The Influence of Ethylene oxide Sterilisation Parameters on hygienic Quality of Spice mixtures

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Preface

The problem of spice contamination, specially in food industry, where it can cause economic looses or, less possibly health hasards, is well resolved by the means of Ethylene oxide sterilisation. On the other hand, the problem of residues may be important to the public health, particulary when unadequate fumigation conditions are applied. By my opinion, the question of parameters is of the basic significance to the residual degree, what made me decide to determine the optimal fumigation conditions.

Literature data

Spice mixtures, regarding from the microbiological point of view, may be the cause of food spoilage and - in extreme cases the cause of infections. Spices are initially contaminated with soil microorganisms like spore forming aerobs and facultative anaerobs. Further contamination takes place during harvesting, fermentation, drying and transportation. The contamination may also take place during spice mixtures production. The determination of different types of microorganisms maynot be applicable to the other spices and may be poorly applicable to the spices of the same consignement. The most common groups of microorganisms in spices are moulds, yeasts and bacterials genera Bacillaceae and Enterobacteriaceae.

The number of aerobic bacteria has no direct relation with public health risk, but may indicate a spoilage risk of the product in which a spices are used (10). Total bacterial number varies from a few hundreds in bay lesves, chili, mustard, cinnam on and cloves to more millions in black pepper, paprika, cumin, coriander etc. Aerobic spore number is approximately hundred times smaller comparing with total plate count (5,9, 11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24).

Bacillaceae are present in an average number of a few ten thousands in majority of spices. The dominate species is Bacillus cereus, present in approximately 30 % of samples. Enterobacteriaceae are also commonly present in spices in levels from a few tens to a few thousands. Salmonella does not appears usually, Proteus sp. and Escherichia coli appears seldom(6, 14, 15, 16, 25, 26, 27, 28, 29).

Some spices act also as antioxidative and antibactericidal agents due to their active components like etheral oils, glucosids, aldehids, kethons and kinons. Therefore, the spices influence the reduction of their own microflora and the reduction of the product microflora. The bactericidal activity is noticed mostly in cloves, cinnamon, garlic and unions (30, 31, 32, 33, 34).

Moulds number differs for instance in black pepper from a few to a few millions per gram, in paprika from a few hundreds to a few ten thousands per gram and in ginger from none to a few thousands per gram. The dominate species are Aspergillus and Penicillium (5, 6, 7, 8, 9).

The microorganisms present in spices have not the ideal conditions for multiplication and toxin synthesis. So, the spices themselves do not represent a direct health hasard, but only when added to the product, where the conditions are much more favorable. Herein, the microorganisms quickly reach the exponental degree of multiplication. In this way their methabolism products (gas, acid) may demage the product, or in the case of toxin formation or multiplication of pathogens they may be of a great health hasard risk.

To eliminate the spoilage and the infection possibility, sterilisation of spices is nowadays widly used all over the world. Fumigation, as one of the most common used sterilisation methods has the advantage in its effectiveness on spices micriflora and disadvantages in dangerous processings and potential health hasards of residues. Epoxides employed in fumigation are Ethylene oxide and the asymmetric Propylene oxide. The epoxide molecula reacts practically with all cell structures, cutting off the cells methabolism, multiplication and genetic system, what results the bacterial death (12, 35, 36,37,38).

On the other hand, epoxide reacts with spice structures physicaly by sorption (diffusion, absorption and adsorption) and chemicaly, forming Ethylene glicol and Ethylene halohydrins. The problem of Ethylene glicol formation is not of the same importance as the one of Ethylene chlorhydrin, which LD/50 is approximately hundred times higher (38). There are also undoubed indicitations of its mutagenicy (35). The question of residual carcinogenity is for the moment still undisolved (35).

In the process of fumigation, there are some factors that influence the effect of sterilisation. Temperature plays a double role. First, it influences the vegetation of spore forming mesophyl microorganisms. The effect of Ethylene oxide on spores is neglecting in comparison on the effect on the vegetative forms of bacteria (39). Secondly, the higher applied temperatures result smaller necessary applied parameters (concentration and exposure) and vice versa (37, 38, 40). The concentration and exposure of Ethylene oxide is in a direct connection with effect on present microorganisms of spices (23, 39, 40). The different groups of microorganisms differ in sensitivity to Ethylene oxide activity what

influences the survival degree of different microorganisms. Bacillaceae are the most resistant to the Ethylene oxid in comparison with Coliforms for instance, where the necessary applied parameters must be much more severe when the same effect is to be obtained (40). The action of Ethylene oxide depends a great deal on Water content of spices, which acts as a solution and transfer media. 14 % moist spices for instance ties app. 50 % higher quantities of present epoxide compared with totaly dried spices (4). The rule of atmosphere relative humidity and relative humidity in the chamber during fumigation is not quite clear. Some authors (37) consider them to have a little influence in the fumigation process, while the others (7) consider the influence to be significant. The water content of majority of spices is satisfactory high to obtain the full effect, only few are not. The moistening would result the improovements but later significant increase of microorganisms would be noticed due to stimulating role of increased water content. Multiplication and toxin synthesis ability of microorganisms depends in a great deal on water activity. The minimal a_w values for moulds for instance are between 0,62 and 0,93, for yeasts between 0,62 and 0,92, for Enterobacteriaceae between 0,95 and 0,96 and for Clostridium between 0,97 and 0,98 (41). The a_w values of spices are much below the minimal necessary a_w values of majority of microorganisms what results the unability of present microorganisms to multiply and to form toxins. Further on, the cell mambrane looses its permeability what results the powered employed parameters to obtaine the satisfying effect (39). The storage of fumigated spices does not influence the microbiological quality when the proper storage conditions are applied. The influence of packing and largeness of units is also of a great importance. The packing materials, common used in distribution of spices and spice mixtures usually do not suite the fumigation because of their unpermeability. The penetration of gas is in proportion with thickness of plastic used as packing material. The best packing media for fumigation purposes is jute for granulated spices and natron paper for grained spices (42, 43). The difussion of gas into the spices depends of largeness of spice seeds respectively the degree of graining and of largeness of units. The bigger the unit is, the longer time is necessary for gas to reach the center of the bag, and the prolonged fumigation is needfull.

Due to Ethylene oxide activity, temperature, vacuum and ventilation conditions, there may proceed some chemical and organoleptical changes. The content of etheral oil for instance depreciates, simultaneoussly the reduction of active components is remarked, what influences the lessening of spice activities (23). Organoleptical changes, when applied parameters are not exagerated, are not significant with an exeption of mustard (12).

Ethylene oxide residues immediately after fumigation are relatively high (a few hundreds ppm) but show the expotential tendency of falling. Two to three weeks afterwards they fall to zero respectively to a few ppm what suits the regulations if they exist, but may even represent the health hasard risk (5, 22, 36, 42, 43, 44, 45). There is an open question of other residues like Ethylene halohydrins which quantity increase compared with Ethylene oxide reduction (11, 23, 38, 44, 45, 46, 47).

Materials and Methods

Source of samples

All spice mixtures were produced, fumigated and analysed in HP Droga Portorož, Yugoslavia in 1980.

Packing

Spice mixtures were packed into 50 kg natron bags.

Fumigation method

Fumigation took place in two Degesch chambers (6,9 m^3) under producers instructions (1).

Fumigation media

T-gas, consisting of 90 % of Ethylene oxide and 10 % of Carbon dioxide.

Microbiological analyses

Samples were analysed for Total plate count, aerobic spore count, moulds, coagulasa positive Staphylococcus, Salmonellae, sulfid reductive Clostridia, spore forming sulfid reductive Clostridia, Proteus sp. and Escherichia coli.

Microbiological analyses were done according to Yugoslave Standards (2, 3).

Microbiological media

All media were purchased from TORLAC Beograd, Yugoslavia except Malonat-Phenylalanin broth, which was purchased by DIFCO, Detroit, USA

Determination of residues

Ethylene oxide residues were determined by El Kishen method (4).

Results and discusion

Coagulasa positive Staphylococcus, Salmonellae, sulfid reductive Clostridia, spore forming sulfid reductive Clostridia, Proteus sp. and Escherichia coli were not present under all examinated conditions of fumigation.

The effect on total plate count (I), aerobic spore count (II) and moulds number (III) varried depending on applied parameters. (I) of spice mixtures, fumigated 5 to 6 hours (51 hours) with 400 g of EO/m^3 at 22°C (31°C), falled approximately for two powers and reached the app. values of a few ten thousands per gram. (II) and (III) showed the similar effect with final contamination degree of app. a few hundreds per gram. Drasticaly prolonged fumigation did not influence the essential improovement of effectiveness.

Using 750 g of EO/m^3 for 17 to 18 hours at 23°C resulted the best effectiveness. (I) falled app. for three powers reaching the level of thousand bacterias per gram and even less. (II) and (III) were absent (less than hundred per gram). All the tested temperatures (13°C, 18°C, 20°C, 21°C, 22°C, 23°C, 25°C, 31°C and 34°C) and durations (5^h, 5 - 6^h, 7 - 8^h, 14^h, 17 - 18^h and 51^h) showed a similar effects. (I) varried from a few thousands to a few hundred thousands with an average value of a few ten thousands bacterias per gram. (II) and (III) varried from less than hundred up to a few thousands per gram. Prolongation and temperature increasings resulted only a slide improovements.

Table 1: total plate count, aerobic spore count and moulds number of natural spice mixtures and spice mixtures fumigated under different conditions (the results are expressed as percents of the samples in the different rangs of contamination)

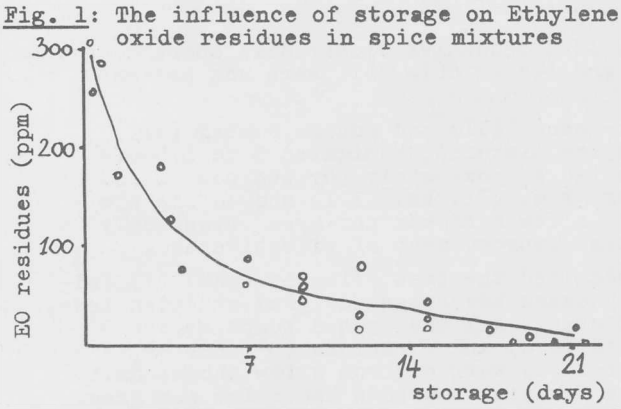
Rang of contamination

Unfumigated spice mixtures

Fumigated spice mixtures

	400 g EO/m^3		750 g EO/m^3							1000 g EO/m^3	
	5-6^h	51^h	5^h		7 - 8^h		14^h	17 - 18^h		5^h	5-6^h
	22°C	31°C	13°C	18°C	23°C	31°C	34°C	20°C	23°C	20°C	22°C
Total plate count											
10^7											
10^6	8										
10^5	30										
10^4	52	10	50	25	42	28	67	10		56	8
10^3	10	60 100	50	63	29	72	33	41		33	84
10^2		20		12	29			39	92	11	8
$<10^2$		10						7	8		
								3			
Aerobic spore count											
10^7											
10^6											
10^5				12						45	
10^4	5	10									
10^3	60	10	15	12	14	28		7		33	25
10^2	30	10 50	75	76	14	28	33	21		22	33
$<10^2$	5	70 50	10		72	44	67	72	100		42
Moulds number											
10^7											
10^6											
10^5								3			
10^4	10	10									
10^3	20	20		12	26			6			28
10^2	50	60 50	75	63	34	14	83	6		88	36
$<10^2$	20	10 50	15	25	50	86	17	85	100	11	36
		22°C 31°C	13°C	18°C	23°C	31°C	34°C	20°C	23°C	20°C	22°C
		5-6^h 51^h	5^h		7 - 8^h		14^h	17 - 18^h		5^h	5-6^h
		400 g EO/m^3	750 g EO/m^3							1000 g EO/m^3	

Fig. 1: The influence of storage on Ethylene oxide residues in spice mixtures



When 1000 g of EO/m^3 was used for 5^h to 6^h at 20°C no improvements were noticed in comparison to fumigation with 400 g of EO/m^3 or 750 g of EO/m^3.

It seems that only one intensified parameter, when others unchanged, cannot essentially improve the effect of fumigation.

The residues of Ethylene oxide were measured immediately after fumigation and in intervals of a few days up to a few weeks. The quantity of residual Ethylene oxide was initially relatively high - a few hundreds ppm. After a few days storage they fall to the values from 70 ppm to 175 ppm and had a constant tendency of falling. Two to three weeks after fumigation they fell to zero respectively to a few ppm.

Being aware that the absence of EO residues do not represent the irrevocability of spice mixtures concerning residues, we believe that the question of EO residues is only a matter of principle but may serve only as a rough indicator of residual contamination in general.

The question of residuals sets up the problem if to apply the fumigation or not. The answer to this is let to the plant technologist, who must estimate the smallest possible demage risk concerning microbiological aspect, economic aspect owing to spoilage of foods, health hasard aspect due to present microorganisms and health hasard aspect due to present residues. The decision is not to be taken in a common way, but must be made with consideration of all the mentioned moments and with full responsibility.

Literature:

1. DEGESCH - Deutsche Gesellschaft für Schädlingsbekämpfung MbH, Frankfurt am Main 1, Neue Mainzer strasse 1, BDR
2. Pravilnik o pogojih, ki jim morajo glede mikrobiološke neoporečnosti ustrezati živila v prometu, Ur.l. SFRJ 2/80, 11.1.1980
3. Pravilnik o metodah mikrobioloških analiz in superanaliz živil, Ur.l.SFRJ 8/77,11.2.77
4. Kishen S.A.El: Determination of Ethylene oxide and methods of its recovery from fumigated substances. J.Sci.Food Agric. 1, 71 (1950)
5. Eschmann K.H.: Gewürze - eine Quelle bakteriologischer Infektionen,Alimenta 4(1965), 83-88
6. Toure B.: Microbiologische Untersuchungen entkeimter Gewürze, Inaugural - Dissertation Institut für Tierärztliche Nahrungsmittelkunde der Justus Liebig Universität,Giesen
7. Horie Y.et al.: Fungi in Spices, J.Food Hygienic Soc.Japan 12(6)(1971),516-519
8. Hadlok R.: Schimmelpilzkontamination von Fleischerzeugnissen durch Naturbelassene Gewürze, Fleischwirtschaft 49(1969),1601-1609
9. Moreau C., Moreau M.: La contamination des epices, ses consequences dans les industries alimentaires. Ind.Alimentaires et Agricoles (1978), 497-502
10. Julseth R.M., Deibel R.H.: Microbial profile of selected spices and herbs at import. J.Milk Food Technol. 37(1974),414-419
11. Coretti K.: Rückstände von Aethylenoxid in begasten Gewürzen und ihre Bedeutung für die menschliche Gesundheit, Meat Ind. Rewiev, 3-4(1971),31-33
12. Mayr G.: Equipment of Ethylene oxide Sterilisation. Pharm.Press London S.1(1961)
13. Gottschalk H.M.: A review on Spices. Present Status of Decontamination techniques such as Gamma Irradiation. Food Irradiation Inf. 7(1977), 7-30
14. Thangamani et al.: Microbial Contamination in Spices,Ind.Food Packer,29(2)(1975),11-13
15. Patel J.D. et al.: Biochemical Characteristics of Some of the Coliforms Isolated from Spices. J.Food Sci. Technol.,India 13(1)(1976),37-40
16. Powers E.M. et al.: Incidence and Levels of Bacillus cereus in processed Spices,J.Milk Food Technol. 39(10)(1976),668-670
17. Coretti K.: Der Keimgehalt von Gewürzen, Fleischwirtschaft, 7(1955),305
18. Coretti K.: Kaltentkeimung von Gewürzen mit Aethylenoxid,Fleischwirtschaft,9(1957), 183-191
19. Hall L.A.: Sterilised Spices: new factor on Food Quality Control,Food Inds.10(1938), 424-464
20. Fabian F.W. et al.: The Role of Spices in Pickled-Food Spoilage. Food Res.4(1939),269
21. Yesair J., Williams O.B.: Spice Contamination and its Control,Food Res.7(1942),118-126
22. Jensen L.B.: Microbiology of Meats, 2nd Ed. Garrard Press,Champaign,Ill.,(1954),345
23. Coretti K.,Inal T.: Rückstandsprobleme bei der Kaltentkeimung von Gewürze mit T-gas (Aethylenoxid). Fleischwirtschaft 5(1969),599
24. Pohja M.S.: Vergleichende Untersuchungen über den Mikrobengehalt fester und flüssiger Gewürze. Fleischwirtschaft 9(1957),547
25. Leitao M.F.de F. et al.: Microbiology of Dehydrated Foods, Coletanea do Inst. de Tech. de Alimentos 5(1973/74),223-241
26. Bodrossy: Verminderung des Infektionskeimgehaltes von Gewürzen der Fleischindustrie, Huisipar, 9(1960),160
27. Nagel V. et al.: Product Specific Spore Counts in Spices. Elelmezesi Ipar 33(6)(1979), 226-229
28. Gerhardt U.: Entkeimung von Gewürzen, Gordian, 9(1969),427

29. Sirnik M. et al.: Mikroflora fumigiranih in nefumigiranih začimb in mešanic začimb, Kongres mesne industrije Jugoslavije, Ohrid, okt.1979
30. Hammer G.F.: Lagerungsstabilisierende Wirkung verschiedener Naturgewürze auf Rohwurst. Die Fleischwirtschaft 11(1977),1944-1946
31. Hitokoto H. et al.: Inhibitory effects of Spices on Growth and Toxin Production of Toxigenic Fungi. App.Envir.Microbiol. 39(4)(1980),818-822
32. Shelef L.A. et al.: Sensitivity of Some Common Food-born Bacteria to the Spices Sage, Rosemary and Allspice, J.Food Sci., 45(4)(1980),1042-1044
33. El Zeany B.A., Abdel-Fattah L.E.: Oxidised Lipids-proteins Browning Reaction.V.Effect of Spices on the Browning of Lipid-protein Mixtures. Rivista Italiana delle Sostanze Grasse 56(11)(1979),438-440
34. Abdel-Fattah L.E., El Zeany B.A.: Effect of Spices on the Antioxidation of Fatty Foods Rivista Italiana nelle Sostanze Grasse 56(11)(1979),140-142
35. Ethylene oxide, Ethylene chlorhydrin and Ethylene glicol. Maximum Residue Limits and Maximum Daily Levels of Exposure. Departement of Health, Education and Welfare, FDA, FR 43(19)(1978),3801-3815
36. Jordy A., Suhr H.: Sorption processes in Gas Sterilisation in the Medical Section. Appl.Microbiol. 26(1973),598
37. Driessen F.M., Duin H.van: Steriliseren met Ethyleenoxyde, Voedingsmiddelentechnologie 8(32/33)(1975),15-19
38. Willson J.E.: Ethylene oxide Sterilant Residues. Bull.Parenteral Drug Ass.24(5)(1970), 226-233
39. Kereluk K. et al.: Microbiological Aspects of Ethylene oxide Sterilisation. Appl. Microbiology 19(1)(1970),146-165
40. Rausher H. et al.: Uber die Verwendung von Aethylenoxid zur Entkeimung, Mitt. der Versuchsstat für das Gärungsgewerbe Wien 7/8(1956),1-4
41. Leistner L. et al.: Microbiology of IM Foods. Short Course on Intermediate Moisture Foods, held in Survilliers (France) on September 22-23,1976 by the Centre de Perfectionnement des Cadres des Industrie Agricoles et Alimentaires (CPCIA)(1975)
42. Jordy A.: Gegenüberstellung verschiedener Methoden zur überprüfung der Gassterilisation mit hochgiftigen Wirkgasen. Mitteilungen der Osterreichischen Sanitätsverwaltung, (1971),4-5
43. Soos K.: Versuche zur Begasung von Lebensmittelen mit Aethylenoxid zum Studium der Gasrückstände. Elelmiszerviszgalati Közlemenyek Bd.XII(1966),132
44. Wesley F.: A Neglected Class of Persistent and Highly Toxic Food Pollutants, R.S.H. 6(1978),226-268
45. Wallace L.G.: Residual Ethylene oxide and Reaction Products in Polymers. Bull. Parenteral Drug Ass. 24(2)(1970),68-75
46. Reinhard L., Karlheinz L.: Akute Vergiftungen. Gustav Fischer Verlag, Stuttgart. (1971),36-39
47. Stijve T. et al.: Determination and Occurrence of Ethylene chlorohydrin Residues in Foodstuffs Fumigated with Ethylene oxide. Trav.Chim.Aliment.Hug.,67(1976),403-428