MOULDS ASSOCIATED WITH MILK DEPENDING ON MACROCLIMATE AND GEOGRAPHICAL LOCATION

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(Received 12th Oct 2004, accepted 28th June 2005)

Abstract. Moulds can be found in milk as contaminants from the environment. The specific qualities of climate, vegetation and land are the important factors affecting the quality of moulds and determinators of genus and species in connection with a certain geographical location. The study was carried out in 297 milk samples taken from different geographical location: A. lowlands, B. hilly-mountainous, C. alluvial plains by the river, D. submountainous and part of basin by the river in course of four seasons. The following media were used for growing moulds in laboratory conditions as: Sabouroud dextrosa agar, Czapek agar, Potato dextrosa agar. Moulds determination was carried out according to their micromorphological properties using moulds determination keys. According to the result of study it was conclude that moulds count in raw milk samples were as follows: A. region: *Fusarium* genus (44.1%) in spring, *Aspergillus* genus (30.8%) in summer, *Penicillium* genus (30.2%) in autumn and the same (32.1%) in winter; B. region: *Fusarium* genus (55%) in spring, *Penicillium* genus (34.8%) in summer, *Penicillium* genus (23.1%) in autumn and *Cladosporium* + *Penicillium* genera (28.6%) in winter; C. region: *Penicillium* genus (69.3%) in spring and the same (31.8%) in summer, *Geotrichum* genus (24.6%) in autumn and *Aspergillus* genus (20.9%) in winter; D. region: *Penicillium* genus (42.9%) in spring and the same (50.73%) in summer, *Cladosporium* genus (43.2%) in autumn and *Penicillium* genus (45.21%) in winter. Finally we concluded that different genus of moulds which were found, are in dependence of geographical locations and seasons.
Keywords. *moulds, raw milk, contamination, ecology*

Introduction

The important influence of environmental factors on fungal growth has been demonstrated in a range of ecosystems. While there are some seasonal variations and certain peak periods most moulds have the capability of living year-round indoors as well as outdoors. Moulds spores established new colonies quickly making elimination difficult. Fungal spores are more abundant than any other airborne particles found in atmosphere including pollen grains. Moulds as inhalant allergen are of primary importance. One of the measures to avoid inhaling them is to remain indoors on windy days after a first frost when spores are abundant. But, it is almost impossible to escape mould spores so prevalent in and out-of-doors [12].

In a study of other authors [6] the seasonal variations had a significant effect on fungal growth. In the harvest period of the year or in such an environment where grain were stored, the *Fusarium* species was mostly found. The important factors for the growth of mould are fungal colonization of cereal grains before and after harvest. Variations in external conditions may not only affect the rate of growth of a mould but, in many cases, can bring about differences in way of growth.

The objective of the presented work was to determine the effect of temperature, moisture, geographical location and seasons of the year on population of moulds in milk samples.

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Materials and methods

The study was carried out on 297 milk samples. Four different geographical locations where the milk samples taken from A. lowlands, B. hill-mountainous, C. alluvial plains by the river, D. submountainous and part by the river in course of four different seasons. For the recovery of moulds the following medium was used: Potato dextrosa agar (PDA) pH 5.6 which was prepared as described in the bacteriological Analytical Manual and supplement with filter sterilized chloramphenicol and chlortetracycline immediately before use. Other media which were used are as follows: Sabouroud dextrosa agar and Czapek agar prepared as described in the Official Method of Analysis. Three dilutions were prepared then they were transferred into sterile plates (1 ml/plate approximately) from samples and from three pepton diluent. Triplicate Sabouroud agar pour plating were made of appropriate dilutions as the percentage of samples contaminated with mould are listed in *Table 1*. In the end of the specified incubation period, the plates were analyzed for fungal population (CFU per gram). Growing colonies were observed at first macroscopically and then microscopically. At first we obtained cultures by sight and then with microscope. We used to isolate a particular mould in order to obtain a pure culture then we described the colony colour and colour changes in the medium texture of surface (described as loose or compact, plane, wrinkled or buckled, velvity, matted, flocose, hairy, ropy, gelatinous etc.) odour of any character of submerged hyphae, full details of spores (colour, shape, septation, size etc.). Mould growth on the surface on the Sabouroud medium was removed to the select medium like PDA or Czapek agar which were selective for some kinds of moulds. These pieces of information were sufficient to place the species in the correct class and order, and consideration of the rest of the date will lead to the family and then genus and species.

Mould was carried out according to their micromorphological properties using mould determination keys [3, 4, 9, 11, 12].

Results and discussion

The results presented in *Table 1* showed in the region C isolation the higest percentage of contaminated samples was 91.30% in autumn. Minor contamination samples isolated in the region B. In the region A, the results showed variations from 64.00% in winter to 100% in autumn. In the region D, the results showed variations from 58.33% in winter, spring and autumn to 83.33% in summer. In the region C, the results showed variations from 57.14% in summer to 91.30% in autumn.

The isolated moulds were shown in the *Table 2*. The different genus of moulds isolated from milk samples during the year in the region A. Most frequently isolated moulds in winter were genus *Penicillium* (30.2%) and in winter were isolated seven different moulds colonies. In spring, most frequently isolated moulds were *Fusarium* (44.1%) beside four different moulds which were isolated. In summer, most frequently isolated mould were *Aspergillus* (30.8%) and eight other different moulds. Most frequently recovered in autumn were *Penicillium* genus (30.2%) and eight different genus of moulds. Most frequently isolated moulds in the region B were genera *Cladosporium* and *Penicillium* (28.6%) in winter, *Fusarium* (55.0%) in spring and *Penicillium* (23.1%) in summer. In the region C, most frequently isolated moulds in winter were members of the genera *Alternaria*, *Aspergillus* and *Geotrichum* (20.9%), in

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Table 1. *The percentage of samples contaminated with moulds depends of macroclimate and geographical location in four seasons of the year.*

location	season	percentage of samples with the colony of moulds
A (lowlands)	winter	64.00
	spring	68.00
	summer	76.00
	autumn	100.00
B (hilly-mountains)	winter	46.67
	spring	75.00
	summer	57.89
	autumn	58.33
C (alluvial plains by the river)	winter	80.00
	spring	80.00
	summer	57.14
	autumn	91.30
D (sub-mountain-ous and part by the river)	winter	58.33
	spring	58.33
	summer	83.33
	autumn	58.33

spring, *Penicillium* and *Cladosporidium* (31.8%) and in autumn, *Geotrichum* (24.6%). In the region C, frequently isolated moulds were *Penicillium* genus (69.3%) in spring, and the same (31.8%) also in the summer, *Geotrichum* genus (24.6%) in autumn, and *Aspergillus* genus (20.9%) in winter. In the D region, frequently isolated mould were *Penicillium* genus (42.9%) in spring and the same (50.73%) in summer, *Cladosporium* genus (43.2%) in autumn and *Penicillium* genus (45.21%) also in winter. *Table 1* presents the percentage of samples contaminated with moulds depends of macroclimate and geographical location in four season of the year.

Table 2 represents the spread and development in milk samples during the year in the four geographical locations.

The highest percentage of contaminated samples was on the alluvial plains by the river in the autumn or in the submountainous region and part by the river in the summer and spring too, but in the lowlands region in summer. The combined effect of medium water activity (a_w) modified atmosphere influenced on the conditional germination of different genus of moulds [3, 5]. A number of techniques for the enumeration and identification of viable mould propagules in the indoor air of houses were researched

Table 2. *Spread and development of mould in milk samples during the year in the geographical location A, B, C and D.*

genus of moulds	season															
	winter (%)				spring (%)				summer (%)				autumn (%)			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Absidia	—	—	—	—	—	—	—	—	7.7	2.2	—	—	7.8	7.7	2.3	12
Alternaria	6.0	6.2	30	—	—	—	—	11	2.6	11	—	—	4.8	7.7	8.4	1.4
Aspergillus	11.7	—	21	—	30	—	—	—	31	6.5	—	2.8	8.5	6.0	—	—
Cladosporium	14.6	28	7.6	9.4	—	—	11	18	5.1	17	32	—	6.0	7.7	13	43
Fusarium	17.5	—	—	—	44	55	3.9	10	23	4.4	7.4	—	18	7.7	8.9	—
Geotrichum	3.6	15	21	38	—	15	—	—	10	10	8	32	6.0	—	24	—
Mucor	14.6	21	15	5.2	—	—	7.7	17	—	11	7.2	5.7	21	7	11	10
Penicillium	32.1	28	4	45	17	30	3	43	15	35	32	51	30	23	24	31
Rhizopus	—	—	3.0	—	—	—	—	—	5.1	—	—	—	—	15.4	—	—
Scopulariopsis	—	—	—	1.6	9	—	—	—	—	2.2	—	2.6	—	7.7	4.5	2.0
Trichoderma	—	—	—	—	—	—	—	—	—	—	—	—	—	7.4	—	—

A = lowlands; B = hilly mountains; C = alluvial plains by the river; D = submountainous and part by the river; — = not found

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by a number of authors [8, 7]. Some authors [1, 2] recommended incubation temperature of enumerations moulds at 25 °C. Incubation time between plating and counting colonies ranges from 5 days for determination of general populations of microflora to 4 weeks of more. The same conditions were used in this experimental work.

In our study, the most different genus of moulds were isolated during summer and autumn. Most frequently recovered moulds could be found in the cereal before harvest, this is why they are called "moulds of field". In our study, most frequently recovered moulds were: *Cladosporium*, *Alternaria* and *Fusarium* in spring and *Fusarium*, *Geotrichum* and *Cladosporidium* in autumn. Insects spread mould spores to great distances [10]. Spores of moulds can disperse in the air with the wind or in combination of wind and rain. Other research [8] analyzed contamination of air surroundings of Kembridge. This author found in the air most frequently spores of moulds from the genus *Penicillium* and *Cladosporium*, their number depended on seasonal variations. In the districts where the most frequent disease was endemic nephritis in the last two years, correlation was found between great mortality [2]. The harvest – according to the work of the some authors – is a process which changes the conditions of ecosystem, because the cereal from the open air are transferred in the indoors where it is stored. As our results presents the highest number of moulds were in summer, spring and autumn.

Conclusion

On the received results we can conclude:

1. The percentage of moulds in the samples of raw milk varies depending on geographical location and season of the year. It begins with 46.64% in winter the region C and the highest number is in autumn 91.30% in the region C, too.
2. From the samples of milk most frequently isolated moulds were as follows: region A: genus *Fusarium* (44.1%), in spring, *Aspergillus* (30.8%) in summer, *Penicillium* (30.2%) in autumn and the same (32.1%) in winter; region B: genus *Fusarium* (55%) in spring, *Penicillium* (34.85%) in summer and the same (23.1%) in autumn, genera *Cladosporium* + *Penicillium* (28.6%) in winter; region C: genus *Penicillium* (69.3%) in spring and the same (31.8%) in summer, *Geotrichum* (27.0%) in autumn, *Aspergillus* (20.9%) in winter; region D: genus *Penicillium* (42.9%) in spring, and the same (50.73%) in summer, *Cladosporium* (43.2%) in autumn and *Penicillium* (45.2%) in winter.

REFERENCES

[1] Association of Official Analytic Chemists (1990): Official methods of analysis. 15th ed. Arlington V.A.
[2] Austwick, P.K.C. (1975): Mikroflora kukuruza pšenice i graha u području endemske nefropatije u Bosni i Hercegovini by Ožegović L., 1982. Simpozijum o mikotoksinima, pp. 55–65.
[3] Beuchat, L.R. (1992): Media for detecting and enumeratiobn yeasts and moulds. – Int. J. Food Microbiol. 17(2): 145–158.
[4] Booth, C. (1971): The genus Fusarium. Commonw. Mycol. Inst. Kew. 273 pp.
[5] Samson, R.A., Ellem, S. & Reenen, H. (1988): Introduction of foodborne fungi. Baarn Institute for Royal Netherland.

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[6] El Halouat, A. & Debevere, J.M. (1997): Effect of water activity, modified atmosphere packing and storage temperature on spore germination of moulds isolated from prunes. – Int. J. Food Microbiol. 3581: 41–48.
[7] Lacey, J. (1989): Pre and post harvest ecology of fungi causing spoilage of foods and other stored products. – Journal of Applied bacteriology Symposium supplement 679, 18: 11–25.
[8] Mislive, P.B., Stack, M.E., Koch, H.A. & Bandler, R. (1992): Yeast, moulds and mycotoxins, ch 18 in Food and Drug Administartion, Bacteriology Analytical Manuel 7th ed. Association of Official Analytical Chemists, Arlington V.A.
[9] Paswey, M. (1964): Meaning in the yeasts and moulds spore contamination in Kembridge air. – Journal of Applied Bacteriology 28(3): 385–389.
[10] Raper, K.B., Stolk, C. & Hadlok, R. (1976): Revision the subsection Fasciculate of Penicillium and some allied species. – In: Samson, R.A., Ellen, S., Reenen, H. (1988): Introduction of foodborne fungi. Baarn Institute for Royal Netherland, pp. 11–45.
[11] Ruize, A.J., Bentabol, A., Gallego, C., Angulio, R. & Jedral, M. (1996): Mycroflora and aflatoxin producing strains of Aspergillus flavus in greenhouse cultivated gren beans. – J. of Food Protect. 58(4): 433–435.
[12] Samson R.A. & Van Reenen-Hoekstra E.S. (1988): Introduction of foodborne fungi. 3rd edn. Centraalbureau voor Schmelaturres.

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