Prod. Sci. 70* (2000) 15–29.

59. J. Brát, J. Pokorný, Fatty acid composition of margarines and cooking fats available on the Czech market, *J. Food Compos. Anal. 13* (2000) 337–343.

60. Z. Cenčič-Kodba, Content of *trans* fatty acids in margarines and selected fatty foods marketed in Slovenia, *3rd Slovenian Congress on Food and Nutrition, Food Processing – Innovation – Nutrition – Healthy Consumers*, P. Raspor, T. Buzeti, L. Gašperlin, M. Jevšnik, B. Kovač, A. Krumpak, P. Medved, S. Oštir, P. Plahuta, M. Simčič, S. Smole-Možina (Eds.), Slovenian Nutrition Society, Ljubljana, Slovenia (2007) p. 119.

61. E. Larqué, M. Garaulet, F. Pérez-Llamas, S. Zamora, F.J. Tebar, Fatty acid composition and nutritional relevance of most widely consumed margarines in Spain, *Grasas y Aceites, 54* (2003) 65–70.

62. Diet, nutrition and the prevention of chronic diseases, *WHO Technical Report Series 916*, Geneva, Switzerland (2003).

63. Crisco® shortening products reformulated to contain zero gram *trans* fat per serving (2008) (*http://www.crisco.com/Promotions_News/Press_Releases/2007/zero_grams_ttran_fat*).

64. J. Farmani, M. Hamedi, M. Safari, A. Madadlou, *Trans*-free Iranian vanaspati through enzymatic and chemical trans-esterification of triple blends of fully hydrogenated soybean, rapeseed and sunflower oils, *Food Chem. 102* (2007) 827–833.

65. L.J. Unnevehr, E. Jagmanaite, Getting rid of *trans* fats in the US diet: Policies, incentives and progress, *Food Policy, 33* (2008) 497–503.

66. R.H. Eckel, P. Kris-Etherton, A.H. Lichtenstein, J. Wylie-Rosett, A. Groom, K.F. Stitzel, S. Yin-Piazza, Americans' awareness, knowledge, and behaviours regarding fats: 2006–2007, *J. Am. Diet. Assoc. 109* (2009) 288–296.

67. World Population to 2300, United Nations, Department of Economic and Social Affairs, Population Division, New York, USA (2004) pp. 4–10.