Volatile and Nonvolatile Nitrosamines in Fish and the Effect of Deliberate Nitrosation under Simulated Gastric Conditions

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Of 63 samples of various fish and seafoods analyzed for volatile nitrosamines only seven contained >1 ppb of *N*-nitrosodimethylamine (NDMA) (highest level, 4.2 ppb) and two contained 1.9 ppb of *N*-nitrosopyrrolidine (NPYR) each. The overall average level of NDMA detected was, however, extremely low (≈0.3 ppb). Approximately 85% of the smoked fish and salted/dried fish were positive for NDMA but most of the canned (n = 16) and all of the 10 fresh fish or seafood samples were negative. The identity of NDMA in two samples was confirmed by high-resolution mass spectrometry. All 14 samples analyzed for *N*-nitrosothiazolidine and another 10 analyzed for nonvolatile hydroxylated nitrosamines gave negative results. Of 14 samples (some smoked) analyzed for nonvolatile nitrosamino acids eight contained traces (average, 6 ppb) of *N*-nitrosoproline and six contained varying levels (27 to 344 ppb) of *N*-nitrosothiazolidine-4-carboxylic acid (not yet confirmed by mass spectrometry). The results of the deliberate nitrosation experiments suggested that the formation of significant amounts of volatile nitrosamines in the stomach, after ingestion of these foods, was very unlikely.

It is well-known that many fish and fish products (e.g., salted and dried fish) as well as various seafoods are rich in amino compounds mainly trimethylamine oxide, trimethylamine, and dimethylamine (Castell et al., 1971; Maga, 1978; Reay and Shewan, 1951). Since all of these compounds, especially dimethylamine, can undergo nitrosation and form *N*-nitrosodimethylamine (NDMA), which is a potent carcinogen, considerable attention has been paid in recent years to the search for the presence of NDMA and other *N*-nitrosamines in these food products. Thus, numerous reports (Fazio et al., 1971; Fong and Chan, 1973; Gadbois et al., 1975; Iyengar et al., 1976; Huang et al., 1977; Maki et al., 1978) have been published describing the finding of traces to fairly high levels (up to 1 ppm) of NDMA and other volatile *N*-nitrosamines in both fresh and processed (salted and dried) fish sold for human consumption. Although the responsible nitrosating agents were not cleary identified in all the cases, they were believed to have originated in a variety of ways: as impurities in crude salt used for the preparation of salted and dried fish (Fong and Chan, 1973), as nitrate and nitrite additives in certain types of smoked fish (Fazio et al., 1971), and as NO_x gases where fish was cooked on an open flame with natural gas or propane as fuel (Kawabata et al., 1980).

Although various studies have confirmed the presence of some volatile nitrosamines (mainly NDMA) in various fish and sea foods, there appears to be some discrepancies as to both the qualitative and quantitative nature of the findings reported in the literature. For example, Maki et al. (1978) in Japan reported the finding of fairly significant levels (10–28 ppb) of *N*-nitrosodi-*n*-butylamine (NDBA) and *N*-nitrosodiisobutylamine (NDiBA) in certain dried fish but in a later study the same group of researchers (Maki et al., 1979) did not observe the presence of these two nitrosamines in similar fish products. As far as it is known no other laboratories have detected the presence of these two nitrosamines in fish and seafoods. Similarly, using a nonspecific gas chromatographic method Fong and Chan (1973) reported the occurrence of unusually high (up to 1 ppm) levels of NDMA in certain types of Chinese salted and dried fish but in a later study Huang et al. (1977) found only 1–35 ppb of NDMA in similar salted fish products. In the latter study, a highly specific and sophisticated gas–liquid chromatographic–mass spectrometric (GLC–MS) technique was used for the analysis of the samples.

More recently, many laboratories have been using the Thermal Energy Analyzer (TEA) technique, which is highly specific for *N*-nitroso compounds (Fine and Rounbehler, 1975), for the analysis of volatile nitrosamines in fish and seafoods. These studies (Bogovski et al., 1982; Groenen et al., 1982; Kawabata et al., 1980; Maki et al., 1979; Pederson and Meyland, 1981) again confirmed the presence of certain volatile nitrosamines (mainly NDMA) in various fish and salted and pickled fish, but the actual levels found in various products seemed to vary widely (from 0.1 to 38 ppb). Such variations could probably be attributed to a number of factors such as type of fish (species, etc.) analyzed, their sources, and processing conditions used to prepare these products. However, no such recent data are available for Canadian and USA products. Also, most of the previous studies dealt only with the levels of volatile nitrosamines; very little data are available for nonvolatile nitrosamine contents of such products. Hence, the present study was initiated.

Furthermore, a recent study by Groenen et al. (1982) suggested that there was a possibility of increased formation of NDMA after incubation of various fish and seafoods with simulated gastric juice and saliva containing thiocyanate (a well-known catalyst for N-nitrosation) and nitrite. These workers noted formation of fairly high levels (24 to 440 ppb) of NDMA after incubation of some samples of cod, mackerel, whitting, herring, shrimp, and crab with simulated gastric juice and saliva. Such formations of NDMA after deliberate nitrosation under simulated gastric conditions were found to be a general phenomenon with most of the samples examined regardless of processing (e.g., smoking, drying, canning) used to prepare the fish and various seafoods. Since fish and seafoods are an important part of man's diet, these findings may have far reaching implications to human health. For this reason, many of the samples in the present study were reanalyzed after deliberate nitrosation to determine the extent of such formations of NDMA or other volatile nitrosamines.

EXPERIMENTAL SECTION

Reagents. Solutions (ca. 100 μg/mL of each in ethanol) of N-nitrosodiethanolamine (NDELA) and the volatile nitrosamine standards were purchased from Thermo

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Electron Corporation, Waltham, MA. *N*-Nitroso-3-hydroxypyrrolidine (NHPYR) was synthesized as described previously (Sen et al., 1976). *N*-Nitrososarcosine (NSAR), *N*-nitrosoproline (NPRO), *N*-nitrosopipecolic acid (NPIC), *N*-nitrosothiazolidine-4-carboxylic acid (NTCA), *N*-nitrosothiazolidine (NThZ), *N*-nitrosobutyl-(4-hydroxybutyl)amine (NBHBA) standards were received as gifts (see Acknowledgements). Dichloromethane and the other organic solvents were all of glass-distilled grades and purchased from Caledon Laboratories, Georgetown, Ontario. Pepsin was obtained from Fisher Scientific Limited. All other reagents used were of analytical grade and were purchased from various commercial suppliers. ***PRECAUTION***! Since *N*-nitroso compounds are potent carcinogens, extreme care should be taken while handling or working with these chemicals.

Reagent Blank. To ensure absence of contamination due to the presence of nitrosamines in reagents, a reagent blank was run as described below under the Analysis of Volatile Nitrosamines section except no food sample was included. Also, each bottle of dichloromethane was tested for nitrosamine contamination before use.

Apparatus. *GLC–TEA.* A Varian gas chromatograph (Model 2700) coupled to a TEA detector (Thermo Electron Corporation, Waltham, MA, Model 502) was used for the analysis of volatile nitrosamines. For details of GLC parameters (e.g., column, carrier gas flow, etc.) and operating conditions for the GLC–TEA system, see Sen et al. (1982). For NThZ analysis a GLC column without any added alkali was used.

Mass Spectrometer. A Varian Mat (Model 311A) mass spectrometer (MS) equipped with an electron-impact ionization source and coupled to a Varian Aerograph gas chromatograph (Model 1400) was used for the MS confirmation. The GLC column was similar to that used for GLC–TEA analysis (Sen and Seaman, 1982).

Fish and Seafood. All samples were purchased locally in the Ottawa–Hull region. Although they were purchased locally most of the products originated from other parts of Canada and some were imported from other countries (e.g., Hong Kong, U.K.). The samples were normally stored at 4 °C and analyzed as soon as possible (usually within a week). Each sample was homogenized thoroughly with a blender before taking an aliquot for analysis.

Analysis for Volatile Nitrosamines. A 20-g aliquot of the homogenized sample was analyzed by the low-temperature vacuum distillation method as described previously (Sen et al., 1979). Briefly, a suspension of the sample and 100 ng of *N*-nitrosodi-*n*-propylamine (NDPA), internal standard, in 200 mL of 3 N potassium hydroxide was distilled under vacuum at 45–50 °C in an all glass flash evaporator, and the aqueous distillate made alkaline and extracted with dichloromethane. The dichloromethane extract was washed successively with an acidic buffer (to remove amines) and dilute alkali, the organic layer was dried by passing through a layer of anhydrous sodium sulfate, and the dried extract was concentrated to 1.0 mL by using Kuderna-Danish (D-D) concentrators (both macro and micro). The concentration of volatile nitrosamines in the final extract was carried out by analyzing 6–8 μL of the extract by gas–liquid chromatography–thermal energy analyzer (GLC-TEA). The experimental conditions allowed the detection and quantitation of the following volatile nitrosamines: NDMA, *N*-nitrosodiethylamine (NDEA), NDBA, NDiBA, *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosomorpholine (NMOR), and *N*-nitrosothiazolidine (NThZ). The detection limit of the method was approximately 0.1 ppb each for the first two compounds and about 0.3–0.5 ppb for each of the other nitrosamines.

GLC–MS Confirmation of the Volatile Nitrosamines. Prior to GLC–MS confirmation the extract obtained from the step above was mixed with 10 mL of *n*-pentane (nitrosamine free), and the mixture cleaned up on a basic alumina (3% water content) column (1 cm × 4 cm) as reported previously under headings 9.3.4 to 9.3.7 (Sen, 1978). The purified extract was carefully concentrated to 0.5 mL (with macro and micro K-D conentrators) and a 2–5-μL aliquot used for GLC–MS confirmation. The MS was operated in the selective ion monitoring mode for the molecular ion of NDMA at a resolution of 5000–7000. Operating conditions: source temperature, 250 °C; emission current, 2 mA; electron voltage, 71 eV; accelerating voltage, 3 kV. The GLC was operated under isothermal (115 °C) conditions.

Determination of Nonvolatile *N*-Nitroso Compounds. Since there is no reliable general method for the determination of nonvolatile *N*-nitroso compounds in foods, the samples were only analyzed for two specific classes of compounds for which reasonably good methods are available at the present time. These two groups of *N*-nitroso compounds referred to are the hydroxylated nitrosamines and the nirosamino acids, both of which are classified as nonvolatile nitrosamines, because (due to their low steam volatility) these compounds cannot be detected by the normal method used for the analysis of the volatile nitrosamines.

Analysis of Hydroxylated Nitrosamines. The methodology used for these compounds was similar to that reported by us previously for the analysis of cured meats and fried bacon (Sen et al., 1982). Briefly, the method consisted of extraction of the sample with acetonitrile, removal of fats and lipids by extraction of the acetonitrile extract with *n*-hexane, cleanup of the defatted extract on acidic alumina column, and final analysis by GLC–TEA after derivatization of the compounds (standards or that present in the extract) with a silylating reagent. The method worked highly satisfactorily for the analysis of NBHBA, NHPYR, and NDELA.

Analysis of Nitrosamino Acids. A 10–20-g aliquot of the sample was analyzed for nitrosamino acids, mainly NSAR, NPRO, and NTCA, by a method described previously (Sen et al., 1985). The method is based on extraction of the sample with methanol in the presence of sulfamic acid (to prevent artifactual formation of *N*-nitroso compounds) and dilute sulfuric acid, removal of fats and lipids by partitioning of the methanol extract with *n*-hexane, removal of methanol by evaporation (rotary evaporator) and transfer of the compounds into dilute aqueous acid, reextraction of the nitrosamino acids into ethyl acetate, esterification of the nitrosamino acids with diazomethane, and GLC–TEA analysis of the ester derivatives. NPIC was routinely added to each sample as an internal standard to monitor the efficiency of the overall method.

Incubation of Fish Homogenates with Nitrite under Simulated Gastric Conditions. *System I.* A 20-g aliquot of the homogenized sample was mixed with 50 mL of water and 1 mL each of potassium thiocyanate (5 mg/mL) and sodium nitrite (1000 μg/mL, prepared fresh daily), and the pH of the mixture was adjusted to 3 to 4 (optimum pH for nitrosation of dimethylamine and other strongly basic amines) with gradual addition of 1 N hydrochloric acid. The mixture was then incubated for 1 h in the dark at 37 °C with gentle shaking. At the end of the incubation period the excess nitrite, which could oth-

Table I. Levels of Volatile Nitrosamines[a] Detected in Various Fish and Seafoods

kind	no. of positive/total	NDMA level, ppb mean	NDMA level, ppb range
various canned products (shrimp, tuna, salmon, mackerel, oysters, sardines, etc.)	4/16	trace	trace
various fresh fish (salmon, sole, perch, haddock, cod, etc.)	0/10		
frozen cod and sole fillet	1/3	0.2	0.7
miscellaneous smoked fish (mackerel, herring, cod, salmon, haddock, etc.)	11/16	0.6	0.3–3.3
miscellaneous salted/dried fish (herring, cod, hake, caplin, turbot, mackerel, etc.)	16/18	0.6	0.2–4.2[b]

[a] The samples were negative for other volatile nitrosamines except two smoked fish each contained 1.9 ppb NPYR (detection limit, 0.1–0.2 ppb). [b] NDMA in two samples (one cod containing 0.9 ppb and one hake containing 4.2 ppb) confirmed by GLC–MS.

erwise form nitrosamines as artifacts during analytical workups, was destroyed by adding 1 mL of 1% sulfamic acid to the mixture and allowing it to react with the nitrite for 5 min. The sample was then mixed with 130 mL of 3 N potassium hydroxide and analyzed for volatile nitrosamines as described above. It should be noted that the concentrations of potassium thiocyanate and sodium nitrite in the incubation mixture were approximately 70 ppm and 15 ppm, respectively.

System II (with Pepsin). This system was similar in composition to that used by Groenen et al. (1982). A 20-g aliquot of the homogenized fish was mixed with 40 mL of simulated gastric juice (0.64 g of pepsin, 0.4 g of sodium chloride, and 1.4 mL of concentrated hydrochloric acid diluted to 200 mL with water) and 10 mL of simulated saliva (5.0 mg of sodium nitrite and 200 mg of potassium thiocyanate dissolved in 100 mL of water), and the mixture adjusted to pH 3 with 1 N hydrochloric acid. The sample was then incubated in the dark for 2 h at 37 °C, the excess nitrite destroyed after incubation with the addition of sulfamic acid as in system I, and the mixture analyzed for volatile nitrosamines by the low-temperature alkaline distillation method as described above. In a few cases, the pH of the incubation mixtures was adjusted to pH 1 (instead of 3). The concentrations of potassium thiocyanate and sodium nitrite in the final incubation mixtures were approximately 80 ppm and 20 ppm which were comparable to those used in system I. It should also be noted that none of the incubated samples (system I or II) were analyzed for nonvolatile nitrosamines or for NThZ—a compound included in our volatile nitrosamine methodology only very recently.

RESULTS AND DISCUSSION

Table I presents the results obtained for the volatile nitrosamine contents of various fish and seafoods analyzed in the study. Although traces of NDMA were detected in quite a few samples, especially in the smoked and salted varieties, the average level of NDMA detected was extremely low (<1 ppb). Only seven samples (one each of smoked bloater and black Alaskan cod, two smoked cod, and one each of salted herring, salted/dried hake, and cod) contained NDMA in greater than 1 ppb level, and two (smoked cod and smoked kippers) contained 1.9 ppb NPYR each. In two of these positive samples, including a salted/dried hake which contained 4.2 ppb NDMA, the identity of NDMA was confirmed by GLC–MS. Most of the canned and all the fresh fish analyzed were negative. It might be worth mentioning that not all the samples in Table I were analyzed for NThZ. Only 14 samples, including eight smoked fishes, at the latter part of the study were analyzed for NThZ. All were negative except one smoked trout which contained extremely low levels (0.9 ppb) of NThZ. It appears, therefore, that unlike the case of smoked cured raw bacons, which have been reported to be positive for NThZ in nearly all cases (Pensabene and Fiddler, 1983), some smoked fish may not contain NThZ. This difference might be attributed to the absence of a nitrosating agent in fish. Nitrite is not permitted in fish and seafoods in Canada but allowed in certain types of smoked fish products in the USA. It will be interesting to determine if such cured smoked fish contain any NThZ.

Table II. Effect of Incubation with Simulated Saliva under Acidic Conditions on the Levels of Volatile Nitrosamines in Various Fish[a]

type of fish	NDMA levels, ppb[b] before incubation	NDMA levels, ppb[b] after incubation
smoked mackerel	0.3	1.2
smoked herring	0.3	0.5
smoked spratts	0.9	1.5[c]
salted mackerel	0.2	1.3[c]
salted herring	0.4	1.3
salted/dried cod	0.9[c]	0.6[c]
salted/dried hake	4.2[c]	6.0
salted turbot	0.8	0.6
salted/dried caplin	0.9	1.0
salted herring	0.4	N[d]
salted mackerel	0.2	0.3
salted cod	0.3	0.5
salted/dried cod	0.4	0.5
smoked bloaters	1.3	1.5
salted herring	1.1	0.7
salted herring	0.4	0.6,[e] 0.4[f]
salted/dried cod	0.9	1.7[e]
salted turbot	0.6	0.4,[e] 0.4[f]
salted herring	1.1	0.4,[e] 0.2[f]
fresh haddock	N	0.3
frozen cod	0.7	1.1
fresh cod	N	0.3
canned mackerel	0.3	1.1
canned tuna	N	0.4

[a] Most samples were incubated under simulated gastric conditions as described under system I (see the Experimental Section). [b] The samples were negative for other volatile nitrosamines except NThZ which was not included in the analytical protocol. [c] Confirmed by GLC–MS. [d] N = negative (<0.1 ppb). [e] These samples were incubated using system II (see the Experimental Section). [f] Same conditions as in system II except the pH of the incubation mixture was adjusted to ≃1.

The results for the incubation experiments involving deliberate nitrosation are presented in Table II. Of 24 samples investigated only in four or five cases was there a noticeable (≥2-fold) increase in the levels of volatile nitrosamines, mainly NDMA. For some samples, the NDMA level was slightly higher before incubation than after. However, the difference was extremely small, and was probably due to experimental variations. The lack of formation of significant amounts of NDMA in these experiments should not be entirely unexpected if one examines the kinetics of nitrosation of the amines found in fish products. The main precursors of NDMA in fish are believed to be dimethylamine, trimethylamine, and trimethylamine oxide (Kunisaki et al., 1977; Ohshima and Kawabata, 1978; Scanlan et al., 1974) all of which are abundant in various fish, especially marine fish (Castell et al., 1971; Maga, 1978; Smith, 1980). However, because dimethylamine is a strongly basic amine its rate of nitrosation is very slow, and that for the trimethylamine and

trimethylamine oxide are even slower (Fiddler et al., 1972; Mirvish, 1975). Substantial amounts of NDMA are formed from these precursors only in the presence of higher levels of nitrite (>100 ppm) and at elevated temperatures (e.g., 100 °C) (Scanlan, 1975).

The nitrite and thiocyanate in the human stomach mainly originate from saliva although considerable amounts of nitrite could also be ingested through nitrite preserved meats and fish. The concentrations of nitrite (as Na^+ salt) and thiocyanate (as K^+ salt) in the gastric juices of human volunteers have been found to vary in the range of 0.7 to 21 ppm and 46 to 145 ppm, respectively, depending on various factors such as type of meal consumed, smoking habits (concentration of SCN^- is higher in saliva of smokers than nonsmokers), etc. (Walters et al., 1976). Therefore, the concentrations of the two chemicals used in our incubation studies reflect situations which could be encountered under realistic conditions. It might be of interest that in an experiment with salted herring we inadvertently used a 20-fold higher concentration of nitrite (285 ppm in the incubation mixture) which resulted in the formation of significant amounts (18 ppb or 260 ng of total) of NDMA. However, such a high concentration of nitrite is unlikely to be found in the human stomach.

It should be noted that adequate control experiments were carried out to guard against the possibility of artifactual formation of nitrosamines or against losses of volatile nitrosamines that might occur while carrying out the analyses. This was done by analyzing a few samples with 5000 ppb of added di-n-butylamine (a marker amine), but no artifactual formation of NDBA was observed. Additionally, a few positive (for NDMA) samples were reanalyzed by a modified low-temperature vacuum distillation method in which the samples were distilled from 200 mL of 1% sulfamic acid (instead of 3 N potassium hydroxide), which is an inhibitor of *n*-nitrosation. The results were similar to those obtained before, thus suggesting the absence of artifactual formation. Furthermore, the unreacted nitrite in the incubation experiments was first destroyed by treatment with excess sulfamic acid before distilling the samples. Therefore, it is highly unlikely that any of the nitrosamines detected in the samples were formed as an artifact.

The percentage recoveries of the method were routinely checked by incorporating NDPA as an internal standard. The recovery values were consistently good (80–90%). In a few cases, the percentage recoveries for all the volatile nitrosamines added to fish at a level of 5 ppb each were checked for both the alkaline and acidic sulfamic low-temperature vacuum distillation methods. For both methods the recovery for all the nitrosamines was excellent (85–100%), except in the case of the sulfamic method in which NPYR and MNOR were recovered only to the extent of 50%. Since the latter method was used only as a check against artifactual formation and since none of the samples contained significant levels of NPYR or NMOR (as determined first by the alakline method), this drawback of the sulfamic method did not deter its usefulness.

In addition to the analysis for volatile nitrosamines nine samples of various smoked or salted fish were analyzed for the hydroxylated nitrosamines. None contained detected levels of NBHBA, NHPYR, or NDELA, although one sample (salted mackerel) gave an indication of the presence of two unidentified TEA positive compounds. Fourteen samples of smoked or salted/dried fish (different from above, but the same as those analyzed for NThZ) were analyzed for the nonvolatile nitrosamino acids. Of these, none contained NSAR, eight contained traces (average, 6 ppb) of NPRO, and six contained NTCA (27, 65, 118, 201, 263 and 344 ppb). However, since none of these compounds has yet been confirmed by GLC–MS the data should be considered as only tentative. Further work is continuing. No data on the carcinogenicity of NTCA are available either. In view of the relatively high levels of this compound recently reported to be present in bacon and other cured meats (Sen et al., 1985) as well as in smoked fish as observed in this study, it will be highly advisable to carry out toxicological studies with this compound.

In summary, it can be concluded that although traces of NDMA occur in various fish and seafoods, especially the salted and dried varieties, its concentration in these products is extremely low. The possibility of formation of significant amounts of volatile nitrosamines in the stomach, due to the interaction of salivary nitrite and amines in the fish, also appears to be remote. Preliminary results on the levels of nonvolatile nitrosamines indicate that significant amounts of NTCA could be present in some products but additional work is needed to determine the extent of the problem.

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Registry No. NDMA, 62-75-9; NPYR, 930-55-2; NPRO, 7519-36-0; NTCA, 94751-62-9.

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Effect of Processing on Flatus-Producing Factors in Legumes

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The effect of various treatments, viz., (1) soaking in plain water and sodium bicarbonate solution, (2) cooking of soaked seeds, (3) autoclaving of soaked seeds, (4) germination, and (5) frying of germinated seeds, was studied by measuring the oligosaccharide contents of five commonly grown legumes, viz., Rajmah (*Phaseolus vulgaris*), Bengal gram (*Cicer arietinium*), black gram (*Phaseolus mungo*), red gram (*Cajanus cajan*), and broad bean (*Vicia faba*). The contents of sucrose, raffinose, stachyose, and verbascose decreased under various treatments. Germination beyond 48 h resulted in complete disappearance of raffinose, stachyose, and verbascose. The 24-h germination is recommended as a reasonably good treatment for legumes for reduction of flatus production as considerable losses of raffinose, stachyose, and verbascose occur during this period.

Legumes consumed by humans in many forms are excellent sources of proteins (20–40%) and carbohydrates (50–60%) and fairly good sources of thiamin, niacin, calcium, and iron (Aykroyd and Doughty, 1977). Some legumes have specific nutrient deficiencies or certain undersirable flavors, flatus factors, and antimetabolites or other toxic substances (Rachie, 1973). Legumes are notorious inducers of flatulence owing to the presence of substantial amounts of flatus-producing oligosaccharides of the raffinose family of sugars (Salunkhe, 1982). Oligosaccharides of the raffinose family of sugars (verbascose, stachyose, and raffinose) are well-known to produce flatus in man and animals (Reddy et al., 1980). Owing to the absence of enzyme (α-galactosidase) capable of hydrolyzing the α1–6 galactosidic linkage, these oligosaccharides accumulate in the lower intestine and undergo anaerobic fermentation by bacteria (Salunkhe, 1982; Gitzelmann and Aurichhio, 1965). Therefore, to utilize legumes as a more acceptable source of inexpensive proteins, it is desirable to reduce the flatulence production. Very limited data are available on the oligosaccharide contents and effects of traditional methods of processing on the oligosaccharide profile of legumes (Iyengar and Kulkarni, 1977; Rao and Balavady, 1978; Reddy and Salunkhe, 1980). The present investigations were, therefore, undertaken (i) to investigate the effect of traditional methods of processing such as soaking, cooking, autoclaving, and germination on the oligosaccharide content of selected legumes and (ii) to evolve a suitable method of processing to decrease the oligosaccharide content of commonly consumed legumes.

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Chart I. Treatments

I	soaking in plain water, A, for 6 h, and then boiling for 60 min and boiling for 45 min soaking in plain water, B, for 12 h, and then autoclaving for 30 min at 15 psi
II	soaking in sodium bicarbonate solution, A, for 6 h, and then boiling for 45 min and boiling for 30 min soaking in sodium bicarbonate solution, B, for 12 h, and then autoclaving for 20 min at 15 psi
III	germination at 24, 48, 72, and 96 h
IV	frying of 24 h germinated seeds for 10 min

MATERIALS AND METHODS

Samples of five common legumes, viz., Rajmah (*Phaseolus vulgaris*), Bengal gram (*Cicer arietinum*), black gram (*Phaseolus mungo*), red gram (*Cajanus cajan*), and broad bean (*Vicia faba*) were obtained from the Department of Plant Breeding, Haryana Agricultural University, Hissar, India.

Processing. The traditional methods of cooking legumes as given in Chart I were followed in this investigation. The samples were soaked in plain water and sodium bicarbonate solution (0.03%) for 6- and 12-h periods at 25 °C. The samples thus soaked were cooked by boiling in 4 times water by weight and autoclaving at 15 psi in double the amount of water for the time specified in Chart I. The soak water was decanted before cooking. The samples to be germinated were surface sterilized with 1% sodium hypochlorite solution, washed thoroughly with distilled water, placed at 30 °C on a damp filter paper, and subjected to analysis at 24, 48, 72, and 96 h of germination.

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