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ORIGINAL ARTICLE

Microbiological contamination of dried and lyophilized garlic as a potential source of food spoilage

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Abstract Garlic is valued more for its flavoring and used in a wide variety of foods. In food technology, fresh garlic is not used, but instead its processed forms, most often dried and lyophilized, are utilized. The quality and safety of the final product largely depends on their microbiological quality. This research has provided information about effect of garlic fixation methods and provided information about effect of microbiological contamination of garlic used as a spice for quality of garlic mayonnaise sauce. The authors decided to undertake studies following a report from one of the manufacturers of garlic sauces on product defects which originated in dried garlic used in the production process. Samples of garlic (n = 26) were examinated using standard cultural methods (counts of fungi, lactic acid bacteria–LAB, spore-producing *Bacillus* sp. and the presence of anaerobic saccharolytic and proteolytic clostridia), automated system TEMPO (total viable count, *Enterobacteriaceae*), immunoenzymatic method using VIDAS tests (occurrence of *Salmonella* sp. and *Listeria monocytogenes*). The number of total viable count was ranged from 3.51 to 6.85 log CFU/g. *Enterobacteriaceae* were detected only in one sample. Comparably low values were recorded for fungi (1.30 to 3.47 log CFU/g). The number of LAB was ranged from 2.34 to 5.49 log CFU/g. *Clostridium* sp. were detected in 22 samples. *Salmonella* sp. and *Listeria monocytogenes* were not detected. It was found that garlic, regardless of th preservation procedure, might be a source of contamination of garlic mayonnaise sauce especially with lactic acid bacteria and *Clostridium* sp. spores.

Keywords Food spoilage · Spices · Garlic · Microbiological contamination

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Introduction

Food industries worldwide have to conform to microbial standards associated with the safety and the quality of their products. Safety aspects are of major importance thus are determined clearly and unambiguously. Microbiological contamination of spice is one of important factor contributing to quality loss, results in spoilage food products. Garlic is one of the most popular spices used in the manufacturing of many food products, e.g. meat preserves, mayonnaise sauces and dressings as well as cottage cheese and flavoured butter. It has been shown that the total mesophilic counts cannot always give a realistic estimation of the microbial contamination levels in food. Many references in the literature stress the importance of psychrotrophic lactic acid bacteria in cases of spoilage since when their population exceeds 10^7 CFU/g they produce off-flavor and off-odor compounds leading to the rejection of the product (Pothakos et al. 2012; Jacxsens et al. 2003).

In food production garlic is appreciated for its taste, smell and commonly-known health properties. It contains sulphurs, arginine, oligosaccharides, flavonoids, and selenium, all substances of which may be beneficial to health (Chia-Wen et al. 2012; Nencini et al. 2011; Sato et al. 2006). Allicin, which has bactericidal and hypoglycaemic activity, is a product of the decomposition of allin which is found in fresh garlic (Park et al. 2009). It is unstable and, after disruption of the garlic structure, is transformed into fat-soluble compounds such as diallyl disulfide (DADS), diallyl sulfide (DAS), diallyl trisulfide (DATS), allylmethyl trisulfide, and diallyl tetrasulfide or other derivatives, e.g. ajoene (Amagase et al. 2001; Iciek et al. 2009). As *Enterobacteriaceae* are very sensitive to allicin, garlic effectively inhibits their growth in the digestive tract (Miron et al. 2004).

In food technology, fresh garlic is not used, but instead its processed forms, most often dried and lyophilized, are utilized. The microbiological quality of spices reflects the

hygienic situation of the region of their production and manufacture. Spices may contain a diverse indigenous microflora. The quality and safety of the final product like garlic mayonnaise sauce, largely depends on their microbiological quality. There are some reports on a potential transfer of numerous microflora, including pathogenic organisms, into dishes and other food products, but the results presented in the literature are varied (De Roever 1999; Witkowska et al. 2011).

Taking the above into consideration, our attention should be drawn to the fact that garlic is not only the source of many beneficial bioactive substances, but it may also be a source of food contamination with microorganisms, including pathogenic flora, and its quality largely depends on conditions during cultivation, harvesting, storage and processing. Poor microbiological quality of garlic and other spices may cause rapid deterioration of the food (Sospedra et al. 2010).

The aim of the study was to determining the microbiological quality of ground, granulated dried and lyophilized garlic used in industrial food production. The authors decided to undertake the studies following a report from one of the manufacturers of mayonnaise sauces on product defects (acidification, separation and accumulation of gases) which appeared only in sauces with garlic.

Materials and methods

Samples preparation

The studies were carried out on industrial samples of garlic obtained from three food producers from the north of Poland. Garlic was delivered to the manufacturers in genuine air-tight packages and was produced by two companies located in Poland. Each of the samples came from other production batches. The studies were performed on 4 samples of ground lyophilized garlic, 3 samples of granulated dried garlic and 19 samples of ground dried garlic. Samples of garlic (10 g) and sterile peptone saline diluent (90 ml) were mixed in plastic bags with a filtered compartment. Each of samples was homogenised by 2 min. in stomacher (Seward 400).

Cultural methods analysis

In the first stage, a series of ten-fold dilutions of tested material was prepared with a 1:10 solution. The microbiological analysis included the determination of:

- number of lactic acid bacteria (LAB)–determined using triplicate pour plates with 1 ml of each dilution in MRS-agar medium (Merck), incubation at 30 °C for 72 h in an incubator with a modified atmosphere (CO_2 incubator, Lab-Line Instruments, INC.) according to ISO 15214:2002,
- number of yeast and mould fungi–determined using triplicate pour plates with 0,1 ml of each dilution in YGC-agar medium (Merck), incubation at 25 °C for 96 h according to ISO 7954:1987,
- number of spore-producing *Bacillus* sp.–determined using triplicate pour plates with 1 ml of each dilution in nutrient agar, incubation at 30 °C for 48 h; before plating the samples were pasteurized at 80 °C for 15 min. in a water bath with controlled temperature (Memmert) in order to eliminate vegetative forms, according to procedures used in the laboratory of Chair of Industrial and Food Microbiology,
- presence of spore-producing *Clostridium* sp.–determined using triplicate pour tubes with 1 ml of each dilution in Meat-Liver medium (Merck), incubation at 37 °C for 48 h in anaerobic conditions (Anaerocult®A, Merck); before incubation the samples were pasteurized at 80 °C for 15 min. in a water bath with controlled temperature (Memmert) in order to eliminate vegetative forms according to PN-A-75052/10:1990

TEMPO® analysis

The automated system TEMPO (Biomerieux) was used for enumeration of total viable count (TVC) and *Enterobacteriaceae* count (EB) in garlic samples. TEMPO uses a dehydrated culture medium and an enumeration card containing 48 wells across 3 different dilutions for the automatic determination of the most probable number (MPN) (User's Manual, Application Guide of TEMPO®, Biomerieux, Available from: https://techlib.biomerieux.com/wcm/techlib/techlib/applications/guidedSearch/cleverLink/CleverLink.jsp?productnumber=tempo./Accessed 06.08.12).

After homogenization, 0.1 cm^3 of the homogenised sample suspension was taken using a sterile pipette from the filtered bag and transferred into the vial containing there constituted culture medium. Vials were mixed by a vortex. The dilutions of the analyzed samples (ground lyophilized garlic, granulated dried garlic and ground dried garlic) was 1/400, 1/4000, 1/400001 / 400 in a single vial. The inoculated medium was moved into the TEMPO card. Cards and reagents were then loaded into the TEMPO Preparation Station according to the manufacturer's instructions, to automatically fill the cards with the appropriate sample dilution. Cards were incubated for 48 h at 30 °C (TVC test) and 24 h at 35 °C (EB test). After incubation the card was placed in an automated reader, which detects fluorescence.

VIDAS® analysis

The presence of pathogenic microflora, i.e. *Salmonella* sp. and *Listeria monocytogenes*, was determined with mini-

Table 1 Number of bacteria in tested samples of lyophilized garlic

No.	Count [log cfu/g] mean ± SE				
	TVC	LAB	*Enterobacteriaceae*	Yeast and moulds	*Bacillus* sp.
1.	5.85±0.02	3.30±0.04	<1.00	<1.00	<2.00
2.	4.95±0.01	2.90±0.03	<1.00	<1.00	3.04±0.02
3.	6.20±0.11	5.49±0.09	<1.00	<1.00	5.07±0.04
4.	5.90±0.09	3.86±0.02	<1.00	<1.00	<2.00

TVC total viable counts; *LAB* lactic acid bacteria; *SE* standard error

VIDAS apparatus (Biomerieux). Vidas tests are used to detect antigens and employ immunoenzymatic technology; the result is based on fluorescent reading (ELFA, Enzyme Linked Fluorescent Assay). Both in the case of detecting *Salmonella* sp. and *Listeria monocytogenes,* this test determines specific antigens present in a tested sample. The implementation of a VIDAS test followed the recommendations of the manufacturer (User's Manual, Application Guide of VIDAS® Biomerieux, Available from: https://techlib.biomerieux.com/wcm/techlib/techlib/applications/guidedSearch/cleverLink/CleverLink.jsp?productnumber=VIDAS./Accessed 06.08.12). An LMO II test was used to identify the presence of *Listeria monocytogenes* in 25 g of samples. After selective enrichment in half-Fraser and Fraser broth, 0.5 ml of culture was transferred into a test strip. The strip was placed in the apparatus and the subsequent procedures were automatic. The results were available after 45 min. An SLM test was used to determine the presence of *Salmonella* sp. in 25 g of sample were preenrichment in 225 ml of buffered pepton water (41.5 °C for 24 h) after that 0.1 ml was taken and transferred into 10 ml of Rappaport-Vassiliadis selective medium (Merck) with soya. After incubation at 37 °C for 48 h, 0.5 ml of culture was placed in a well of a test strip and heated for 15 min in a Vidas Heat and Go heat block, then Vidas SLM test was performed.

Statistical analysis

Data were analyzed by determining standard error of the mean and simple correlation after converting the microbial counts to a logarithmic scale (StatSoft Inc. STATISTICA (data analysis software system), Version 6. http://www.statsoft.com, 2001).

Results

The number of total viable count in the tested samples of garlic was 4.95–5.90 log CFU/g in the case of lyophilized garlic; 6.60–6.85 log CFU/g for the granulated garlic; and 3.51–6.53 log CFU/g in the samples of ground dried garlic. *Enterobacteriaceae* were detected only in one sample (out of 26), it was sample of ground dried garlic. In this case, the number of Gram-negative rods amounted to 2.07 log CFU/g. In the other samples, the number of *Enterobacteriaceae* was <1.00 log CFU/g (Tables 1, 2 and 3). The comparably low values were recorded for fungi. In all analysed garlic samples the number of yeast was <1.00 log CFU/g of product. However, mould fungi were detected in all three samples of granulated garlic and their number ranged from 1.30 to 3.47 log CFU/g (Table 2). Moulds were also identified in 5 out of 19 samples of ground dried garlic (Table 3); their number amounted to 2.00–3.47 log CFU/g. The number of lactic acid bacteria was varied regardless of the type of garlic. The number of these rods was 2.34–5.49 log CFU/g. Anaerobic spores of *Clostridium* sp. were detected in all samples of granulated and ground dried garlic in 0.1 or 0.01 g. The spores of saccharolytic clostridia were identified in only one sample (in 0.1 g) of lyophilized garlic (Table 4). *Bacillus* sp. was detected at <2.00–5.07 log CFU/g (Tables 1, 2 and 3). Pathogenic *Salmonella* sp. and *Listeria monocytogenes* were not detected in 25 g of any garlic samples.

Discussion

The analysis of data from the literature on the microbiological quality of garlic found varied results. There are reports on 36

Table 2 Number of bacteria in tested samples of granulated garlic

No.	Count [log cfu/g] mean ± SE				
	TVC	LAB	*Enterobacteriaceae*	Yeast and moulds	*Bacillus* sp.
1.	6.85±0.06	3.92±0.02	<1.00	1.30±0.01 (moulds)	2.00±0.03
2.	6.60±0.07	3.69±0.04	<1.00	3.47±0.04 (moulds)	2.54±0.02
3.	6.72±0.11	3.57±0.02	<1.00	2.30±0.03 (moulds)	2.69±0.06

TVC total viable counts; *LAB* lactic acid bacteria; *SE* standard error

Table 3 Number of bacteria in tested samples of ground dried garlic

No.	Count [log cfu/g] mean ± SE				
	TVC	LAB	*Enterobacteriaceae*	Yeast and moulds	*Bacillus* sp.
1.	5.46±0.11	2.34±0.02	<1.00	<1.00	4.39±0.05
2.	5.41±0.08	3.51±0.04	2.07±0.03	<1.00	4.25±0.11
3.	3.51±0.04	3.54±0.06	<1.00	<1.00	4.50±0.12
4.	5.62±0.07	4.69±0.07	<1.00	2.00±0.02(moulds)	4.71±0.05
5.	5.79±0.12	2.84±0.02	<1.00	2.47±0.04(moulds)	4.47±0.07
6.	6.41±0.11	5.27±0.11	<1.00	<1.00	<2.00
7.	6.07±0.04	5.23±0.08	<1.00	<1.00	<2.00
8.	6.51±0.13	4.99±0.08	<1.00	<1.00	<2.00
9.	6.20±0.11	5.49±0.02	<1.00	3.47±0.07(moulds)	5.07±0.07
10.	5.93±0.09	5.47±0.03	<1.00	<1.00	<2.00
11.	5.60±0.06	4.94±0.11	<1.00	<1.00	<2.00
12.	5.44±0.04	2.81±0.13	<1.00	<1.00	<2.00
13.	5.60±0.07	2.90±0.02	<1.00	<1.00	<2.00
14.	5.82±0.02	3.65±0.02	<1.00	<1.00	4.90±0.05
15.	5.69±0.01	3.68±0.03	<1.00	<1.00	4.39±0.07
16.	6.53±0.06	4.84±0.05	<1.00	2.69±0.06(moulds)	3.93±0.04
17.	6.30±0.08	4.07±0.06	<1.00	2.30±0.02(moulds)	4.17±0.09
18.	6.07±0.11	5.30±0.07	<1.00	<1.00	4.47±0.10
19.	5.79±0.11	3.91±0.02	<1.00	<1.00	4.30±0.11

TVC total viable counts; *LAB* lactic acid bacteria; *SE* standard error

cases of botulism type B due to the consumption of garlic butter made with bottled and chopped garlic mixed with soya oil and stored at room temperature which was contaminated with *Clostridium botulinum* (De Roever 1999). The authors (St Louis et al. 1988) indicated that a pH-reducing substance had to be added to such a preparation of garlic which had to be kept in a refrigerator to limit the potential growth of *Clostridium botulinum*. Our studies revealed the presence of saccharolytic clostridia in 22 analysed samples of garlic (Table 4), including all four samples of granulated garlic and only one sample of lyophilized garlic. The initial confirming studies proved the presence of *Clostridium butyricum* in the tested samples as opposed to the results reported by Ghoddusi and Sherburn (2010) who analysed 25 samples of garlic and did not detect *Clostridium butyricum* in any of them. This bacterial species may be responsible for defects in food products associated with the accumulation of butyric acid fermentation products (CO_2 and H_2). In our studies, proteolytic clostridia were detected in 0.1 g in 21 samples of garlic. The presence of anaerobic bacilli in the majority of tested samples may result from the contamination of garlic with soil, which is the source of clostridia. Therefore, special attention should be paid to preparative procedures during the processing of garlic (washing, peeling and chopping) since the temperature of preservation process does not kill bacterial spores.

Brużewicz and Malicki (2007) tested 40 samples of dried garlic. The total count of bacteria in these samples was on average 2.34 log CFU/g and decreased during 6-month storage at 20 °C to 1.76 log CFU/g. The number of coliform bacteria was 1.71 log CFU/g (in fresh samples) and 1.48 log CFU/g (after 6 months of storage), whereas the number of fungi was 2.21 log CFU/g at the beginning of the experiment and 2.23 log CFU/g at the end of storage period. These authors did not detect pathogenic microorganisms in any of the tested garlic samples, which was consistent with the results of our studies. Reduction of microflora in powdered garlic during 6 months of storage was related, according to these authors, to the presence of natural antibacterial substances. The similar results are cited by Schweiggert et al. (2007). In this case, the following microorganisms were detected: total number of bacteria −4.69 log CFU/g and number of coliform rods and fungi from <2.00 to 2.69 log CFU/g. In our studies, the number of total viable count was lower, ranging from 3.51 to 6.85 log CFU/g (Tables 1, 2 and 3). In the case of yeast, their number in the majority of tested samples was <1 log CFU/g and moulds were detected in minor amounts only in 8 samples. The lack of fungal growth in the tested garlic samples confirms the high fungistatic activity of compounds found in garlic (Corzo-Martínez et al. 2007). While performing tests with garlic, attention should be paid to the fact that the presence of biologically active fungistatic substances may influence their results. If only a low dilution (10^{-1} g) is used in cultures, a high concentration of the compounds that inhibit fungal growth may yield false results.

Table 4 Presence of *Clostridium* sp., *Salmonella* sp. and *Listeria monocytogenes* in tested garlic samples

No.	Presence (P)/no presence (NP) *Clostridium* sp.		Presence (P)/no presence(NP) in 25 g	
	Saccharolytic in 0.1 g	Proteolytic in 0.1 g	*Salmonella* sp.	*Listeria monocytogenes*
Lyophilized garlic				
1.	P	NP	NP	NP
2.	NP	NP	NP	NP
3.	NP	NP	NP	NP
4.	NP	NP	NP	NP
Granulated garlic				
1.	P	P	NP	NP
2.	P	P	NP	NP
3.	P	P	NP	NP
Ground dried garlic				
1.	P	P	NP	NP
2.	P	P	NP	NP
3.	P	P	NP	NP
4.	P	P	NP	NP
5.	P (in 0,01 g)	P (in 0,01 g)	NP	NP
6.	P	P	NP	NP
7.	P (in 0,01 g)	P	NP	NP
8.	P	P	NP	NP
9.	P (in 0,01 g)	P (in 0,01 g)	NP	NP
10.	P (in 0,01 g)	P (in 0,01 g)	NP	NP
11.	P	P	NP	NP
12.	P	P	NP	NP
13.	P (in 0,01 g)	P (in 0,01 g)	NP	NP
14.	P	P	NP	NP
15.	P	P	NP	NP
16.	P	P	NP	NP
17.	NP	NP	NP	NP
18.	P	P	NP	NP
19.	P	P	NP	NP

It is therefore always recommended to use higher dilutions of garlic. In our studies, this phenomenon was observed in several tested samples of garlic; after culturing of 10^{-1} g dilution, fungal growth was not detected on the plates, while the cultures of the same samples diluted at 10^{-2} g or 10^{-3} g yielded mould growth on the plates.

The results of our studies also confirm high hygiene during the storage and processing of garlic. *Enterobacterieceae* at 2.07 log CFU/g were detected only in one sample of ground dried garlic and it could have resulted from secondary contamination during the marketing of this spice. Other authors have reported similar results in their studies. Witkowska et al. (2011) detected *Enterobacteriaceae* in the tested garlic samples at 1.86 log CFU/g, while in the experiment carried out by Schweiggert et al. (2007) the count of coliform rods was <2.00–2.69 log CFU/g.

An analysis of the results revealed that the number of *Bacillus* sp. (Tables 1, 2 and 3) varied (from <2.00 to 5.07 log CFU/g). The initial analysis confirmed that, in the majority of cases, these bacilli belonged to *Bacillus subtilis* species. The tests did not identify *Bacillus cereus* in the samples of garlic, which was consistent with the results reported by Valero et al. (2002) who attempted to characterize *Bacillus cereus* isolated from vegetables. Those authors tested 6 samples of fresh garlic (out of 56 samples) and did not find pathogenic *Bacillus cereus* strains in any of them. They explained that garlic contained phenol compounds, protocatechuic acid and catechol which showed antibacterial activity.

Lactic acid bacteria were detected in all 26 samples of garlic (regardless of their type) and their counts amounted to 2.34–5.49 log CFU/g. The presence of these bacteria may be responsible for a taste defect (acidification) in the final product, i.e. garlic sauce. Shim and Kyung (1998) reported that garlic was a natural and excellent environment for the growth of lactic acid bacteria (indicating *Leuconostoc* genus as the

main representative). In addition, these authors emphasized that in the case of ineffective inactivation of lactic acid bacteria during the processing of garlic, they could have unrestrictedly continued fermentation and contributed to the occurrence of defects of food products. The garlic tested in our studies was contaminated with lactic acid bacteria (regardless of preservation method) which were probably responsible for acidification of garlic mayonnaise sauces.

Pathogenic *Salmonella* sp. and *Listeria monocytogenes* were not detected in 25 g in any of the tested garlic samples (regardless of their type). The decrease of the inhibiting effect of garlic extract on *Salmonella* sp. growth may be related to adaptation of *Salmonella* sp. to the presence of the specific inhibiting activity of the garlic extract (Oliveira et al. 2005).

Conclusions

The results of our studies suggest that garlic, regardless of the preservation method, may be a source of contamination with technologically detrimental microflora, mainly lactic acid bacteria and anaerobic spore-producing bacilli. Metabolism of this microflora may lead to numerous defects during storage in the final product. The most widely applied parameter used to determine the microbial quality of raw materials and food products are largely based on the total viable mesophilic counts which can't always give a realistic estimation of the microbial contamination. It means that in food product like mayonnaise sauces with garlic lactic acid bacteria and anaerobic spore-producing bacilli seems to be better parameter is the determination of the microbiological quality than total viable mesophilic counts.

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