207

The Microbial Ecology of Prepared Raw Vegetables

P.C. KOEK, Y. DE WITTE AND J. DE MAAKER

Sprenger Instituut, Wageningen, The Netherlands

Contents

3/9 82 Laatste versie van publikatie

1. Introduction

Cleaned and cut raw vegetables are used in great quantities in the kitchens of hospitals, hotels, old people's homes and army units, and also in private households. Working people, especially, often buy such prepared vegetables. In view of the known spoilage and health hazards associated with food processing, the Microbiological Section of the Sprenger Instituut, Wageningen, is conducting applied research on the storage and processing of horticultural produce, especially fruits and vegetables. Some investigations on prepared cut, sliced and diced vegetables have already been completed.

During 1978 investigations on the microbial loading of vegetables were started using sliced endive bought from shops in and around Wageningen. Some of the samples were stored at four different temperatures (1, 8, 14 and 20°C) for 24 or 48 h and others were investigated microbiologically at once. These experiments were set up in summer with endive crops grown in the open field, and in winter with endive grown in greenhouses or imported from Italy or France.

In all cases high counts of mesophilic aerobic colonies (ca. 10^7/g), coli-aerogenes group (ca. 10^7/g) and non-fermentative Gram negative rods (ca. 10^6/g) were found: the the storage temperatures in this experiment were clearly too high. There was a correlation between the numbers of Escherichia coli, and the storage time and temperature. In this experiment there were too many different factors beyond our control, such as the origin of the product, mostly unknown, and the use of different machines for slicing the

endive. The following year a line study was made at two important plants for preparing vegetables. Their machines and methods differed completely so the results could not usefully be compared. Despite these difficulties our impression was that preparation, cooling and the latest date of sale should be better standardized.

2. Good Manufacturing Practice is Recommended

In the USA and Canada the Food and Drug Administration has, in co-operation with the food industries, developed the concept of Hazard Analysis and Critical Control Points. Recommended Codes of Practices is being developed in the United Nations Food Standard Program, and the term Good Manufacturing Practice (GMP) can also be used for the same set of principles.

One of the main points of GMP is that water, not of drinking quality, should not be brought into contact with the food being processed. Another important principle is that the machines should reach the specified hygiene requirements (Anema 1978). It is desirable, therefore, that the designers of machines for slicing or dicing vegetables should co-operate with micro-biologists. GMP concerns the whole industrial process, not simply the machines (Mossel 1977): hygiene of the staff, the plant, packing materials and stores are also important (Shewan 1976).

3. The Quality of Vegetables to be Prepared

In preparing raw, sliced or diced vegetables the primary material quality must be properly controlled; only the best quality produce should be bought for processing.

However good the quality, the produce will already have

been naturally contaminated with various bacteria and fungi (Mossel & Westerdijk 1949; Tamminga et al. 1978). Leafy vegetables are not only susceptible to bacterial and mould diseases; they can also be infected by secondary bacteria such as Erwinia and Pseudomonas species (Webb & Mundt 1978). For instance, Green et al. (1974) found soil to be a reservoir of Pseudomonas aeruginosa and discovered that this bacterium has the capacity to colonise the upper parts of plants. Splashing water can carry ~~the~~ this bacteria um to aerial parts of plants where they enter water-~~soaked~~ congested areas.

Bulb vegetables, such as onions, and root vegetables, such as carrots, are more likely to be contaminated by fungi and bacteria than are leafy vegetables. Root vegetables are usually cleaned with water to improve their appearance at auction. It is important, however, to use good quality water or the GMP rules will be infringed. In the Netherlands it is now forbidden by law to use ditchwater for this purpose (Tamminga et al. 1978).

4. Experiments with Four Vegetables

A. Machines used to prepare the vegetables

Different slicing and dicing machines were used in the microbial ecological study of prepared vegetables to be described later. For slicing endive and leek an in-plant machine unit, consisting of a slicing machine, a washing machine, a container and a spin drier was used (Fig.1).

F → In ~~this~~ machine ~~unit~~ the bottom ~~of each plant was first~~ cut off and the outer leaves removed. The product was then transported by a conveyor belt to an S-shaped knife which sliced it. The sliced product was then passed to the washing machine containing 2000 litres of tap water in which it was agitated by metal blades which moved forwards in the washing ~~vessel~~ basin. At

F The bottom of each plant was first cut off and the outer leaves remove

the end of ~~the basin~~ the blades returned above the water to the starting point where they were lowered into the water again, thereby making a circulating movement. In the meantime, recirculated water was sprayed on top of the floating sliced product. At the end of the basin the sliced product was transferred to a conveyor belt made of small stainless-steel chains and simultaneously washed from above with tap water. At the end of the conveyor belt the slices fell into a stainless-steel container which, when filled with the sliced product, was put in a spin drier to remove surplus water. The product was collected in polyethylene bags and put in cleaned plastic boxes.

Carrots were scraped mechanically with carborundum, then diced and cut in a special machine, using different cutting discs. Diced carrots were collected in a plastic box. A similar machine was used for slicing leeks. At the plant used for the experimental work the plastic boxes were brushed and cleaned by a special machine. This method which was very effective and hygienic and ~~which~~ should be introduced into other plants where vegetables are prepared. For commercial use the product is normally packed in new, clean polyethylene bags; large quantities are intended for use in hospitals, restaurants etc.

B. Materials and methods

The experiments on endive, leek, carrots and cauliflower were conducted according to GMP rules which meant that every part of the machines had to be cleaned before use. In most cases this was satisfactory, but the construction of the washing machine prevented thorough cleaning.

Contamination of the machines by micro-organisms was monitored according to the following criteria:

1, colony counts of the Enterobacteriaceae, as a tentative taxonomic grouping of psychrotrophic and thermotrophic micro-organisms; 2, colony counts of the mesophilic and psychrotrophic groups of Gram negative and oxidase positive rods, such as members of the Pseudomonadaceae; 3, numbers of Lancefield group D streptococci, yeasts and moulds; 4, numbers of lactobacilli (as these also occur naturally on vegetables they were enumerated separately).

(i) Sampling

Ten grams were taken at random from each sample of vegetable, mixed with 90 g of tryptone-soya broth and macerated in a Colworth Stomacher for 2.5 min.

(ii) Monitoring micro-organisms in samples

As there is no standardized method for the microbial monitoring of foods, particularly prepared raw vegetables, we used the system of Mossel et al. (1977) to isolate Gram negative bacteria, using tryptone-soya agar containing 2 mg/l of crystal violet following repair of the cells by pre-incubation in tryptone-soya broth. The strains isolated by ourselves, using this method, were identified by the staff of Professor Mossel's laboratory at Utrecht. Most isolates were pseudomonads.

For isolating the Enterobacteriaceae a dilution of 10^{-1} was poured on a solid repair medium of tryptone-soya agar and incubated for 5 h, then overlaid with violet-red-bile-glucose agar (Mossel 1978). Strains isolated from the colonies were then identified to genus level.

Two methods were used to monitor E.coli in the samples. In the first method a dilution of the sample was poured into tryptone-soya broth, as a liquid repair medium, and incubated for 3 h. Tryptone-soya broth to which a double strength solution of brilliant green-ox bile had been added, was then

added to the culture and incubation continued.

In the second method the dilution was poured on tryptone-soya agar, as a solid repair medium, and after 5 h of resuscitation was overlaid with McConkey agar.

For isolating Lancfield group D streptococci the same methods were used for resuscitation of the organisms, with tryptone-soya broth and tryptone-soya agar as liquid and solid repair media, respectively. Afterwards, kanamycin-aesculin-azide medium was used to establish their presence or absence and to isolate from the colonies.

Oxytetracycline-gentamicin yeast agar was used to isolate yeasts and fungi. Yeasts were present in fewer cases than expected.

Lactobacilli were resuscitated on a solid repair medium of tryptone-soya agar for 5 h. Rogosa agar was then overlaid on the solid medium and the plates placed in GasPak jars for anaerobic incubation (Mossel & Tamminga 1980).

C. Results

The results of the work on sliced endive and leek treated with standard and controlled industrial methods are shown in Fig.2. For each vegetable the results of microbiological analysis for the standard industrial method are shown on the left (a) of the Figure, and the results after applying the GMP rules as completely as possible are shown on the right (b). Each column represents the $\log_{10}$/g of the total numbers of micro-organisms indicated. Lancfield group D streptococci and E.coli are not shown in the histograms. Escherichia coli was identified only once, in the sliced endive prepared in the standard way, even then they were not numerous.

One of the reasons could be that carrots and leeks grow in or partly in the soil in contrast with endive and cauliflower.

There was a difference between the numbers of bacteria on the sliced endive prepared in the standard way and that prepared by the GMP controlled method. More bacteria of the Enterobacteriaceae and Pseudomonadaceae were isolated from the former product than from the latter. A completely different result was obtained with sliced leek. Compared with the sliced endive the total numbers of Enterobacteriaceae and Pseudomonadaceae were higher, and unexpectedly, the controlled sliced leek had exceptionally high numbers of pseudomonads. The total number of Enterobacteriaceae was the same in both cases, and the numbers of yeasts and lactobacilli were very low.

In Fig.3 the results for the four sliced or diced vegetables prepared by the GMP controlled method are compared. The highest counts of Enterobacteriaceae were given by carrots, the next highest by leeks, and the lowest by sliced endive. Neither Lancefield group D streptococci nor E. coli were isolated from any product. Cauliflower was an exception in this investigation; no appreciable numbers of micro-organisms in any of the groups tested for were found.

Cauliflower, which is related botanically to cabbage, merits special attention. Tamminga et al. (1978) and Yildiz & Westhoff (1981) reported that cauliflower produced substances which partially inhibited the growth of bacteria in media. Pederson & Fisher (1944) had already mentioned this phenomenon with the juice of cabbage and other vegetables, and Clapp et al. (1959) listed isothiocyanates as the main sulphur compounds in cabbage. Although there is some evidence that cauliflower tissue contains inhibitory substances they were not detected in experiments to test the effect of a cauliflower suspension, juice of cauliflower stem and juice of cauliflower

* Question: Have you mentioned in table 1 the differences in standard-processed and GMP-controlled foods concerning endive and leek? Carrots and cauliflower are GMP-processed.

sprigs on seven different bacteria, a yeast and two fungi (Hossel & Cornelissen 1960).

D. Ecological survey of bacteria from the prepared raw vegetables

In Table 1 the identified species of bacteria are listed. * The following general comments can be made:

(1) In all the products pseudomonads SH I and bacteria of the genus Enterobacter were found. See Hendrie & Shewan (1979) and Hugh (1981) for identification of pseudomonads, and Brocklehurst & Lund (1981) for occurrence of these organisms on vegetables.

(2) In the standard-processed endive E. coli was isolated only once. In GMP-controlled sliced endive the new genus Kluyvera and, on one occasion, Flavobacter meningosepticum were identified (Kleeberger et al. 1980; Farmer et al. 1981). Yeasts and lactobacilli were isolated only from the standard-processed endive.

(3) Erwinia organisms were isolated from both the leek products, the GMP-controlled diced carrots and from the sliced endive prepared by the standard industrial method.

(4) Kluyvera was also identified among isolates from the GMP-controlled diced carrots.

(5) Lactobacilli and yeasts were isolated from GMP-controlled diced carrots.

(6) Diced cauliflower carried the lowest microbial load.

(7) In this survey pathogenic bacteria seemed to be of no importance.

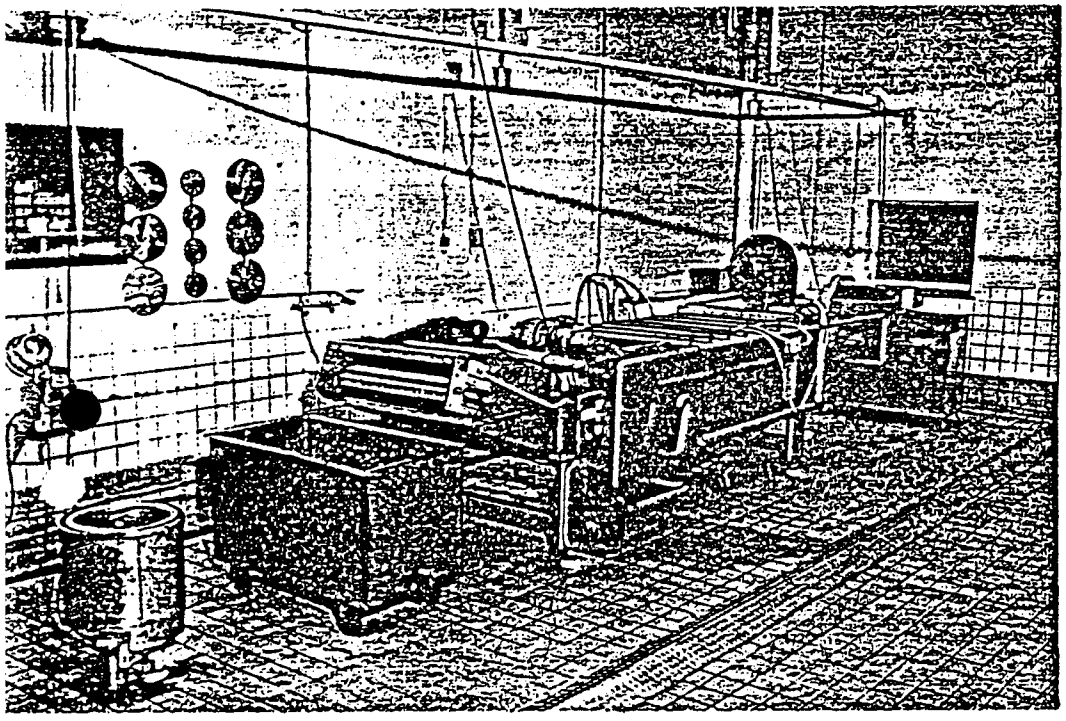
E. Storage

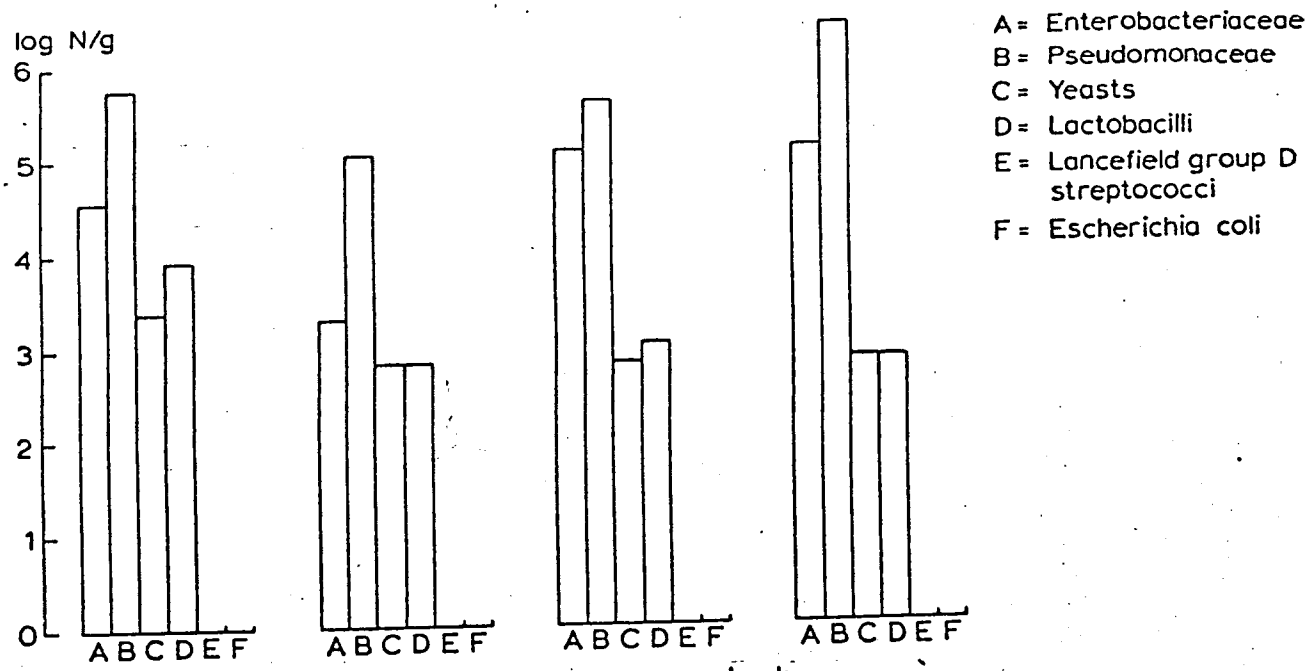
To prevent growth of bacteria in the period between preparing

and consuming the product, it is necessary to store it at low temperatures; an average temperature of 0-1°C is advisable (Anon. 1981). In the plant where our experimental work was done the products were normally stored ~~in the~~ cold and later distributed by vehicles with refrigeration units. In shops the products should, of course, be displayed in refrigerated showcases.

show-cases

Fig. 1. The machine unit for prepared raw vegetables. From right to left: the slicing machine, the washing machine, the container and the spindrier.





a b a b

Fig. 2. Survey of micro-organisms in sliced endive and leek after standard and controlled industrial methods.
The histograms on the left side of each product mentioned, are the results of the standard industrial method; those on the right side are the results after applying the G.M.P.-rules.

a = industrial method

b = G.M.P.

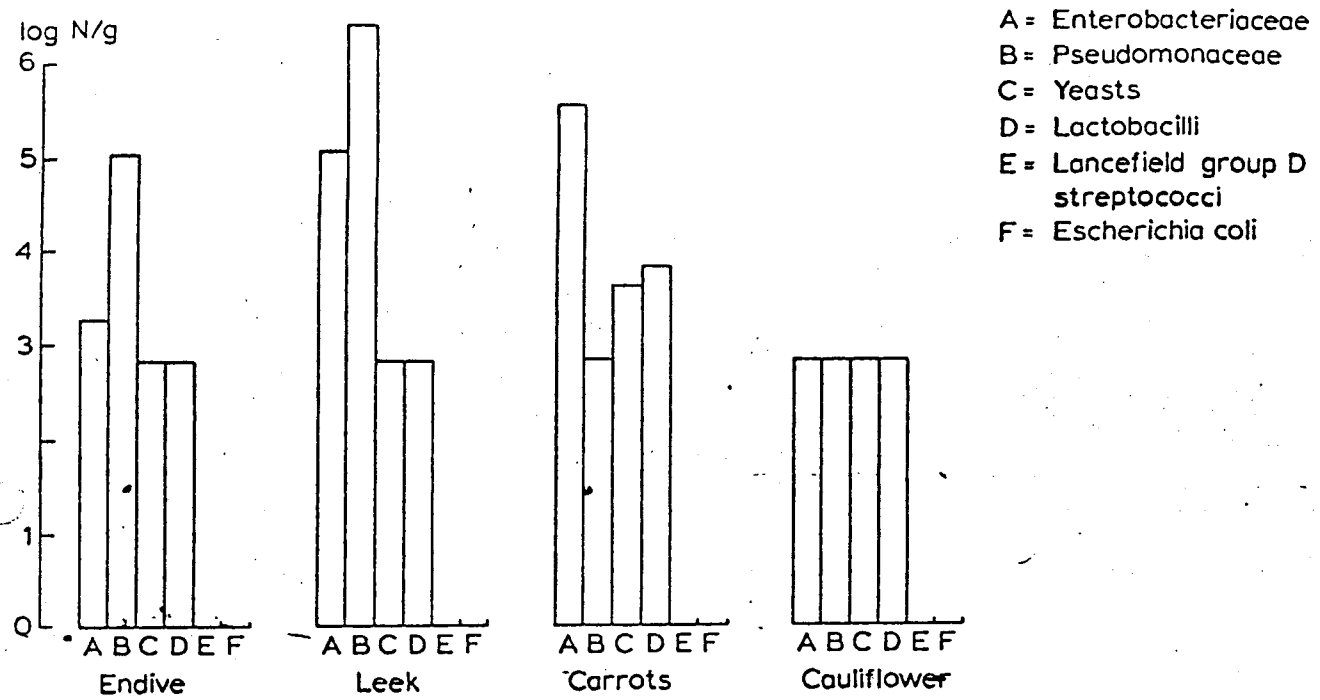


Fig. 3. Survey of micro-organisms in sliced endive, leek, diced carrots and cauliflower after G.M.P.-controlled, industrial method.

	Endive a	Endive b	Leek a	Leek b	Carrots b	Cauliflower b
Pseudomonas Sh. I	x	x	x	x	x	x
Pseudomonas Sh. II - IV		x				
Pseudomonas maltophilia	x	x				
Xanthomonas	x					
Acinetobacter		x	x			
Citrobacter			x			
Enterobacter	x	x	x	x	x	x
Enterobacter atypical	x		x			
Erwinia	x		x	x	x	
Kluyvera		x			x	
Hafnia					x	
Serratia					x	
E.coli	x					
Yersinia	x					
Enteropathogenes						
Flavobacter meningosepticum		x				
Lancefield D streptococci						
Yeasts	x				x	
Lactobacilli	x		x		x	

Table 1. An ecological survey of identificated micro-organisms in different sliced and diced raw vegetables.

a = industrial method

b = field