INFLUENCE OF POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METALS ON THE METHOD OF BREAD BAKING

By Ogbeche K A*, and Anyakora C *

*Dept. of Pharmaceutical Chemistry, School of Pharmacy, College of Medicine, University of Lagos.

Abstract

The influence of smoke on various method used to bake bread and the concentrations of some polycyclic aromatic hydrocarbons (PAHs) and heavy metals in baked bread was assessed. Bread samples from different bakeries operated by either electricity , gas, charcoal or fire wood ovens and their residue content of PAHs and heavy metals were assessed. The total concentrations of PAHs detected in electriccity, gas, charcoal and fire wood ovens/bakeries had an average of 5.5, 66.0, 125.3 and 188.7ug/kg respectively. Samples collected from charcoal and fire wood bakeries had a wide spectrum of PAHs in comparison with that collected from gas and electricity operated bakeries. Lead had the highest concentration in the four group of bread sampled, as compared to nickel. The concentration of lead detected in bread samples produced from electricity, gas, charcoal and firewood bakeries were 224.6, 784.7, 1006.9 and 1243.8ug/kg respectively, while the estimated intake of PAHs and lead in electricity operated bakeries are 5.5ug and 224.6ug per person per day. The present study has indicated the comparatively high level of daily intake of benzo(a)pyrene and lead in comparisons to levels reported from many other countries and those recommended by international regulatory bodies.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are generated by incomplete combustion of organic materials arising in part from natural combustion such as forest fires and volcanic eruptions. For the most part, human activities, such as industrial production, transportation(smoke pollution) and waste incineration generate significant levels of PAHs.(1-3). Several PAHs such as benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene are known to be potential human carcinogens(4). These organic pollutants are ubiquitous in the environment and due to their physical and chemical properties they migrate through food chain into hydrophobic compartments and accumulate in lipids at the end of the food chains(5-9). Human exposure to these compounds occurs through the diet and therefore knowledge of the transfer pathways through the food chain is a major issue in food safety. Similarly heavy metals are among most frequently encountered contaminants in the environment. Several reports have focused on residues of numerous heavy metals in foodstuffs(10). Other reports have delineated on the contamination of cereal products, including bread with heavy metals. In Nigeria, bread is a major component of people's diet most especially breakfast. The per capita consumption of bread is among the highest in the sub-region. Local bread is produced in a number of different ways in the country, for instance in the cities or urban areas most of the bakeries are either fully or partially automated using electricity and gas as the main source of energy to operate these bakeries, while in the rural areas bakeries are less well equipped and many of them use the charcoal or fire wood heat generated oven for baking. The concentration level of PAHs and some heavy metals in bread and cereal have been studied because of the paramount importance of these food materials and the heavy reliance of some section of the society on them(11,12). In this present study, the influence of PAHs and heavy metals on the method of bread baking was investigated.

Materials and Method

Sampling: Bread samples were collected from four types of bakeries, electricity and gas mainly from Ikeja and Lagos Island while charcoal and fire wood heat generated bakeries from Sango Ota in Ogun state of Nigeria. 10 loaves were collected from a minimum of three bakeries each, kept in a clean container, labeled and taken to the laboratory for easy identification. Samples were taken directly after being baked not to let them be exposed to any form of contamination. Bread samples of each bakery were separately cut into small pieces and mixed thoroughly, before three sub-samples each of 50g were taken from each bakery for analysis.(13).

Reagents: All solvents used were of analytical grade, potassium hydroxide pure pellets were reagent grade(BDH). Aluminum oxide 90 and silica gel 60 (70-230mesh) for column chromatography were obtained from Merck. Authentic PAHs were purchased from Alltech (USA) as a mixture dissolved in acetone, containing 13 compounds.

Extraction: Samples extraction and clean up was conducted according to the method reported by Ahmed et al.(13). Bread subsamples, each of 50g were reflux extracted for 10h in a soxhlet unit with 200ml of n-hexane. The extract was concentrated to 2ml using rotary evaporator. The concentrated extract was transferred to a round bottom flask and saponified using 100ml of aqueous methanolic KOH (30g KOH dissolved in 30ml of distilled water and 270ml of methanol), to eliminate possible fat material and the mixture was refluxed again for 2h. Saponified material was quantitatively transferred into a separating funnel using 100ml of methanol-distilled water mixture and 100ml of n-hexane, shaken for 5min and left to settle. Aqueous layer was drained out in a beaker and extracted again with n-hexane 100ml. The organic layer was combined and evaporated using rotary evaporator and residue was quantitatively transferred on top of glass column. Adsorption column chromatography was performed to separate the aliphatic hydrocarbons from aromatics and other compounds. The column was topped with 2g of anhydrous Na_2SO_4 and eluted with 50ml of toluene. Polycyclic aromatic hydrocarbon fractions were concentrated to 1ml and transferred to a glass tube, wrapped in aluminum foil ready for analysis.

Capillary gas liquid chromatography: A pye Unicam 304 model gas chromatograph, equipped with a flame ionization detector was used in this study. An analytical capillary column (AT 5, 30m X 0.53), with film thickness of 1.2mm was used to separate polycyclic aromatic hydrocarbons. Column conditions, temperature programme, quantification were performed according to the method reported by Ahmed et al (14).

Validation study: Control bread samples were fortified with a standard solution of PAHs in hexane (10mg/l) at three levels to 50g of bread control sample, 5, 50, and 100ml standard solution to give a final concentration of control samples as 1,10 and 20ml. Each fortification level was replicated three times and the extraction of the control sample was performed as earlier mentioned. The average rate of recovery was 82%.

Heavy metal analysis : Bread samples were left to air dry and tested for contamination by leaching with 5% nitric acid and stored in a high density polyethylene bottles before subsamples of 50g each were taken from each group. 0.5g finely ground samples was weighed into a test tube and 3ml of nitric acid added into each tube and allowed to remain over night at room temperature and protected from dust. Tubes were heated up to 130° C for 4h using a metal block thermostat unit(Liebisch, Germany) and left to cool down before 2ml of nitric acid and 0.7ml of perchloric acid and 43ml distilled water were added to each tube. A programmable circuit was used to raise temperature up to 230° C within 34h. The clear wet ash in each tube was dissolved in 2ml of distilled water and analyzed for element measurement with flame atomic absorption spectrophotometer (Perkin - Elmer, model 3030 equipped with HGA-600 transverse heated graphite furnace, AS-60 auto sampler) in a close circuit cooling system with an electrodeless discharge lamp power supply.(14)

Table 1

Residues of PAHs(mg/kg) detected in bread baked with different type of method

Compound	Fire wood	Charcoal	Gas	Electricity
Fluorene	28	4.7	2.5	0.2
Phenanthrene	10.3	7.6	5.7	0.2
Anthracene	20.3	13.9	11.3	0.3
Pyrene	25.7	17.5	12.8	0.0
Benzo(a)pyrene	56.6	34.7	17.1	4.4
Benzo(a)anthracene	47.8	46.9	16.6	0.4

Results and discussion:

Residues of polycyclic aromatic hydrocarbons detected in bread samples collected from bakeries using different methods are shown on Table 1. The total concentration of PAHs detected in bread samples from electricity, gas, charcoal and fire wood operated heated oven bakeries had an average of 5.5, 66.0, 125.3 and 188.7μg/kg respectively. Samples collected from gas, charcoal and fire wood operated bakeries had a wide spectrum of PAHs, but concentration of individual compounds varied to a considerable extent.

On the other hand residues of PAHs detected in bread from electricity operated bakeries had a much smaller spectrum and concentrations while high concentration of higher molecular weight PAHs such as benzo(a)pyrene and benzo(a)anthracene were detected in fire wood and charcoal operated bakeries. This may indicate that combustion gases emitted from fire wood and charcoal during bread baking are the major source of such high concentrations of PAHs. However the presence of such heavy PAHs residues in smaller concentration in bread samples baked with electricity operated bakery may reflect in the ingredients. This finding would give some support to the assumption that some part of the residues detected in all samples are cereal borne residues and not necessarily the baking process especially in the case with electricity bakery. Various studies have indicated that wheat and maize flour contain appreciable levels of PAHs impinge on crop during growing season.(15-17). Residues of PAHs detected in all bread samples in this study are much higher than residues reported from European countries. For instance in England, Dennis et al (18) reported that the level of benzo(a)pyrene was less than 0.1μg/kg in white flour and similar amount was found in bread, while in Canada, Lawrence and Webber (19) showed that breakfast of wheat and corn product contained a range of 2.5-3.9ug/kg PAHs. Residues of lead and nickel detected from bread samples collected from the various method of heat generated oven bakeries are shown on Table 2. Lead had the highest concentration of 1243.8ug/kg in fire wood

Table 2.Residue of lead and total PAHs detected from different method of baking

Method of baking	Lead content ug/kg	Nickel ug/kg
Electricity	224.6	3.7
Gas	784.7	15.6
Charcoal	1006.9	34.8
Fire wood	1243.8	78.8

followed by charcoal with 1006ug/kg while gas and electricity had 784.7 and 224.6ug/kg respectively, this decreasing order though in lesser amount was indicated with nickel. The variation in the lead content might be due to the baking process and smoke emission rather than the atmospheric lead during cereal growth or storage in open heaps before baking.

Cabrera et al (20) reported that atmospheric contamination, excessive use of fertilizers and pesticides and irrigation with residual water were among causes of contamination of raw food stuffs. Table 1. also indicates the estimated daily intake of total PAHs based on consumption of bread baked with different methods, benzo(a)pyrene, the most regulated PAH compound by law in most countries has often been measured as an indicator of PAH level in food stuffs. In this present study the estimated intake of PAHs based on bread consumption with electric oven only is 5.5ug/kg per person per day, while that of lead is 224.6ug/kg, however reports of daily intake of benzo(a)pyrene from other countries based on bread consumption vary between 0.12 - 3.4ug/kg.

This study therefore suggest that some special care should be taken in bakeries using fire wood and charcoal as heat generated ovens to minimize bread contamination with PAHs and heavy metals.

Acknowledgement

The authors are grateful to Dr Yetunde Soile of Cadbury Nig. Plc., Agindingbi, Ikeja, Lagos, for her technical assistance and Mrs Juliana Abayomi of National Agency of Food, Drug Administration and Control (NAFDAC), laboratory, Lagos, Nigeria, for the use of their atomic absorption spectrophotometer to analyze the heavy metals. We are indebted to the Bakers who kindly agreed to supply the bread used in this investigation. For confidentiality the bakers and exact locations of these bakeries are not disclosed.

References:

1. Baek S.O, Field R.A, Goldstone M.E, Kirk P.W, Lester J.N and Perry R.A.(1991): A review of atmospheric polycyclic aromatic hydrocarbons: sources, fate and behaviour. Water, air soil pollut., 60, pp 279-300.
2. Lorber M, Cleverly D, Schaum J, Phillips L, Schweer G and Leighton T. (1994): Development and validation of an air to beef food chain model for dioxin-like compounds. Environ. Sci. Technol., 156, pp 39-65.
3. Yang H.H, Lee W.J, Chen S.J and Lai S.O. (1998): PAH emission from industrial stacks. J. Hazard mater., 60, pp 159-174.
4. Internal Agency for Research on cancer. (1983): IARC Monographs on evaluation of polynuclear aromatic compounds, Part 1, chemical, environmental, and experimental data; IARC Lyon, France.
5. Madhavan N.D, Naidu K.A. (1995): Polyaromatic hydrocarbons in placenta, maternal blood, umbelical cord blood and milk in Indian women. Hum. Exposure toxicol., 14, pp 503-506.
6. Malachlan M.S (1997): A simple model to predict accumulation of PCDD/Fs in agricultural food chain. Chemosphere, 34, pp, 1263-1276.
7. Basset J.O, Butikofer U, Dafflon D, Koch H.(1998): Type of polycyclic aromatic hydrocarbons on fragnants and perfumes. Sci. aliments, 18, pp 347-359.
8. Roeder R.A, Garber M.J, Schelling G.T.(1998): Assessment of dioxins in foods from animal origins. J animal sci., 76 pp 142-151.
9. Feidt C, Grova N, Laurent C, Rychen and Laurent F.(2000): Le transfert des micropolluants organiques dans la chaine alimentaire. Etat et perspectives de recherche. Oleagineux corps gras lipides. 5, pp 431-435.
10. Llobet J.M, Granero S, Schumacher M, Corbella J and Domingo J.L.(1998): Biological monitoring of environmental pollution and human exposure to metals in Tarragona, Spain. IV. Estimation of dietry intake, Trace elements, 15(3), pp 136-141.
11. Hubbard A.W and Lindsay D.G.(1979): Dietary intake of heavy metals by consumers in the UK, in: Proceedings of the International conference on management and control of heavy metals in the environment, London., pp 52-55.
12. De vos R.H, Van dokkum W and Schouten A.(1990): Polycyclic aromatic hydrocarbons in Dutch total diet samples(1984-1986), Food chem.toxicol. 28, pp 263-268.
13. Ahmed M.T, El-sayed A.H, El Samahy and Youssof K.(2000): The influence of baking fuel on residues of polycyclic aromatic hydrocarbons and heavy metals in bread. J. hazardous materials, A80, pp 1-8.
14. Ahmed M.T, Abdelall G.A Elrasbi S and Asker A.A.(1998):Residues of polycydic aromatic hydrocarbons in fish and water from sultanate of Oman, Int. J. environ. Health res. 8(77), pp 84-86.
15. Stahl W, and Eisenbrand G.(1988): Determination of polycyclic aromatic hydrocarbons and nitrosamines, in: R Macera (E.d.), HPLC in food analysis, Academic press, London, pp 277.
16. Tricker A.R, Perussmann T.J.(1990): Chemical and food contamination in the initiation of cancer, proct. Nutr. Soc., 49, pp 133-144.
17. Alexandra K, Rios M, Kadubar F, Lang N.P and Bartsch H.(1996): Evidence of anti benzo(a)pyrene diol epoxide-DNA adduct formation in human colon mucosa, Carcinogens 17(9), pp 2081-2083.
18. Dennis M.J, Massey R.C, Cripps G, Venn I, Howarth N and Lee G.(1991): Factors affecting the polycyclic aromatic hydrocarbon content of cereals, fats and other food products, Food addict. Contam. 8(4), pp 517-530.
19. Lawrence J.F, Webber D.F.(1984): Determination of polycyclic aromatic hydrocarbon in Canadian samples of processed vegetables product by liquid chromatography with fluorescence detection. Food chem. toxicol. 21, pp 569-574.
20. Cabrera C, Lorenzo M.L and Lopez M.C.(1995): Lead and cadmium contamination in dairy products and its repercussion on total dietary intake, Food chem.,43, pp 2081-2083.
21. Pfannhauser.W.(1991): Polycyclic aromatic hydrocarbons in food and selected vegetables in Austria, Mittel lebensmittelunters Hygiene. 82, pp 66-79.