trans Fatty Acid Content of Canadian Margarines Prior to Mandatory trans Fat Labelling

W. M. N. Ratnayake · C. Gagnon · L. Dumais · W. Lillycrop · L. Wong · M. Meleta · P. Calway

Received: 5 October 2006/Revised: 27 April 2007/Accepted: 26 June 2007/Published online: 8 August 2007 © AOCS 2007

Abstract Dietary trans fatty acids (TFA) are of major concern because of their adverse effects on blood lipid levels and coronary heart disease. In Canada, margarines were significant sources of TFA during the 1980s and 1990s. However, this is expected to change with increased public awareness over their adverse health effects and the introduction of new legislature to include TFA content on the Nutritional Facts table of food labels. In this study, the TFA content of the top-selling 29 Canadian margarines, which represented 96.3% of the market share, was determined by capillary gas-liquid chromatography in order to assess the influence of regulatory development during the 3-year transition period between the announcement of new food labelling regulations in Canada that require mandatory declaration of the trans fat content in most prepackaged foods in January 2003 and its enforcement on 12 December 2005. The 29 margarines included 15 tub margarines made from non-hydrogenated vegetable oils (NHVO-tub margarines), 11 tub margarines made from partially hydrogenated vegetable oils (PHVO-tub margarines) and three print margarines, which were also made from partially hydrogenated vegetable oils (PHVOprint margarines). The 15 NHVO tub-margarines accounted for 71% of the total margarine market share and generally contained less than 2% TFA (mean value $0.9 \pm 0.3\%$ of total fatty acids). The mean total TFA contents of PHVO-tub margarines and PHVO-print margarines, were $20.0 \pm 4.5\%$ and $39.6 \pm 3.5\%$, and their market shares were 19.3 and 6.0%, respectively. Although during the last 10 years, increasing number of soft tub margarines that contained very little trans fats have been made available in Canada, the PHVO-tub- and -print margarines still contain high levels of trans fats similar to those margarines that were sold in the 1990s. The market share data suggest that the margarines prepared using NHVO and containing almost no TFA were preferred by Canadians over those margarines prepared using PHVO, even before the mandatory declaration of TFA content came into effect on 12 December 2005.

Keywords Linoleic acid \cdot α -Linolenic acid \cdot Margarine \cdot Partially hydrogenated vegetable oils \cdot Saturated fatty acids \cdot *trans* fatty acids \cdot *trans* fat labelling in Canada

W. M. N. Ratnayake (⋈) · C. Gagnon
Nutrition Research Division, Food Directorate,
Health Products and Food Branch, Health Canada, PL 2203C,
251 Sir Frederick Banting Driveway,
Ottawa, ON, Canada K1A 0L2
e-mail: nimal_ratnayake@hc-sc.gc.ca

L. Dumais Nutrition Evalua

Nutrition Evaluation Division, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada

W. Lillycrop \cdot L. Wong \cdot M. Meleta \cdot P. Calway Ontario Region Office, Health Products and Food Branch, Health Canada, Scarborough, ON, Canada

Abbreviations

CVD Cardiovascular disease **EPA** Eicosapentaenoic acid Docosahexaenoic acid **DHA FAME** Fatty acid methyl esters **HDL** High density lipoprotein Low density lipoprotein LDL **MUFA** Monounsaturated fatty acids **NHVO** Non hydrogenated vegetable oils Partially hydrogenated vegetable oils **PHVO**



PUFA Polyunsaturated fatty acids

SFA Saturated fatty acids TFA trans fatty acids

Introduction

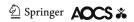
Evidence from a variety of metabolic studies indicates that trans fatty acids (TFA) are undesirable components in the diet, because their consumption increases blood levels of total cholesterol, low density lipoprotein (LDL)-cholesterol, and triglycerides, and decreases high density lipoprotein (HDL)-cholesterol [1]. These effects are strongly associated with increased coronary heart disease [2]. Increased intakes of saturated fatty acids (SFA) may also contribute to coronary heart disease, but they are considered to be less damaging than TFA, because SFA elevate both LDL cholesterol and HDL-cholesterol [1]. Epidemiological studies also point to a greater risk of coronary heart disease from TFA than from SFA [3, 4]. Several professional health organizations such as the American Heart Association [5], the Institute of Medicine [6], the Danish Nutrition Council [7], and the Food and Agricultural Association of the United Nations [8] have recommended limited intakes of TFA and SFA in order to reduce the risk of coronary heart disease. Most of the organizations recommend limiting the TFA intake to less than 1% of overall energy intake.

In 1995, it was estimated that the average Canadian consumed about 8.4 g TFA per day, which represented 10% of the total fat intake [9]. Intakes were variable with some Canadians consuming up to 38 g per day. These levels of TFA consumption are among the highest in the world. In view of the adverse health effects of TFA and the high level of these fatty acids in the Canadian diet, Health Canada in January 2003 undertook a number of actions, one of them being the amendment of the Food and Drug Regulations to require the mandatory declaration of TFA content of foods on the labels of most pre-packaged foods [10, 11]. The mandatory label regulations came into effect on 12 December 2005. It was anticipated that these labelling regulations would be an incentive to the food industry to decrease the TFA content of foods and also help consumers to make food choices aimed at decreasing their TFA intake. Besides labelling, another approach to decrease the TFA intake is a restriction on the level of trans fat in the food supply. In November 2004, the Canadian House of Commons passed a motion directing Health Canada and the Heart and Stoke Foundation of Canada to co-chair a multi-stakeholder task force with a mandate to develop recommendations and strategies to effectively

eliminate or reduce processed trans fats in Canadian Foods to the lowest level possible. The Task Force was formed in early 2005 and its membership composed of individuals from the food manufacturing and food services sectors, federal government representatives from a number of departments and agencies, professional associations, academia, consumer groups, population health experts and oilseed producers and processors. To ensure that its recommendations would be based on the best available evidence the Task Force commissioned a comprehensive scientific literature review on health effects of dietary fats, sought advice from internationally recognized experts on health implications of substituting other fatty acids for trans fats, consulted the food industry to assess the feasibility of reducing the use of processed trans fats and also review data on the available alternatives to partially hydrogenated fats. The final report of the Task Force was released in June 2006. The Task Force recommended limiting the total TFA amount to 2% of total fat content in all vegetable oils and soft, spreadable (tub-type) margarines and to 5% of total fat content in all other foods sold to consumers or used as an ingredient in the preparation of foods on site by retailers or food service establishments [12]. These limits do not apply to food products, for which the fat originates exclusively from ruminant meat or dairy products. If these recommendations are implemented, Canada will be the second country after Denmark [7] to restrict trans fat content in the food supply. In the meanwhile, a few major cities across North America are already setting rules to limit the trans fat content in menu items of restaurants and food services within their jurisdiction.

TFA are principally formed during the conversion of liquid vegetable oils into solid fats via the industrial process of partial hydrogenation. Partially hydrogenated solid fats are used in the production of margarines, shortenings, frying fats and in various types of bakery products. The TFA content of these foods may vary considerably (from less than 1 up to 50% of total fat) depending on the type of fat used [11, 13]. TFA also found at low levels (generally 2–5% of fat content) in ruminant-based foods such as dairy products and beef [11, 13]. These TFAs are the result of the bacterial transformation of unsaturated fatty acids in the rumen of ruminant animals. In addition to partially hydrogenated vegetable oils and ruminant fats, small amounts of TFA found in refined, unhydrogenated edible oils that been known since 1974 [14].

Margarines were known to be a significant source of TFA in the Canadian diet. Since 1965, Canadian margarines were found to contain very high levels of TFA, with average values generally ranging from 20 to 25% of total fatty acids [15–21]. Some print margarines had values as high as 50% TFA. However, during the last 10 years in response to the increased knowledge of the negative health



effects of TFA, progress has been made to decrease the TFA content of Canadian margarines [20, 21]. In particular, an increasing number of *trans*-free margarines, prepared using inter-esterified non-hydrogenated liquid oils, have been made available to the Canadian consumers.

The new labelling regulations that were announced thorough a Canadian Government Gazette Notification in January 2003 allowed declaration of the TFA content on food labels on a volunteer basis until mandatory declaration came into effect on 12 December 2005. This study determined the TFA content of the top-selling margarines that were sold in Canada soon after the start of third year of the 3-year transition period from the initial publication of the new labelling regulations in January 2003 and its enforcement in December 2005. This would help to assess the impact of the 3-year transition period of labelling regulation on the TFA content of margarines and the consumer selection of margarines during this period.

Materials and Methods

Margarine Selection

National market share data for margarines were obtained from a marketing research organization. The information included the sales volumes generated at retail for a 52-week period ending on 12 June 2004. Using this information, the 29 top-selling margarines, which represented 96.3% of the market share were selected for analysis. Six samples from each brand were purchased in March 2005 from retail stores in Toronto, Ontario. The 29 samples included 26 tub- and three print-margarines. Among the tub-margarines, there were 15 brands made from non-hydrogenated vegetable oils (NHVO). The other tub-margarines and also the print margarines were made from partially hydrogenated vegetable oils (PHVO).

Analysis

Approximately 1 g sample from three of the margarine samples of each brand was taken to create a composite sub sample. A similar composite sub sample was prepared using the other three margarine samples of the same brand. Each of the composite sub samples was placed in a separatory funnel and extracted with hexane. The hexane extract was washed with water, dried over sodium sulfate and hexane was evaporated under a gentle stream of nitrogen. Then a 20–30 mg portion of the extracted fat was taken and converted to fatty acid methyl esters (FAME) by heating with 1 mL of 14% BF₃-methanol at 100 °C for 1 h. The FAME were recovered using hexane and analyzed

according to the AOCS Official Method Ce 1h-05 [22] using an SP-2560 capillary column (100 m \times 0.25 mm i.d., 20 μ m thickness, Supelco, Bellefonte, PA) in an Agilent 6890 GC System equipped with a flame ionization detector and a ChemStation (Agilent Technologies, Little Falls, PA). The column oven temperature was 180 °C. Ultra high purity hydrogen was the carrier gas and run at constant flow rate of 1.0 mL min⁻¹. The injector and detector temperatures were 250 °C. The injector was set up for a split ratio of 100:1.

The fatty acid methyl ester (FAME) peaks on the gas chromatographic trace were identified by comparison with published traces [22–25] and also using a standard FAME mixture (GLC-463; Nu-Chek-Prep, Inc., Elysian, MN). Identification of some of the individual *trans* isomers of oleic and linoleic acids were further established by comparison of retention times with FAME standards of 6t-18:1, 7t-18:1, 9t-18:1, 11t-18:1, 12t-18:1, 13t-18:1, 15t-18:1, 9t,12t-18:2, 9c,12t-18:2 and 9t,12c-18:2 (Sigma, St. Louis, MO). The fatty acid composition data shown in the Tables are the average for the analysis of the two sub samples.

In the direct single step GC technique used in this study there was some minor overlaps of the *cis* and *trans* isomers [24, 25]. Most importantly, all the *trans*-18:1 isomers were well separated from the *cis*-18:1 isomers, except that 15t-18:1 overlapped with 9c-18:1 and that the GC peak for 6c,7c and 8c-18:1 isomers overlapped with that for 13t + 14t-18:1 [24]. The 15t-18:1 and 6c,7c,8c-18:1 are minor components in partially hydrogenated oils [24] and therefore, in the calculation of the fatty acid composition of the margarine samples, it was assumed that the peak for 9c-18:1 contained only *cis* 18:1 isomers and the peak for 13t + 14t-18:1 contained only *trans* 18:1 isomers [24, 25].

Results and Discussion

The relative proportions (% of total fatty acids) of total TFA, lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0), total SFA, total *cis*-oleic acid isomers (cis-18:1), linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3) of the NHVO tub margarines, PHVO tub margarines and of the print margarines are presented in Tables 1, 2 and 3, respectively.

The mean total TFA for the 15 NHVO-tub margarines was 0.8% (range 0.5–1.7%) (Table 1). For the PHVO-tub margarines and print margarines the mean TFA content was 20.0% (range 17.0–32.6%) (Table 2) and 39.3% (range 35.9–42.9%) (Table 3), respectively. These TFA data for the present study are compared in Table 4 with those reported for Canadian margarines during the previous 15 years [19–21]. The most notable change is the large increase in the number and market share of the NHVO-tub

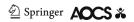


Table 1 Fatty acid composition (% total fatty acids) of tub margarines made from non-hydrogenated vegetable oils

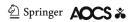
Sample ID	TFA	C12:0	C14:0	C16:0	C18:0	Total SFA	Cis-18:1	18:2n-6	18:3n-3
T1	0.5	2	0.7	8.9	3	15.2	49.1	29	4.9
T2	0.5	1.3	0.8	9	3	15.4	49.1	28.9	4.8
T3	0.5	1.8	0.9	9.8	2.4	16.1	58.3	16.9	6.7
T4	0.7	1.7	0.8	9.6	2.3	15.7	57.9	17.3	7.1
T5	0.7	1.4	0.8	9.2	3	15.6	48.9	28.8	4.8
T6	0.5	1.3	0.7	9.2	3.1	15.5	49	28.8	5.1
T7	1.7	1.4	0.8	9.4	2.5	15.2	53.1	19.4	8.8
T8	0.9	1.3	0.7	8.3	2.7	14.5	59.2	17.6	6.2
T9	0.9	1.4	0.8	8.4	2.6	14.6	59.1	17.4	6.5
T10	0.7	4.2	1.6	12.8	8.5	28.3	19.7	44.5	6.6
T11	0.7	4.4	1.7	13.2	8.6	29.1	19.8	43.9	6.3
T12	0.7	4.5	1.7	13.1	8.5	28.9	19.8	43.9	6.5
T13	1.2	1.8	0.9	15.1	4.6	23.3	22.9	45.4	7
T14	0.6	4.3	1.7	13.2	8.5	28.8	19.9	43.9	6.5
T15	1	2	0.9	15.3	4.8	24	21.3	46.6	6.9
Mean \pm SD $(n = 15)$	0.8 ± 0.3	2.3 ± 1.3	1.0 ± 0.4	11.0 ± 2.5	4.5 ± 2.6	20.0 ± 6.0	40.5 ± 17.2	31.4 ± 12.0	6.3 ± 1.1
Range	0.5-1.7	1.3-4.5	0.7-1.7	8.3–15.3	2.3-8.6	14.5–28.8	19.7–59.2	16.9–46.6	4.8-8.8

Table 2 Fatty acid composition (% total fatty acids) of tub margarines made from partially hydrogenated vegetable oils

Sample ID	TFA	C12:0	C14:0	C16:0	C18:0	SFA	Cis-18:1	18:2n-6	18:3n-3
T16	17.3	0.1	0.1	10.5	8.1	19.6	23.8	33.9	4.6
T17	22.3	0.1	0.2	5.2	6.3	12.9	49	11	3.2
T18	17.1	0	0.1	10.3	7.8	19.1	24.3	34	5.2
T19	32.6	0	0.1	4.8	7	13.1	48.8	3.1	0.5
T20	18.2	0.4	0.2	10.3	8	20	23.5	33.1	5
T21	17.4	0.6	0.3	10.2	7.9	19.9	22.4	34.4	5.6
T22	19.8	0.4	0.2	5.1	5.9	15.7	34.6	23.7	5.2
T23	19.3	0	0.1	7.5	6.8	15.5	38.8	21.3	4
T24	17	0	0.1	10	7.9	18.9	24.9	34	4.9
T25	20.6	0	0.1	5.1	5.9	12.4	48.3	12.7	4.5
T26	18.5	0.2	0.2	10.2	7.9	19.4	24.1	32.3	5.3
Mean ± SD	$20.0 \pm 4.5 \ 55$	0.2 ± 0.2	0.2 ± 0.1	8.1 ± 2.6	7.2 ± 0.9	17.0 ± 3.1	33.0 ± 11.3	24.9 ± 11.3	4.4 ± 1.4
Range	17–32.6	0-0.6	0.1-0.3	5.1-10.5	5.9-8.1	12.4-20.0	24.1–49.0	11.0-34.4	0.5-4.6

Table 3 Fatty acid composition (% total fatty acids) of print margarines made from partially hydrogenated vegetable oils

Sample ID	TFA	C12:0	C14:0	C16:0	C18:0	SFA	Cis-18:1	18:2n-6	18:3n-3
P1	42.9	0.1	0.1	9.3	5.3	15.6	34.8	5.3	0.6
P2	35.9	1.2	0.5	10	9.4	22	32.5	8.3	0.3
P3	39.2	0.5	0.3	10.5	5.8	17.9	32.7	8.4	1.1
Mean	39.3 ± 3.5	0.6 ± 0.6	0.3 ± 0.2	9.9 ± 0.6	6.8 ± 2.2	18.5 ± 3.2	33.3 ± 1.3	7.3 ± 1.8	0.7 ± 0.4
Range	35.9–42.9	0.1-1.2	0.1-0.5	9.3–10.5	5.3-9.4	15.6–22.0	32.5–34.8	5.3-8.4	0.3-1.1



margarines in Canada. The number increased from one in 1989 to eight in 1994–1995. At that time, there was no consumer awareness of TFA and declaration of TFA content on food labels was not allowed. Communication of the nutritional benefits of NHVO-margarines was done through the use of claims such as "low in saturated fat" and "made using non-hydrogenated vegetable oil." However, these margarines were more expensive and were targeted to health conscious consumers who were ready to pay a premium price for the extra nutritional quality. Currently there are fifteen different brands of NHVO-tub margarines sold in Canada.

In contrast to NHVO-margarines, the total TFA content of PHVO tub- and print-margarines has not changed during the last 15 years. Moreover, the TFA content has considerably increased in print margarines produced in 2005 and 1999 relative to those produced in the earlier years. The high TFA content of current print margarines is surprising considering that the adverse health effects of TFA were clearly demonstrated during the 1990s [1, 3, 4] and that during the same period, many national and international health organizations have called for reduction of *trans* fat levels in the human diet [5–8].

The mean *trans* isomer details of oleic, linoleic and α -linolenic acids for the three margarine categories are presented in Table 5. As expected, the *trans* isomers of oleic acid (*trans* 18:1) were the major isomer group of margarines prepared from partially hydrogenated vegetable oils. The NHVO-tub margarines contained almost no *trans* 18:1 isomers. In both PHVO tub-and print- margarines the isomers ranged from 4t-18:1 to 16t-18:1. The 10t-18:1 isomer was the predominant isomer in all PHVO-margarines. 9t-18:1 was the second most abundant isomer in PHVO tub margarines, whereas in PHVO print-margarines it was 11t-18:1 (i.e., vaccenic acid). In contrast to other *trans* isomers

of oleic acid, vaccenic acid is proven to be a unique trans isomer because it also occurs in ruminant fats [13] and it could be converted to cis-9, trans-11 conjugated linoleic acid isomer (CLA) in humans by the action of delta-9 desaturase [26, 27]. Studies performed on different animal species have shown that the consumption of CLA leads to reduction of adipose tissue fat mass and total body weight, reduces the plasma concentrations of total-and LDL-cholesterol, and has an anti-inflammatory effect (reviewed in Ref. [28]). However, the few human studies conducted so far have not confirmed the beneficial health effects shown in animal studies (reviewed in Ref. 29). If CLA and its precursor vaccenic proven to be beneficial in future human trials then the PHVO-print margarines can also be considered as a good source of vaccenic acid. From the data presented in Table 4, it can be calculated that 10 g serving of PHVO-print margarine could provide about 536 mg of vaccenic acid, whereas 10 g of butter would provide only about 168 mg. However, the presence of large proportions of other trans isomers, especially 10t-18:1 and 9t-18:1, in PHVO-print margarines, which are considered to be hypercholesterolemic trans 18:1 isomers [27] would outweigh the potential beneficial effects of vaccenic acid.

Compared to the wealth of data on the health and biological effects of *trans* 18:1, there is paucity of data on *trans* 18:2 isomers. However, two recent case controlled studies have shown that higher levels of *trans*-18:2 in plasma phospholipids are associated with higher risks of fatal ischemic heart disease and sudden cardiac death [30, 31]. These studies have suggested that in addition to *trans*-18:1, closer attention should also be paid to the levels of *trans*-18:2 in foods. All the margarines analyzed in the present study showed varying levels of several *trans*-18:2 isomers and *trans*-18:3 isomers (Table 5). In NHVO-margarines, the main *trans* fatty acids were the geometrical

Table 4 Comparison of the total TFA content of current margarines with those reported for Canada for the last 15 years

Year of production (Reference)	Margarine type (number of samples)	Total TFA content (% total fatty acids)		
		Mean	Range	
1989 [19]	Print $(n = 19)$	34.2	20.9–49.9	
	Tub-partially hydrogenated $(n = 30)$	21.0	10.1-35.2	
	Tub-non hydrogenated $(n = 1)$	0.0	_	
1994–95 [<mark>20</mark>]	Print $(n = 30)$	34.3	16.3-47.7	
	Tub-partially hydrogenated $(n = 71)$	21.4	10.5-44.8	
	Tub-non hydrogenated $(n = 8)$	2.2	0.9-5.0	
1999 [21]	Print $(n = 14)$	39.8	31.1-44.6	
	Tub-partially- $+$ non-hydrogenated ($n = 14$)	16.8	1.1-44.4	
2005 (Present study)	Print $(n = 3)$	39.3	39.2-42.9	
	Tub-partially hydrogenated $(n = 11)$	20.0	17.0-32.6	
	Tub-non hydrogenated $(n = 15)$	0.8	0.5-1.7	



Table 5 Composition (% total fatty acids) of trans isomers of oleic, linoleic and α -linolenic acids of tub-and print-margarines

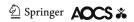
trans fatty acid isomer	Non-hydrogenated tub margarines (n = 15) mean Range		Partially hydrogenated tub margarines $(n = 11)$ mean range		Partially hydrogenated print margarines $(n = 3)$ mean range	
4t-18:1			0.1-0.1	0.0-0.1	0.1-0.0	0.0-0.1
5t-18:1	_	_	0.1-0.0	0.1-0.1	0.2-0.1	0.1-0.2
(6t + 7t + 8t)-18:1	_	-	3.2-0.8	2.4-4.5	4.2-0.4	3.9-4.6
9t-18:1	0.1-0.1	0.1-0.3	3.4-1.7	2.3-8.1	5.5-1.2	4.4-6.7
10t-18:1	_	-	4.0-0.7	3.3-5.6	8.4-0.7	7.7–9.0
11t-18:1	_	-	2.7-0.3	2.4-3.6	6.7-0.4	6.5-7.2
12t-18:1	_	-	1.7-0.2	1.5-2.1	3.9-0.5	3.4-4.4
13t + 14t-18:1	_	-	2.6-0.2	2.3-2.9	5.0-0.6	4.4-5.5
16t-18:1	_	-	0.3-0.1	0.2-0.3	0.6-0.2	0.4-0.7
Total t-18:1	0.1-0.1	0.1-0.3	18.0-3.4	15.6-27.2	34.8-3.7	31.0-38.4
tt-18:2	_	-	0.1-0.1	0.1-0.3	0.4-0.2	0.2-0.6
9t,12t-18:2	_	-	0.1-0.1	0.0-0.5	0.3-0.1	0.2-0.3
9c,13t-18:2	_	-	0.5-0.3	0.3-1.4	1.4-0.1	1.3-1.5
9c,12t-18:2	0.1-0.1	0.0-0.2	0.4-0.3	0.2-1.2	1.2-0.2	1.1-1.4
9t,12c-18:2	0.1-0.1	0.0-0.2	0.3-0.3	0.1-1.1	0.9-0.3	0.6-1.2
9t,15c + 10t,15c-18:2	_	-	0.1-0.1	0.0-0.5	0.4-0.1	0.3-0.4
9c,12c,15t-18:3	0.2-0.1	0.1-0.5	0.2-0.1	0.1-0.4	0.0 – 0.0	0.0 – 0.0
9c,12t,15c-18:3	0.0 – 0.0	0.0-0.1	0.1-0.1	0.0-0.2	0.0 – 0.0	0.0 – 0.0
9t,12c,15c-18:3	0.2-0.1	0.1-0.4	0.2-0.1	0.0-0.4	0.0 – 0.0	0.0 – 0.0
Total t-PUFA	0.6-0.3	0.3-1.6	2.1-1.3	1.3-5.8	4.5-0.4	4.1-4.9

isomers of linoleic (9c,12t-18:2 and 9t,12c-18:2) and α -linolenic acids (primarily 9c,12c,15t-18:3 and 9t,12c,15c-18:3). The levels and types of these *trans*-PUFA are typical of those found in refined, unhydrogenated canola and soybean oils [11]. Most of the PHVO-margarines, especially the print margarines contained almost no *trans*-18:3 isomers, but there were considerable amounts of *trans*-18:2 isomers. The most predominant *trans*-18:2 isomers were 9c,13t-18:2, 9c,12t-18:2 and 9t,12c-18:2.

In addition to the low content of TFA, the NHVO-tub margarines have in general a nutritionally favourable fatty acid profile. The mean proportions of cis-18:1 (primarily oleic acid; 40.5%), linoleic acid (31.4%) and α -linolenic acid (6.3%) (Table 1) were considerably higher than those for PHVO-tub margarines (33.0, 21.1 and 3.6%, respectively; Table 2) and PHVO-print margarines (33.3, 7.3 and 0.7%, respectively; Table 3). The mean total SFA content of the NHVO-tub margarines was 20.0% and it was slightly higher than that for PHVO-tub-margarines (mean 17.8%) and PHVO-print margarines (mean 18.5%). Human clinical studies have consistently shown that substituting TFA and SFA with cis-MUFA (i.e., oleic acid) and polyunsaturated fatty acids (PUFA) improves serum lipid and lipoprotein patterns. In particular, it lowers serum total and LDLcholesterol levels and results in a more favourable total/ cholesterol ratio [1], which can

cardiovascular disease (CVD) risk [5, 6]. Therefore, in general, the NHVO-tub margarines, relative to PHVO-tub and print margarines offer a more favourable fatty acid profile for protection against CVD.

Since SFA can provide functional properties comparable to those of TFA, there is concern that SFA would be used as a convenient substitute for TFA in some foods. Examination of the fatty acid profiles of NHVO tub-margarines allows one to split this group of margarines into two subgroups. One group (samples from T1 to T9 in Table 1) has low proportions of total SFA (range: 14.5–16%) and 18:2n-6 (range 16.9-29.0%) and a high proportion of cis-18:1 (range 48.9-59.2%) compared to the other sub-group (samples from T10 to T15) (ranges: SFA 23.3-29.1%, linoleic 43.9-46.6%, oleic 19.7-22.9%). Both sub-groups, however, have similar proportions of 18:3n-3 and TFA (Table 1). The SFA proportions of the second NHVO-tub are also much higher than those seen for the PHVO-tub margarines (Table 2) and print-margarines (Table 3). The high content of total SFA in the second sub-group of NHVO is due to high proportions of all the long-chain SFA (i.e., C12:0 to C18:0), except for samples T13 and T15. In these two samples the high proportion of SFA is primarily due to a high proportion of C16:0. The fatty acid profiles of the NHVO-margarines samples T1–T9 suggest that in these margarines, a blend of high-oleic vegetable oils (such as



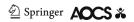
canola oil) and small amount of palm oil have been used as the primary replacement for partially hydrogenated fats as a mean to achieve the desired melting and functional characteristics. For the second group of NHVO margarines (samples T10–T15), the desired characteristics were most likely achieved by blending fully hydrogenated vegetable oil (e.g., cottonseed oil) with coconut oil, palm kernel oil or palm oil.

It is well known that the various fatty acids have different effects on serum lipid concentrations. SFA in general are thought to increase CVD risk because they elevate serum total- and LDL-cholesterol relative to cis-MUFA and PUFA [32, 33]. Currently, however, it is recognized that the individual SFA do not have an equal effect on serum lipids [33, 34]. There is general agreement that when considering effect of SFA on LDL-cholesterol, C12:0, C14:0 and C16:0 are hypercholesterolemic and that C18:0 is neutral when these fatty acids replace equivalent energy levels of *cis*-MUFA or PUFA in the diet [1, 35]. Because of the variable effects of fatty acids on serum lipids, the ratio of all the cholesterol-lowering cis-unsaturated fatty acids (i.e., sum of *cis*-oleic acid isomers, linoleic and α -linolenic acid) to all the cholesterol-raising fatty acids (i.e, sum of C12:0, C14:0, C16:0 and TFA) is a useful index for comparing the nutritional quality of different dietary fats. Table 6 lists the ratios calculated for all the margarine samples of the present study. The ratio for all the tubmargarines was greater than 1 and that for print margarines was less than 1. The first sub-group of NHVO-margarines (samples T1-T9) had the highest ratio, suggesting that these margarines would be nutritionally superior to all the other tub-margarines. The second sub-group of NHVO-margarines (T10–T15), despite having a high proportion of SFA, had a higher ratio than that for PHVO-tub margarines, suggesting that the T10–T15 NHVO-margarine might lead to a better serum lipid profile than PHVO-tub margarines even without considering the additional negative effect of TFA on HDL-cholesterol and CVD risk.

Although linoleic and α-linolenic are important components of a cholesterol-lowering and more healthful diet, the benefits depend to some degree on consuming an appropriate balance of these two fatty acids. There is some concern that North Americans consume more than the required amount of n-6 fatty acids (mostly as linoleic acid) but not enough n-3 fatty acids [36, 37]. A high dietary content of 18:2n-6 is not desirable because it can interfere with the metabolic conversion of 18:3n-3 to eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) and thereby decrease the tissue amount of these two long-chain n-3 fatty acids [36-38]. High tissue contents of EPA and DHA are desirable because they can exert cardioprotective effects in patients with coronary heart disease and in healthy individuals [36–38]. The Canadian Trans Fat Task Force members also recognized the importance of having a good balance in the dietary levels of n-6 and n-3 fatty acids and therefore, in their report they emphasized that oils high in cis-MUFA rather than n-6 PUFA should be considered as alternatives to partially hydrogenated oils [12]. In this context it is important to note that the fatty acid profiles of the NHVO-tub margarine samples from T1 to T9 (Table 1)

Table 6 Ratio of total of cholesterol-lowering C18 unsaturated fatty acids (i.e., sum of oleic, linoleic and α -linolenic acids) to total cholesterolraising fatty acids (i.e., sum of lauric, myristic, palmitic acids and TFA) of tub- and printmargarines

NHVO tub-mar	garine	PHVO tub-marg	garine	PHVO print-margarine		
Sample ID	Ratio	Sample ID	Ratio	Sample ID	Ratio	
T1	6.9	T16	2.2	P1	0.8	
T2	7.1	T17	2.3	P2	0.9	
T3	6.3	T18	2.3	P3	0.8	
T4	6.4	T19	1.4			
T5	6.8	T20	2.1			
T6	7.1	T21	2.2			
T7	6.6	T22	2.5			
T8	7.4	T23	2.4			
T9	7.2	T24	2.4			
T10	3.7	T25	2.5			
T11	3.5	T26	2.1			
T12	3.5					
T13	4.2					
T14	3.6					
T15	3.9					
Mean ± SD	5.6 ± 1.6	Mean ± SD	2.2 ± 0.3	Mean ± SD	0.8 ± 0.05	
Range	3.5–7.4	Range	1.4–2.5	Range	0.8-0.9	



meet the recommendations of the Canadian Trans Fat Task Force. These margarines have high levels of *cis*-MUFA (primarily as oleic acid) and moderate level of 18:3n-3 (range: 4.8–8.8%) as well as a favourable ratio of 18:2n-6 to 18:3n-3 (range: 2.2–6.0, note: individual ratios for each margarine are not shown in Table 1). The second subgroup of NHVO-tub margarines (T10-T15; Table 1) are also good sources of 18:3n-3 (range 6.3–7.0%), however, their high content of 18:2n-6 and consequently, the high ratio of linoleic acid to α -linolenic acid (range: 6.5–6.7) makes them less desirable in addition to their high proportion of SFA.

Public opinion surveys conducted over the last 6 years have shown that the Canadian consumers have an increased awareness of the negative health effects associated with trans fats [12]. A survey on consumer awareness of trans fats conducted in September 2005 reported that 79% of Canadians have heard about trans fats [12]. This high degree of awareness seem to have a key influence on the purchasing decisions of Canadians regarding margarines. From the 29 margarines analyzed in the present study, the NHVO-tub margarines accounted for approximately 71% of the margarine market share. Whereas, the PHVO-tub margarines and print margarines accounted for approximately 19.3 and 6% of the margarine market share, respectively. These large differences show that the margarines prepared using non-hydrogenated vegetable oils that contain almost no trans fats were preferred by Canadians over those margarines prepared using partially hydrogenated vegetable oils. It is important to note here that the Canadians have made these margarine choices even before the mandatory trans labelling came into effect on 12 December 2005.

The provision of voluntary declaration of TFA content in food labels encouraged the appearance on the market of a second group of NHVO margarines. Although these margarines contain almost no TFA, they have less desirable fatty acid profiles because of the high content of saturated fat and high ratio of n-6 to n-3. It will be interesting to see the evolution of the margarine market once the new nutrition labelling regulations are more fully implemented.

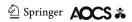
Taking into account margarine disappearance from retail outlets (83 million kg), the market share (individual data not shown here), the *trans* content of each of the 29 margarines, and the Canadian population (> 2years) in 2004 (31.28 million), the per capita intake of *trans* fat by Canadian margarines sold in retail outlets is estimated to be 0.53 g/day. This value is considerably lower than the value of 0.96 g/day estimated from a consideration of margarines sold in Canada in 1995 [21]. This change is most likely due to increased availability of zero-*trans* margarines in recent years and consumers preference for these margarines.

In summary, the fatty acid data presented here shows that the currently available top- selling margarines in Canada include15 different tub margarines that contain almost no TFA. Included among them were nine tub-margarine samples with nutritionally favourable fatty acid profiles. These nine margarines have low-levels of SFA, high levels of cis-MUFA and moderate levels of linoleic and of α -linolenic acids in a nutritionally desirable ratio. On the other hand, there still exist several margarines in the Canadian market that contain very high levels of trans fats. Fortunately, it appears that most Canadians are aware of the negative health effects of trans fats. The new mandatory TFA labelling regulations in Canada will be helpful for Canadian consumers to select margarines with better fatty acid profiles.

Acknowledgments Maya Villeneuve and Josee Deeks, Nutrition Research Division, Health Canada, are thanked for organizing the collection of market share data of margarines.

References

- Mensink RP, Zock PL, Kester ADM, Katan MM (2003) Effects of dietary fatty acids and carbohydrate on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr 77:1146– 1155
- American Heart Association (1997) trans fatty acids. Plasma lipid levels, and risk of developing cardiovascular disease. Circulation 95:2588–2590
- Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC (1997) Dietary fat intake and the risk of coronary heart disease in women. N Eng J Med 337:1491– 1493
- Oomen CM, Ocké MC, Fesken EJM, van Erp-Baart MA, Kok FJ, Kromhout D (2001) 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 357:746– 751
- Lichtenstein AH, Appel LJ, Brans M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosett J (2006) Diet and lifestyle recommendations revision 2006. A scientific statement from the American Heart Association Nutrition Committee. Circulation 114:82–96
- 6. Institute of Medicine, Report of the panel on macronutrients of the standing committee on the scientific evaluation of dietary reference intakes (2002) Dietary reference intakes of energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. The National Academy Press, Washington, pp 8.1– 8.97
- Stender S, Dyerberg J (2003) The influence of trans fatty acids on health. A report, from the Danish Nutrition Council 4th edn. Publication no. 34
- Food and Agriculture Organization of the United Nations. Joint WHO/FAO expert consultation on diet, nutrition, and the prevention of chronic diseases. WHO technical report series 916, Geneva (2002)
- Ratnayake WMN, Chen ZY (1995) trans fatty acids in Canadian breast milk and diet. In: Przybylski R, McDonald BE (eds)



- Development and processing of vegetable oils for human nutrition. AOCS Press, Champaign, pp 20–35
- Regulations amending the food and drug regulations (Nutrition labelling, nutrient content claims and health claims), Department of Health, Canada Gazette, Part 11, 1 January 2003
- Ratnayake WMN, Zehaluk C (2005) trans fatty acids in foods and their labelling regulations. In: Akoh CC, Lai OM (eds) Healthful lipids. AOCS Press, Champaign, pp 1–32
- Transforming the food supply. Report of the *trans* fat task force submitted to the Minister of Health. June 2006 (Internet address: http://www.healthcanada.ca/transfat)
- Fritsche J, Steinhart H (1998) Analysis, occurrence, and physiological properties of *trans* fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA)—A review. Fett/Lipid 100:190–210
- Ackman RG, Hooper SN, Hooper DL (1974) Linolenic acid artifacts from the deodorization of the oils. J Am Oil Chem Soc 69:42–49
- Beare JL, Tovel D, Murray TK (1965) The total cis methylene interrupted fatty acids in Canadian margarines. Can Med Assoc J 93:1219
- Beare-Rogers JL, Gray LM, Hollywood R (1979) The linoleic acid and trans fatty acids of margarines. Am J Clin Nutr 32:1805–1809
- Saharsrabudhe MP, Kurian CJ (1979) Fatty acid composition of margarines in Canada. J Ins Can Sci Technol Aliment 12:140– 144
- 18. Beare-Rogers JL, Hollywood R, O'Grady E (1985) transFatty acids in Canadian margarines. Can J Public Health 76:276–277
- Ratnayake WMN, Hollywood R, O'Grady E (1991) Fatty acids in Canadian margarines. Can Inst Sci Technol J 24:81–86
- Ratnayake WMN, Pelletier G, Hollywood R, Bacler S, Leyte D (1998) trans fatty acids in Canadian margarines: recent trends. J Am Oil Chem Soc 75:1587–1594
- 21. Innis SM, King DJ (1999) trans fatty acids in human milk are inversely associated with concentrations of essential all-cis n-6 and n-3 fatty acids and determine trans, but not n-6 and n-3, fatty acids in plasma lipids of breast-fed infants. Am J Clin Nutr 70:383–390
- 22. AOCS, Determination of cis-, trans-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method, official and recommended practices of the AOCS, 5th edition Revisions and corrections. AOCS Press, Champaign, Official Method Ce 1h-05, approved 2005, revised 2005
- Ratnayake WMN, Pelletier G (1992) Positional and geometrical isomers of linoleic acid in partially hydrogenated oils. J Am Oil Chem Soc 69:95–105
- 24. Ratnayake WMN (2004) Overview of methods for the determination of *trans* fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. J AOAC Int 87:523–539

- 25. Ratnayake WMN, Hansen SL, Kennedy MP (2006) Evaluation of the CP-Sil 88 and SP-2560 columns used in the recently approved AOCS official method Ce 1h-05: determination of cis-, trans-m saturated, monounsaturated, and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method. J Am Oil Chem Soc 83:475–488
- Turpeinen AM, Mutanen M, Aro A, Salminen I, Basu S, Palmquist DL, Grinari JM (2002) Bioconversion of vaccenic acid to conjugated linoleic acid in humans. Am J Clin Nutr 76:504–510
- Lock AL, Parodi PW, Bauman DE (2005) The biology of *trans* fatty acids: implications for human health and the dairy industry.
 Aust J Dairy Technol 60:3–12
- Belury M (2002) Dietary conjugated linoleic in health: physiological effects and mechanisms of action. Annu Rev Nutr 22:505–531
- Salas-Salvadó J, Márquez-Sandoval F, Bulló M (2006) Conjugated linoleic acid intake in humans: a systematic review focussing on its effect on body composition, glucose, and lipid metabolism. Crit Rev Food Sci Nutr 46:479–488
- Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH, Tracy RP, Siscovick DS (2006) Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults. The cardiovascular health study. Circulation 114:209–215
- Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH, Copas MK, Cobb LA, Siscovick DS (2002) Cell membrane *trans*-fatty acids and the risk of primary cardiac arrest. Circulation 105:697–701
- Keys A, Anderson JT, Grande F (1965) Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metabolism 14:776–778
- Hegsted DM, McGandy RB, Myers ML, Stare FJ (1965) Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 17:281–295
- Hodson L, Skeaff CM, Chisholm WM (2001) The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. Eur J Clin Nutr 55:908–915
- 35. Thijssen MA, Mensink RP (2005) Small differences in the effects of stearic acid, oleic, and linoleic acid on the serum lipoprotein profile of humans. Am J Clin Nutr 82:510–516
- Lands WEM (1992) Biochemistry and physiology of n-3 fatty acids. FASEB J 6:2530–2536
- 37. Hibbein JR, Nieminen LRG, Blasbalg TL, Riggs JA, Lands WEM (2006) Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity. Am J Clin Nutr 83(suppl):1483S–1493S
- Calder PC (2004) n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. Clin Sci (Lond) 107:1–11

