Overview of SALATRIM, a Family of Low-Calorie Fats

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INTRODUCTION

The desire for lower body weight and healthier eating has stimulated interest in foods with reduced calories and/or less fat. The richest source of calories in the diet is fat (9 kcal/g for fat vs 4 kcal/g for protein and carbohydrate); therefore, control of calories from fat provides the most effective means of controlling total calories. The Surgeon General has recommended that no more than 30% of the calories in the diet should come from fat (U.S. Department of Health and Human Services, 1988). Currently, American and Western European diets frequently contain in excess of 40% of calories from fat. In addition to calories, fats frequently impart improved sensory properties to food including richness or smoothness. Many flavor components are soluble in fats; therefore, changes in the fat content of a food can significantly change the flavor balance of the food. A fat substitute that provides the physical properties of fat, but with reduced calories, could provide a significant benefit to the consumer.

SALATRIM is a family of structured triacylglycerols developed by Nabisco Foods Group, which provides the physical properties of fat but with approximately half of the calories of a normal edible oil. The SALATRIM (*s*hort *a*nd *l*ong *a*cyl*tri*glyceride *m*olecule) triglycerides are composed of mixtures of long-chain saturated fatty acids (predominantly stearic) and short-chain fatty acids (acetic, propionic, and/or butyric) esterified to the glycerol backbone. Thus, the SALATRIM family members can include an infinite number of low-calorie fat products resulting from these mixtures of long-chain saturated fatty acids and the short-chain fatty acids. The papers following this overview describe the chemical characterization, the safety studies, and the clinical evaluation for the SALATRIM family of low-calorie fats, all with a view of demonstrating the versatility and efficacy of SALATRIM fats.

BACKGROUND

Most protein- and carbohydrate-based fat mimetics, such as Simplesse or dextrins, can be used with varying degrees of success in high-moisture foods but have very limited application in baked or other low-moisture products. A water-based system, while providing proper mouth feel, can alter the distribution of flavors in food requiring substantial reformulation of flavor mixtures. At the other extreme, zero-calorie nonabsorbable fat replacements can contribute to anal leakage (if fat is liquid at body temperature) and significantly reduce the absorption of fat-soluble vitamins. These problems can be overcome by altering the fatty acid composition to raise the melting point of the fatlike molecule to be higher than body temperature, but while this change helps to correct the physiological disturbance, it also limits the range of functionality of the fat substitute.

Nabisco's approach to developing lower calorie fats was to provide a functional series of fat substitutes that are based on two principles. First, short-chain fatty acids provide fewer calories per unit weight than long-chain fatty acids, which is discussed in detail by Finley et al. (1994a). Second, because stearic acid is poorly absorbed, also discussed by Finley et al., stearic acid-containing fats impart less energy. Normally, fats containing high levels of long-chain saturated fatty acids would have melting temperatures too high for food applications. However, the melting range of SALATRIM is controlled by incorporation of various preparations of short-chain fatty acids on the triacylglycerol with the long-chain saturated fatty acids. With SALATRIM, the ratio of short-chain fatty acids to long-chain fatty acids is used to obtain even greater flexibility in the functional range of the fat substitute.

Many natural and modified food fats are commercially available to provide a variety of functional properties in foods. Because selection of fatty acid distribution is possible with the SALATRIM family of fats, the potential for providing an extensive range of functional properties in foods is high. Specifically, the ratio of short-chain (i.e., acetic, propionic, butyric) to long-chain fatty acids (i.e., stearic acid) allows the physical properties of common food fats to be closely matched. As an example, cocoa butter melts around 32 °C and thus provides the cooling mouth feel and smooth melt associated with chocolate. Importantly, SALATRIM preparations have been made to closely emulate the cocoa butter melting profile. Other SALATRIM preparations are useful in baked products and in filled dairy products.

CHEMISTRY OF SALATRIM

SALATRIM is a "family" of triacylglycerols produced by the interesterification of highly hydrogenated vegetable oils with triacylglycerols of acetic and/or propionic and/or butyric acids. The resulting mixture of triacylglycerols contains fatty acid distributions representative of the starting material randomly distributed on the glycerol backbone. The conditions of the synthesis and the random nature of the triacylglycerols are described in detail by Klemann et al. (1994a). The actual distribution of triacylglycerides in various SALATRIM preparations has been measured and compared to the expected random triacylglycerol distribution. This comparison clearly shows that the triacylglycerol composition of the mixtures is close to the theoretically predicted random distribution, and the results support the highly predictable interesterification chemistry used to produce SALATRIM (Softly et al., 1994). The chemical characterization of these unique triacylglycerols required some modification of existing IUPAC procedures reported by Huang et al. (1994a). This paper provides a detailed description of how a complex triacylglycerol mixture, such as SALATRIM, can be fractionated and analyzed to quantify specific triacylglycerols whose identification can be confirmed through mass spectroscopy. Structures are further elucidated by Henderson et al. (1994), who applied both proton and one- and two-dimensional ^{13}C NMR to confirm the positional isomers of SALATRIM triacylglycerols. Analyses were also conducted to determine the amounts of mono- and diglycerides, unsaponifiables, tocopherols, and phytosterols in the SALATRIM preparations (Softly et al., 1994).

CALORIC AVAILABILITY

Caloric availability of food ingredients can be estimated on the basis of predictable pathways of digestion, but

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substantiation of the predicted caloric availability is a more difficult task. To address this problem, Finley et al. (1994d) developed a simple rat assay for measuring the caloric bioavailability of food fats. The method is based on a 2-week growth assay with rapidly growing young rats, which provided caloric bioavailability values that agree with the literature values for lard, tallow, and cocoa butter. Caloric bioavailability ranges for SALATRIM preparations were established using this method. Hayes et al. (1994a) report metabolic studies using radiolabeled material to confirm the fate of all portions of the SALATRIM molecule in rats and to verify the caloric bioavailability. Klemann et al. (1994b) developed a hypothesis explaining the caloric data and suggested likely models for the digestibility of SALATRIM molecules. In balance studies conducted as part of the human clinical trials with SALATRIM, the limited availability of stearic acid was confirmed, and the calculated caloric availability was essentially the same as that reported in the rat bioassay and metabolism studies of Finley et al. (1994a). The caloric availability of the tested SALATRIM molecules was determined to be approximately 5 kcal/g.

SAFETY STUDIES

On the bais of the literature review reported by Hayes et al. (1994b), toxicological effects would not be expected from a triacylglycerol with stearic acid and short-chain fatty acids. Rapid hydrolysis of the short-chain fatty acids, with subsequent conversion to carbon dioxide, would be expected, but stearic acid, the predominant long-chain fatty acid, would be only partially absorbed. The literature suggests that the stearic acid in the 1- and 3-positions of the triacylglycerol would be hydrolyzed by lipases, and the free stearic acid would be poorly absorbed. The stearic acid in the 2-position would be likely to remain on the glycerol, and the monoglyceride would be absorbed. A substantial portion of the absorbed stearic acid would then be converted to oleic acid. Klemann et al. (1994b) proposed that about 50% of the total stearic acid should be absorbed, and in metabolic studies by Hayes et al. (1994b), this proposal was confirmed. The conversion of absorbed stearic acid to oleic acid was also confirmed with approximately 50% of the absorbed stearic acid being converted to oleic acid (Hayes et al., 1994a). The fate of the radiolabeled portions of the SALATRIM molecule followed predicted routes and therefore did not suggest any safety issues with SALATRIM.

To further test the hypothesis of the safety of SALATRIM, five different preparations were tested in 13-week rat assays (Hayes et al., 1994d–f). In all of the assays in which SALATRIM was fed at levels up to 15% of the diet, no toxicologically significant effects were observed. In one of the studies, cecal contents were collected to determine if any unusual effects would be observed in intestinal microflora of rats (Scheinbach et al., 1994). Scanning electron micrographs of the cecal contents failed to reveal any significant change in intestinal microfloral morphology due to ingestion of SALATRIM. In addition, several microbially associated characteristics were analyzed including cecal pH, determination of primary and secondary bile acids, conversion of cholesterol to coprostanol, and conversion of primary phytosterols to secondary phytosterols. Again, as expected, SALATRIM did not elicit any detrimental changes in the animal.

Hanford minipigs were fed SALATRIM at 10% of the diet for 13 weeks by Hayes et al. (1994g) to provide toxicological data from a nonrodent species. As with other animal tests, no toxicologically significant effects were observed when SALATRIM was consumed.

The literature did not suggest that a triacylglycerol or any of the components of SALATRIM would illicit a mutagenic effect, but two studies were undertaken to assess the potential of mutagenic response. Reverse mutation assays were conducted with five strains of *Salmonella typhimurium*, both with and without metabolic activation. As expected, no evidence of cytotoxicity or mutagenicity from the SALATRIM triacylglycerols was found (Hayes and Riccio, 1994). To test for potential toxicity in mammalian cells, Hayes et al. (1994c) conducted chromosomal aberration assays using Chinese hamster ovary cells, unscheduled DNA synthesis in rat hepatocytes, and hypoxanthine–guanine phosphoribosyltransferase assay. In addition, *in vivo* micronucleus assays were conducted in the bone marrow of rats fed 10% SALATRIM in their diets for at least 13 weeks. As predicted, no genotoxic activity was found as a result of exposure to SALATRIM.

A clinical testing program based on the chemical, genetic toxicology, and animal studies was initiated. Five separate clinical studies were conducted to assess the safety of SALATRIM in humans (Finley et al., 1994b,c). An initial single-day exposure (45 or 60 g) of subjects to SALATRIM did not reveal any biochemically measurable changes or clinical events. In subsequent studies exposure to SALATRIM (45 or 60 g/day) for either 4 or 7 days duration resulted in slight increases in serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST). In addition, some subjects reported gastrointestinal discomfort. Krajcovicova-Kudlackova and Dibak (1985) have shown similar increases of serum enzymes in rats fed a high-fat diet. In their studies, the diets contained as much as 36.5% fat, which is higher than used in our studies, which may explain why we did not see the effect in our animal studies. The changes in ALT and AST were not of a magnitude to elicit any clinical concern; however, we felt it was important to determine if they were transitory or not. In a 28-day clinical study in which subjects were exposed to 60 g of SALATRIM a day, both AST and ALT returned to normal levels after an initial increase. In every study the changes in serum enzymes did not occur in all subjects on the test diets. As in the initial clinicals, greater incidence of nausea and bloated feeling was reported in subjects consuming SALATRIM vs controls. Since the subjects were receiving 60 g of fat a day in addition to their regular diet, and since the doses were approximately twice the estimated consumption of the 90th percentile predicted user of foods containing SALATRIM, the 60 g a day consumption was considered excessive yet still did not result in any severe adverse reactions.

CONCLUSIONS

SALATRIM has been shown to be a completely predictable low-calorie fat in all aspects of our investigations. The chemical synthesis results in predictable distributions of triacylglycerols. The caloric availability of SALATRIM was very close to the predicted value and was verified in rats and humans and in metabolic studies using radiolabeled material. No toxic effects were observed in animal studies of up to 13 weeks. In clinical studies, SALATRIM was well tolerated at doses of up to 30 g a day. However, some individuals reported nausea and discomfort after ingestion of 60 g a day, which was not unexpected. Transient low-level increases in serum AST and ALT were observed in humans but were not considered to be clinically significant. All studies conclude that the SALATRIM family offers strong potential as a reduced-calorie fat substitute in several food applications.

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