

# Microbial infestation in black pepper and its control measures

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## ABSTRACT

The information available on nature and extent of microbial contamination in black pepper and various processes developed for their elimination are reviewed.

Key words : black pepper, microbial contamination, control measures.

## Introduction

Black pepper constitutes the most important spice of the world and is a major source of foreign exchange for India. It is, therefore, important to maintain all aspects of its quality including freedom from microbial contamination. The information available on microbial infestation of black pepper and their control measures are reviewed.

## Nature and extent of microbial contamination

Most spices including black and white pepper used in food products are contaminated to varying degrees with mould spores, yeasts, bacteria and insects. They also cause spoilage occasionally in canned foods to which they are added (Anonymous 1990). Spore-forming organisms present in a spice mixture containing coriander and white pepper were responsible for swells in canned chopped hems. Black pepper contaminated with

aerobic spores enormously increased the spore count of sausages (Silliker 1963).

Five per cent of 60 spices (including black pepper) examined were positive for *Clostridium perfringens* (Strong, Canada & Griffiths 1963). Warmbred & Fry (1966) found that black pepper had a microbial load ranging from  $3 \times 10^3$  to  $23 \times 10^6$  per gram and a mould count from  $3 \times 10^3$  to greater than  $10 \times 10^6$  per gram but coliforms were fewer than 100 per gram. Prepared sausage seasonings had greater total counts of coliforms than individual ingredients. According to Kinner, Kotula & Morcuri (1968) onion, celery and white pepper powders were contaminated with aerobic spore formers, the maximum being in celery. Black pepper contained fewer than 10 coliforms per gram.

*Bacillus subtilis* and *B. mesentericus* were present in 94 per cent of spices examined (Goleiz 1969), coliform contamination being high in black pepper, paprika,

cardamom and caraway. In many countries, a majority of spices are imported and in many cases by the time they reach the consumer, they have deteriorated in quality, owing to loss of volatiles and microbial and insect infestation (Clark 1970).

The total microbial load in eight spices and three spice mixtures ranged from  $20 \times 10^3$  per gram (refined salt) to  $55 \times 10^6$  per gram (turmeric). Coliforms were present in black pepper, coriander, mustard, fenugreek, cumin, fennel and curry powder; coriander had the maximum load (2400 per gram) and fenugreek the minimum (130 per gram). Yeast and mould infestation were present in all spices, black pepper being heavily infested (9800 per gram). Aerobic mesophiles were the highest in turmeric ( $20 \times 10^5$  per gram) and the lowest in fennel ( $6 \times 10^2$  per gram). The incidence of mesophilic putrefactives ranged from 26 per gram (fenugreek) to 920 per gram (coriander and fennel) but refined salt was free from it. Thermophilic flat sour were present in limited numbers in cumin and refined salt only. A non-coagulate type of *Staphylococci* was present in minimum numbers in some spices. *C. perfringens* was present in black pepper, turmeric, coriander, mustard and fenugreek (30 to 170 per gram). Sulfide stinkers and *Salmonella* were completely absent (Krishnaswamy, Patel & Parthasarathy 1971; Krishna-swamy *et al.* 1973). The subject of microbial contamination in spices including black and white pepper was reviewed by Pruthi (1980).

### Inactivation of microflora

To eliminate spoilage due to natural contamination of black pepper and other spices with spoilage micro-organisms, it is desirable to treat them either at

the source of production or prior to their use in perishable foods such as meat and sausages.

Several processes are described in literature for elimination of organisms causing spoilage in spices. They can be classified broadly as: (i) cold sterilization by irradiation, (ii) vacuum fumigation with or without treatment and (iii) thermal inactivation.

### Cold sterilization by irradiation

The various factors to be studied in any consideration of ionizing radiations as sterilizing agents for food include: (i) the best type of radiation, (ii) appropriate packaging, with special regard to size, (iii) dose required for sterilization, (iv) effect of treatment on flavour, odour, texture, enzymes, nutrients and storage life of foods and (v) choice and cost of equipment and building. Work on these aspects were reviewed by Proctor & Goldblith (1951).

The first study on cold sterilization of spices and other foods by irradiation was conducted by Proctor, Goldblith & Fram (1950), who observed that cathode rays reduced markedly or eliminated completely the bacterial content of a number of spices and herbal spices at a dosage of  $1.33 \times 10^6$  rep; more than 99.9 per cent of organisms were killed by exposure to the cathode rays from a Van de Graff instrument.

Robison, Overbeck & Porter (1957) obtained similar results with soft X-rays. They further reported that black pepper lost some of its flavour with a dose of  $1.5 \times 10^6$  rep. In a series of eight papers by Hall (1955-1958), the effects of ionizing radiations on spices were presented. This useful research work, done under contract with the US Army Quarter-

master Corps, is not generally available. The sterilizing action of ionizing radiation on spices as well as change in the sensory characteristics of black pepper and cinnamon was reported.

Lerke & Farber (1966) studied the effect of a dose of 2 Mrep of irradiation from a resonant transformer electron beam generator on the content of bacteria, yeasts and moulds of 12 spices (including black pepper). The microbial population of the products (bacteria, moulds and yeasts) were either completely eliminated or reduced to insignificant levels. However, in the 12 spices studied, there was a considerable change in the odour complexes that was noticeable sensorily, as well as in their content of volatile reducing substances (Table 1). Black pepper lost most the aromatic component. Cinnamon also lost the pleasing aromatic component, but retained a more medicinal type of odour. Cloves had a weaker characteristic 'off' odour.

The earlier work on irradiation of spices was reviewed by Pruthi (1980). However, Coretti (1955) reported that exposure of spices to ultraviolet light is not effective for inactivation of microflora. It is, however, possible to prepare nearly 'bacteria-free' extracts from spices and to reduce the bacterial content in them by heating, treatment with ethylene oxide and possibly also by means of beta or gamma radiation. A major advantage of irradiation is that it is a 'cold' treatment. At a maximum dose recommended for treating spices, i.e. 10 KGy, the increase in temperature of the product is only 2-4°C. Thus, it is unlikely that loss of volatile oil components of the spice could occur by using this method. Moreover, ionizing radiations cannot add radioactivity to the food regardless of

the length of time the food is exposed or amount of energy 'dose' absorbed. In other words, these radiations have too low an energy to induce radioactivity in any material including food, spices or black pepper. The effectiveness of irradiation as a method for microbial decontamination and insect disinfection was reviewed by Farkas (1984), Farkas & Andrassy (1985), Kiss and Farkas (1988) and Sharma, Padwal-Desai & Nair (1989). Medium-dose irradiation (up to 10 KGy) is effective to reduce microbiological level in raw or ground black pepper and other spices to acceptable levels. Irradiation can best be applied to pre-packaged spices to avoid possibility of recontamination. Technological and sensory qualities of black pepper and other spices treated up to 10 KGy are not significantly affected.

#### *Commercial applications of irradiation*

Following the positive recommendation of the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Foods in 1980, many governments recognised food irradiation as a physical process for treating foods similar to heating and freezing preservation of foods. As a result, 37 governments introduced regulations allowing use of irradiation for treating one or more food items. Among these, 24 countries are using the process for treating several food items including black pepper and other spices for commercial purposes. The details of approval and commercial application of irradiated spices are included in Table 2 (Leaharanu 1990).

The quantities of spices treated by irradiation in various countries to ensure hygienic quality have also increased in

Table 1. Effect of beta irradiation on microbial content of spices

Spices	Micro-organisms per gram x 10 <sup>-3*</sup>								Odour (VRS** reduction as meq/g)
	Before irradiation				After irradiation				
	Bacteria		Yeasts & moulds		Bacteria		Yeasts & moulds		
	Range	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	
Allspice	207.56-584.80	365.570	2.44-5.20	3.76	0	0	0	0	115.9
Black pepper	18,999-49,998	29,000	0.516-1.130	0.858	0	0	0	0	58.8
Cayenne pepper	1.15-3.47	2.32	0.048-0.132	0.084	0	0	0-0.003	0.001	15.5
Chilli pepper	3.9-9.0	6.87	0-0.003	0.001	0-0.006	0.003	0	0	35.9
Cinnamon	0.096-1.540	0.74	24	24	0	0	0	0	132.8
Clove	0.50-4.00	1.92	0	0	0	0	0	0	147.0
Ginger	0.72-2.76	1.62	0-1	1	0	0	0	0	—
Nutmeg	0.42-4.67	2.55	0.30-4.68	2.13	0	0	0	0	86.3
Oregano leaf	0.16-16.40	8.28	5.84-49.64	24.54	0	0	0-0.024	0.012	53.9
Paprika	71.95-155.78	101.90	0.048-0.216	0.098	0	0	0	0	24.7
Sage	1.40-5.68	2.31	0.80-1.36	1.24	0	0	0	0	65.5
Sweet basil	6.48-62.72	27.52	0.720-1.280	1.07	0	0	0-0.072	0.034	69.2

\* For ease of presentation, the microbial counts represent the actual count divided by 1000

\*\* Volatile reducing substances: 10 minute aeration period for all samples. A 0.025 N KMnO<sub>4</sub> solution was used. Results are expressed as milliequivalents of KMnO<sub>4</sub> reduction in VRS

Table 2. Approval and commercial use of irradiated spices

Country	Type of approval	Max. dose (KGy)	Year	Commercial use*	Starting year
Argentina	Unconditional	10	1983	Yes	1988
Bangladesh	Unconditional	10	1983	No	—
Belgium	Unconditional	10	1983	Yes	1981
Brazil	Unconditional	10	1985	Yes	1985
Canada	Unconditional	10	1984	Yes	1989
Chile	Unconditional	10	1982	Yes	1983
Denmark	Unconditional	10	1985	Yes	1986
Finland	Unconditional	10	1987	Yes	1987
France	Unconditional	11	1983	Yes	1983
Germany	Provisional	10	1982	No	—
Hungary	Unconditional	8	1986	Yes	1982
India	For export	10	1986	No	—
Indonesia	Unconditional	10	1987	Yes	1988
Israel	Unconditional	10	1985	Yes	1986
Korea	Unconditional	10	1988	Yes	1988
Netherlands	Unconditional	10	1988	Yes	1978
Norway	Unconditional	10	**	Yes	1982
Pakistan	Unconditional	10	1988	No	—
South Africa	Unconditional	10	1983	Yes	1981
Syria	Unconditional	10	1986	No	—
Thailand	Unconditional	10	1986	No	—
USA	Unconditional	30	1986	Yes	1986
Yugoslavia	Unconditional	10	1984	Yes	1986

\* Irradiated spices, regardless of quantity which have entered commercial channels

\*\* Permission given on case by case

recent years. There is a unique situation in the Federal Republic of Germany which prohibits use of ethylene oxide for fumigating spices, while not approving irradiation on this commodity. In addition, the regulation in this country allows irradiation of spices for export only. Indeed, commercial quantities of spices are irradiated from time to time in this country. The total world-

wide quantity of irradiated spices per annum is estimated to be 10,000 t (Leaharanu 1990). However, the statistics of actual quantities of black pepper irradiated world-wide are not available yet.

#### *Economics of irradiation*

Like other physical treatments of food eg. canning, freezing, drying etc., irra-

diation has an economy of scale i.e. larger the volume of product, lesser the unit cost of treatment. Similar to other physical treatments, the capital cost of an irradiation facility is relatively high ranging from approximately US \$ 1 million for a large pilot irradiator to US \$ 6-8 million for a fully automatic commercial irradiator. However, unlike other processes, irradiation has a low operating cost because of its self-contained source of energy in the case of Co-60 irradiator and the efficient energy transfer in case of treatment by electron machine. The actual cost of irradiation of spices varies from country to country depending on local situations (Leaharanu 1990).

In general, cost of irradiation of spices (including black pepper) varies from US \$ 0.02 to 0.10 per kg using a Co-60 irradiator. The variation in cost is mainly due to volume and radiation dose required for treating different types of spices. Cleland (1984) estimated that the cost of electron machine treatment of spices having a yearly output of 1,00,000 t at 0.7 US cents per kg. One major advantage of irradiation of spices is that the facility, either with radio isotopic or electron source of radiation can also be used for treating other food ingredients (eg. enzyme preparation, starch etc.), food and non-food products (eg. disposable medical and pharmaceutical products, food packages etc.). Thus the overall economics of irradiation facilities would be even more attractive as the volume of products treated increases.

A feasibility of installing a single purpose irradiator for treating black pepper was conducted in Malaysia. They concluded that such a facility was not economically feasible in view of the

uncertainties concerning volume of products available for treatment and marketability of irradiated black pepper. However, when a multi-purpose irradiator for treating black pepper, cocoa beans and frozen shrimps was considered, using the facility for either 6000 or 8000 h per annum, the economics of such a facility become feasible (Arshad & Juri 1987).

### **Vacuum fumigation (Chemical treatment)**

Chemical fumigation with ethylene oxide and propylene oxide is the most widely used method. The degree of decontamination which may be achieved by this method depends essentially on: (i) concentration of gas, (ii) temperature, (iii) length of time allowed and (iv) water content of spice (Gottschalk 1977). Essentially, fumigation is a batch treatment and additional time is required to aerate the gas after treatment.

Fumigation is generally recognised as an effective method for decontamination black pepper and other spices. In terms of quality of treated spices, volatile and non volatile oil content of black pepper and other spices were reduced severely by fumigation treatment. In addition, a marked reduction in colour intensity of paprika was noted. Losses in volatile oil of ginger, mace, pimento and cloves and a noticeable alteration of aroma of mustard, even at low gas concentrations, were also reported (Coretti 1957).

The major problem with ethylene oxide (ETO) fumigation is worker safety. ETO itself is toxic to man in the vapour concentration range used for fumigation and forms flammable and explosive mixtures with air. This danger, however,

can be reduced by use of a mixture of ETO and carbon dioxide. Another disadvantage of ETO fumigation is the danger of chemical residues in food. ETO may react with inorganic constituents in food to form ethylene chlorohydrin and ethylene bromohydrin which persists as toxic residues (Wesley, Rouke & Darbishire 1963; Reith 1967; Stijve, Kalsbach & Eyring 1976).

### **Ban on chemical fumigation**

Because of worker safety and residue problems, use of chemical fumigation is banned or restricted in most countries. The EC has introduced a directive to ban the use of this technique beyond 1990. Belgium, Denmark, Federal Republic of Germany and the Netherlands have already banned fumigation of spices.

The microorganisms of natural spices were reduced by treatment with 1000 to 20,000 ppm of  $\text{ClO}_2$  in air or inert gas followed by heating the treated spices from 100°F (35°C) to about 225°F (107.2°C). Thus, black pepper containing 32,50,000 bacteria per gram had only 750 gram when treated for 30 min at 225°F (Woodward 1949).

A patented process is described in which spices or other vegetable materials were first heated under vacuum and then impregnated with ethylene oxide in sufficiently high concentrations to destroy bacteria, yeast and mould spores. Practically, any ground spice can be treated by this process without loss of more than 1% of volatile oils and without the occurrence of residual ethylene oxide in the products (Hall 1951). Sterilization by the ethylene oxide process is very effective in destroying spores, especially when the material treated is in a normally dry state. Dry treated

spices can be kept for a long time without danger of the spore count increasing. Foods (for example meat) containing sterilized spices would also have better keeping quality than those containing untreated spices.

Another cold sterilization process, using ethylene oxide gas, kills bacteria in spices and other dry food ingredients. The process is superior to fumigation and the prolonged heating method of infestation control (Anonymous 1954). Ethylene oxide gas was very effective as a sterilizing agent for spices. However, strict control should be kept on the sterilization process or the colour and flavour can be adversely affected. Spices sterilized in this manner are considered quite non-toxic as the gas is completely removed after sterilization. Bacterial limits of 3000 organisms per gram for black pepper and 1000 organisms per gram for other spices were suggested (Coretti 1957).

Vacuum sterilization of spices, using pure ethylene oxide and Etox (90% ethylene oxide and 10%  $\text{CO}_2$ ) was investigated. It was concluded that ethylene oxide could be used for disinfecting many food stuffs including spices. The best results were obtained by a vacuum of 95% decreasing gradually during sterilization to 20%, a temperature between 25° and 35°C and in most cases, 500 g of ethylene oxide per cubic metre is sufficient. Fluctuations in moisture in Hungarian climate did not influence the sterilizing effect. Information on effect of ethylene oxide on various species of bacteria at different concentrations and temperatures and varying periods of exposure are provided (Rauscher, May & Kaemmerer 1957).

The US Food and Drug Administration prescribed a maximum tolerance lim-

ited of 50 ppm for residues of ethylene oxide in whole spices/whole black pepper (Anonymous 1957). Pruthi (1964 & 1968) reviewed the subject of sterilization of black pepper and other spices.

### Physical or thermal sterilization

A number of experiments using varying temperatures on the organoleptic quality of spices were conducted. Flavour of spices was affected at 90°C and became progressively more distinctive with increasing temperatures. Black pepper was among the most stable and marjoram the most labile of the spices (Theissen 1970; Theissen & Scheide 1970). Where heat is used as a sterilization method, a temperature of 110°C is usually required, either by steam injection or by oil emulsion (Bartels & Hadlock 1967). Heat is generally recognised as inapplicable for sterilizing spices, as the temperature to ensure microbiological safety always leads to a loss of some of the volatile oil and consequently, to a marked reduction in aroma and product quality (Gottschalk 1977).

### *Sterispice sterilization process*

A process of 'thermal sterilization' of herbs and spices known as 'Sterispice' was developed in Denmark. This process is achieved by steam injection at temperatures above 100°C. The full sterilization cycle is different for various spices and takes account of the heat stability of flavour components. To prevent loss of flavour, a coating system based on protein extracts from food grade bones, is added to spices during the process. This method is applicable for treating herbs and spices only in their original form i.e. unground. Some 350 t of black pepper kernels and other

original form of herbs and spices are treated by this process annually in Denmark. The cost of heat sterilization was not reported. Some spices, however, cannot tolerate the high temperatures used. For example, paprika turned black and some herbs such as dill lost their green colour. Onions and garlic had a tendency to develop either roasted or cooked taste. Black pepper was, however, comparatively stable (Sorenson 1989).

Misra, Pruthi & Siddappa (1962) and Muthu *et al.* (1962) attempted disinfection of spice products by direct heating under an infrared lamp (stationary heating) and for canned ground spices and curry powder, heating with agitation in a spin pasteurizer developed by Pruthi and associates (Pruthi 1957; Pruthi, Ramanathan & Lal 1959). Schonberg (1955) claimed that by suitable heat treatment of black pepper and other spices, products free from bacteria and spores could be obtained. A process was developed by which dry particulate food was sterilized by a process consisting of vapourizing a pyrocarbonic acid diester by heating the mixture throughout the dry food. Thus, diethyl pyrocarbonate was heated to 30° to 50°C while a flow of air was maintained through it to carry the vapourized diethyl pyrocarbonate through a conduit to penetrate black pepper. After 15 min black pepper was sterilized (Eolkin & Bouthilet 1967).

### Conclusion

Most of the microbial contamination of black pepper and other spices is by aerobic spore forming bacteria. Spices do not support growth or long term survival of non-sporic pathogenic bacteria such as *Salmonella*. However, contamination by these is not uncommon.



There are reports of incidences of salmonellosis attributed to consumption of black pepper (Laidley et al. 1974; Anonymous 1973 & 1974). In 1981 and 1982, Norway reported incidences of over 120 patients infected by *S. oranienberg*. Since then, Norway introduced regulations requiring treatment of imported spices by irradiation (Anonymous 1982 a & b).

Mould contamination of spices does not always correlate with bacterial contamination. Certain spices eg. black pepper, chilli and coriander seem to be highly contaminated with moulds. Toxicogenic moulds are detected in spices. Aflatoxins are reported in black pepper, ginger, turmeric, celery seeds and nutmeg, although levels of toxins recorded were generally low (Scott & Kennedy 1973 & 1975; Suzuki, Daninius & Kilbuck 1973; Flannigan & Hui 1976).

With increasingly strict regulations on quality of spices in international trade and with the growing concern of the food industry and consumers about risks from microbiological contamination in food, irradiation is likely to become a standard technique for 'pasteurizing' solid food such as meat, poultry, seafood and spices in a similar manner to the effective use of heat for pasteurizing liquid food eg. milk (Vajdi & Pereira 1973).

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