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XEROPHILIC MYCOPOPULATIONS OF TEAS IN BULK*

ABSTRACT: Other the water, tea is the most popular beverage in the world today. They are used for ages, in the beginning as refreshing drinks, and later more for their healing properties. Teas have been demonstrated to show antioxidative, anti-carcinogenic, and anti-microbial properties. Considering that the teas, during the production, are not treated with any temperature, there is high risk for contamination with different type of microorganisms, especially with moulds. Moulds are ubiquitously distributed in nature and their spores can be found in the atmosphere even at high altitudes and under favorable conditions of temperature and humidity, moulds grow on many commodities including cereals, oil seeds, nuts, herbs and spices. Most of them are potential producers of mycotoxins which present a real hazard to human health.

The aim of this work was to investigate total mould count and to identify moulds isolated from teas in bulk, than from teas treated with hot, sterile, distilled water and from the tea filtrates. Tested teas were peppermint, sage, yarrow, black tea, bearberry, lemon balm, mixture of teas from Zlatibor. In teas in balk was observed high contamination with different kinds of moulds (1.84-4.55 cfu/g), such as *Aspergillus awamori, A. lovaniensis, A niger, A. phoenicus, A. repens, A. restrictus, A. sydowii, A. versicolor, Eurotium amstelodami, E. chevalieri, E. herbariorum, Penicillium chrysogenum,* and *Scopulariopsis brevicaulis*. The most frequent were species from Aspergillus and *Eurotium* genera. Thermal treatment with hot, sterile, distilled water reduced the number of fungal colonies. *Aspergillus awamori* was the most resistant and appeared in six samples of filtrates of tea, *Aspergillus niger* in one sample and *Penicillium chrysogenum* in one sample.

KEY WORDS: teas, mould contamination, thermal treatment

INTRODUCTION

Other the water, tea is the most popular beverage in the world today. They are used for ages, in the beginning as refreshing drinks, and later more for their healing properties. Tea and tea products mainly contain tea polyphe-

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nols, which are natural antioxidants and have been demonstrated to show antioxidative, anti-carcinogenic and anti-microbial properties by many researchers (McKay and Blumberg, 2002; Rietveld and Wiseman, 2003).

Considering that the teas, during the production, are not treated with any temperature, there is high risk for contamination with different sort of microorganisms, especially with moulds, mostly xerophilic moulds.

Moulds are ubiquitously distributed in nature and their spores can be found in the atmosphere even at high altitudes, carried and disseminated by wind and air currents, or spread by insects, rodents, and other animals (Kungulovski et al., 2011) and under favourable conditions of temperature and humidity, moulds grow on many commodities including cereals, oil seeds, nuts, herbs, and spices (Brera et al., 1998).

Most of moulds are potential producers of mycotoxins. Mycotoxins are secondary extracellular metabolites of moulds. After biosynthesis, they diffuse from mycelium into the substrate (Višić, 2007). They, as potential mutagenic, teratogenic, cytotoxic, and carcinogenic present a real hazard to human health. Mycotoxins are, also, very thermostable. Even the temperature of sterilization is too low to cause destruction of mycotoxin structures (Škrinjar, 1997).

Because of those reasons the aim of this work was to investigate total mould count and to identify moulds isolated from teas in bulk, than from teas treated with hot, sterile, distilled water, and from the tea filtrates.

MATERIALS AND METHODS

Mycological investigations were carried out in seven samples of tea. The samples were collected from health food store (6) and from the market (1). The names and families of each sample are presented in Table 1.

Tab. 1 – Names and families of studied teas

No.	English name	Latin name	Family
1.	Peppermint	*Menta piperita*	*Lamiaceae*
2.	Sage	*Salvia officinalis*	*Lamiaceae*
3.	Yarrow	*Achilea millefolium*	*Asteraceae*
4.	Black tea	*Camellia sinensis*	*Theaceae*
5.	Bearberry	*Arctostaphylos uva ursi*	*Ericaceae*
6.	Lemon balm	*Mellissa officinalis*	*Lamiaceae*
7.	Mixture of teas from Zlatibor		

In further investigation, the influence of thermal treatment on surviving of the present moulds during the preparation of beverages was investigated. Twenty grams of seven samples of tea were treated with 180 ml of hot sterile distilled water for 15 minutes. After filtration, total mould count in dry residues were determined using dilution method by Koch in duplicates, while total mould count in 1ml of filtrate of teas were determined by membrane filter method (MFM).

Media used for total mould count determination were: **MY10-12** (maltose extract, 20 g/l; yeast extract, 5 g/l; NaCl, 100 g/l; glucose, 120 g/l; chloramphenicol, 0.05 g/l; agar, 20 g/l) and **MEA (**maltose extract, 20 g/l; pepton, 1g/l; glucose, 20 g/l; NaCl, 170 g/l; chloramphenicol, 0.05 g/l; agar, 20 g/l).

Inoculated Petri dishes were incubated for 7 (MY 10-12) and 14 days (MEA) at 25ºC.

Identification of isolated fungal species was done according to T h o m and R a p e r (1945), K l i c h (2002) and S a m s o n et al. (2004).

RESULTS AND DISCUSSION

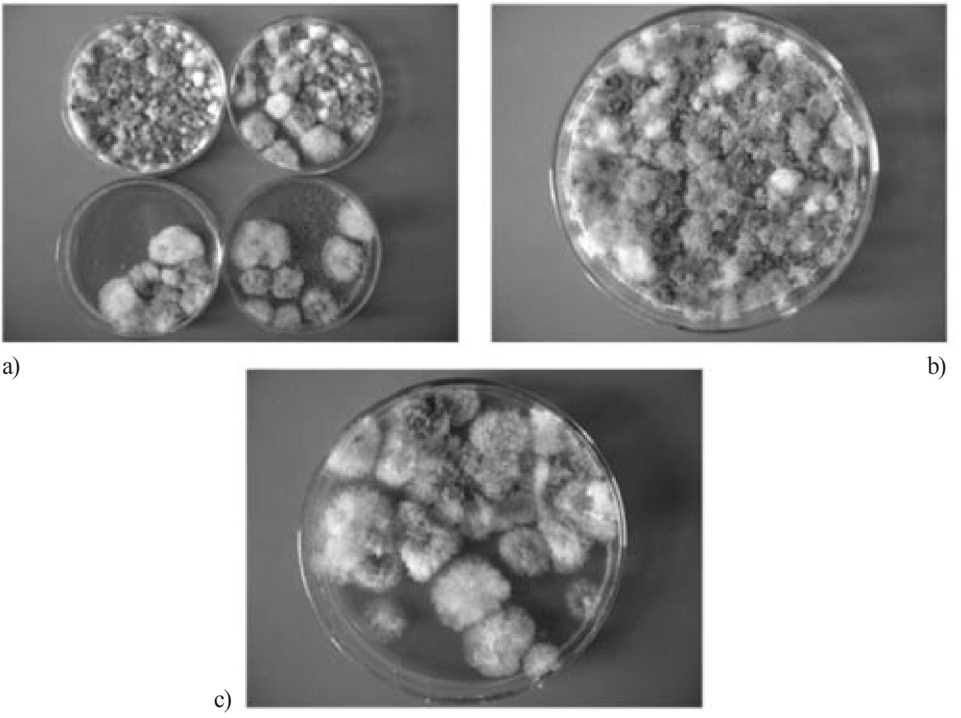
Mycological investigations of tea samples in bulk

Results for total mould count per gram of teas in bulk, expressed as log of cfu/g, are presented in Table 2 and in the Figure 1.

The most contaminated was a sample of mixture of teas from Zlatibor what is determined by applying MY10-12 medium (4.55 cfu/g), while on MEA medium, for the same tea, moulds were not isolated. Black tea was the least contaminated on both media (1.84 cfu/g on MY10-12; 1.00 cfu/g on MEA medium).

Tab. 2 – Total mould count per g of teas in bulk [log_{10}cfu/g] determined by using MY10-12 and MEA media

Sample	MY 10-12	MEA
	Total mould count [log_{10}cfu/g]	Total mould count [log_{10}cfu/g]
Peppermint	2.77	2.66
Sage	2.602	2.95
Yarrow	3.56	2.93
Black tea	1.84	1.00
Bearberry	2.83	1.70
Lemon balm	3.04	2.42
Mixture of teas from Zlatibor	4.55	-



a) b) c)

Fig. 1 – Mould contamination of teas in bulk

From studied samples a number of different moulds were isolated which are classified to 4 genera and 13 species as can be seen in Table 3.

Tab. 3 – Fungal species isolated from teas samples

Genus	**Subgenus**	**Section**	**Species**
Aspergillus	*Circumdati*	*Nigri*	*Aspergillus awamori* Nakazawa *Aspergillus niger* v an Tieghem *Aspergillus phoenicus* (Cda) Thom
Aspergillus	*Nidulantes*	*Versicolores*	*Aspergillus versicolor* (Vuill.) Tiraboschi *Aspergillus sydowii* (Bain and Sart.) Thom and Church
Aspergillus	*Aspergillus*	*Restricti*	*Aspergillus restricrus* G. Smith
Aspergillus			*Aspergillus lovaniensis* Biourge *Aspergillus repens* DeBary
Eurotium	*Aspergillus*	*Aspergillus*	*Eurotium amstelodami* (Mangin) Thom ans Church *Eurotium chevalieri* (Mangin) Thom ans Church *Eurotium herbariorum* (Wiggers) Link
Penicillium			*Penicillium chrysogenum* Thom
Scopulariopsis			*Scopulariopsis brevicaulis* (Sacc.) Bain

Fungal species isolated from teas in bulk are presented in Table 4. The most frequent were species from genera *Aspergillus* and *Eurotium*. Also, some species from genus *Penicillium* were presented.

Tab. 4 – Fungal species isolated from teas in bulk

Me-dium	Samples						
	Peppermint	**Sage**	**Yarrow**	**Black tea**	**Bearberry**	**Lemon balm**	**Mixture of teas from Zlatibor**
MY 10-12	*Aspergillus awamori Aspergillus versicolor Eurotium amstelodami*	*Aspergillus awamori Eurotium amstelodami*	*Eurotium amstelodami Eurotium chevalieri*	*Aspergillus awamori*	*Aspergillus awamori Aspergillus niger*	*Aspergillus flavus Aspergillus lovaniensis Aspergillus niger Scopulariopsis brevicaulis*	*Aspergillus restrictus Eurotium amstelodami Eurotium herbariorum Penicillium chrysogenum*
MEA	*Penicillium chrysogenm*	*Aspergillus versicolor*	*Penicillium chrysogenum*	*Aspergillus repens*	*Aspergillus versicolor Eurotium herbariorm*	*Aspergillus versicolor Aspergillus sydowii Eurotium amstelodami Eurotium herbariorum*	

The most of isolated moulds are xerophilic and minimum of water activity for their growth is presented in Table 5.

Tab. 5 – Minimum temperature and water activity for growth of different fungal species

Fungal species	Temperature [ºC]	a_w
Aspergillus flavus	10	0.81
Aspergillus niger	12	0.77
Aspergillus versicolor	6	0.70
Eurotium amstelodami	10	0.71
Eurotium chevalieri	6	0.72
Eurotium herbariorum	4	0.78
Penicillium chrysogenum	4	0.84
Scopulariopsis brevicaulis	*	0.90

*not determined

Moulds in dry residues and in tea drinks after thermal treating

Results for total mould count per gram in dry residues after thermal treating, expressed as log of cfu/g are presented in Table 6. It is observed reduction of total mould count in all samples what can also be seen in the Figure 2. On MY10-12 medium number of colonies was ranged from 1.00 cfu/g on peppermint to 2.02 cfu/g on lemon balm, while on MEA medium growth didn't appear in all samples.

Tab. 6 – Total mould count in dry residues after thermal treating [log_{10}CFU/g]

Sample	MY 10-12	MEA
	Total mould count [log_{10}CFU/g]	**Total mould count [log_{10}CFU/g]**
Peppermint	1.00	-
Sage	1.78	-
Yarrow	1.30	-
Black tea	1.60	-
Bearberry	1.81	-
Lemon balm	2.02	-
Mixture of teas from Zlatibor	1.30	-

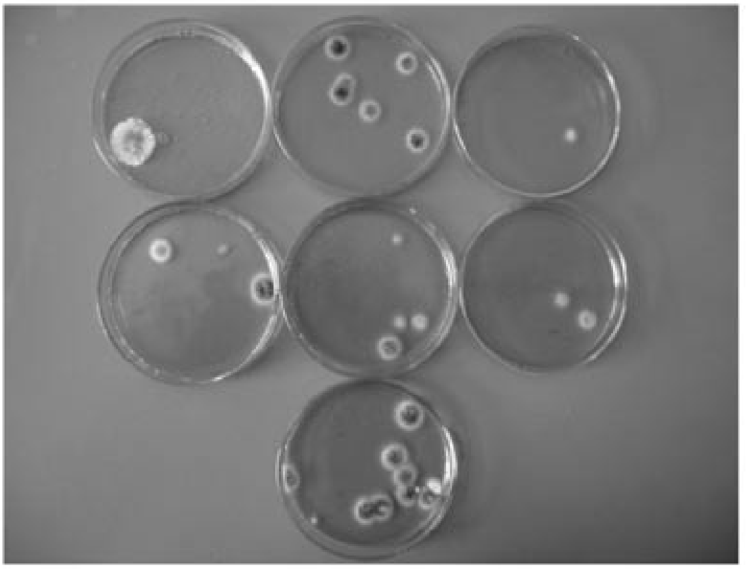


Fig. 2 – Mould contamination of dry residues after thermal treating

Isolated fungal species from dry residues after thermal treating were from genera *Aspergillus* and *Eurotium* what is presented in Table 7.

Tab. 7 – Isolated fungal species from dry residues after thermal treating

Medium	Samples						
	Peppermint	Sage	Yarrow	Black tea	Bearberry	Lemon balm	Mixture of teas from Zlatibor
MY 10-12	*Eurotium herbariorum*	*Eurotium amstelodami*	*Aspergillus awamori*	*Aspergillus awamori*	*Aspergillus awamori*	*Aspergillus niger*	*Aspergillus niger* *Eurotium herbariorum*
MEA	-	-	-	-	-	-	-

Regarding filtrates of tea, growth was observed in all samples on MY10-12 medium, while on MEA medium there was no growth (Table 8, Figure 3). The least contamination was in filtrate from peppermint (0.70 cfu/g) and the highest in filtrate from mixture of teas from Zlatibor (1.84 cfu/g).

From all filtrates, except from lemon balm, was isolated *Aspergillus awamori.* In filtrate of lemon balm appeared *Aspergillus niger* and *Penicillium chrysogenum*, what is presented in Table 9.

Tab. 8 – Total mould count in filtrate of tea obtained by membrane filter method

Sample	**MY 10-12**	**MEA**
	Total mould count [$\log_{10}$cfu/ml]	**Total mould count [$\log_{10}$cfu/ml]**
Peppermint	0.70	-
Sage	1.33	-
Yarrow	1.69	-
Black tea	1.43	-
Bearberry	1.23	-
Lemon balm	1.56	-
Mixture of teas from Zlatibor	1.84	-

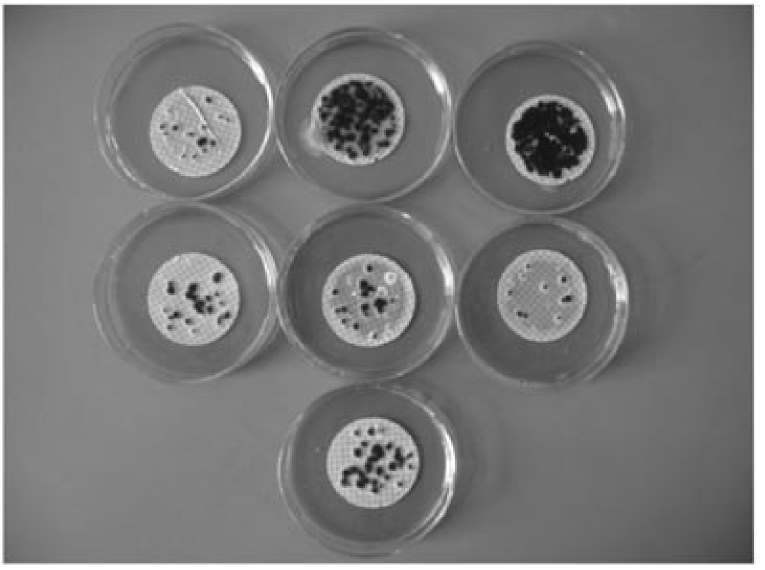


Fig. 3 – Mould contamination of filtrate of tea obtained by membrane filter method

Tab. 9 – Isolated fungal species from filtrate of tea obtained by membrane filter method

Medi-um	Samples						
	Peppermint	Sage	Yarrow	Black tea	Bearberry	Lemon balm	Mixture of teas from Zlatibor
MY 10-12	*Aspergillus awamori*	*Aspergillus awamori*	*Aspergillus awamori*	*Aspergillus awamori*	*Aspergillus awamori*	*Aspergillus niger Penicillium chrysogenum*	*Aspergillus awamori*
MEA	-	-	-	-	-	-	-

The presence of different mould genera in teas in bulk, dry residues after thermic treatment and in filtrates of teas can be seen in the Figure 4.

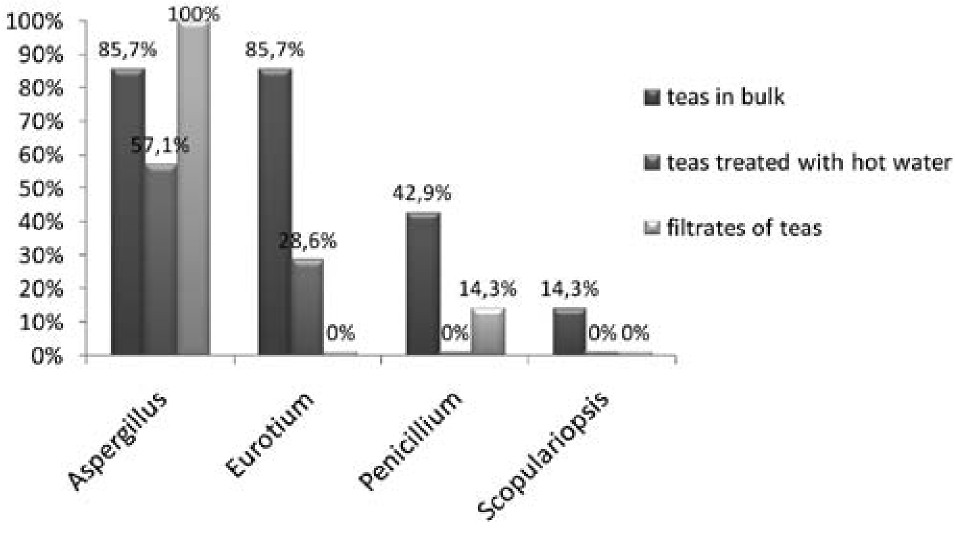


Fig. 4 – Presence of different mould genera in teas in bulk, dry residues after thermic treatment and in filtrates of teas

It is obvious that *Aspergillus* species were present in all three cases. *Eurotium* species was not present only in tea filtrates, *Penicillium* species were present in teas in bulk and in tea filtrates and *Scopulariopsis brevicaulis* was present only in teas in bulk.

All of isolated fungal species, according to different allegations, are potential producers of mycotoxins. In the Table 10 can be seen some of isolated moulds whose are potential producers of toxins.

Tab. 10 – Some of isolated moulds whose are potential producers of toxins

Moulds	Toxins
Eurotium amstelodami	Sterigmatocystin (traces)
Eurotium herbariorum	Sterigmatocystin (traces)
Eurotium shevalieri	Emodin, gliotoxin, physicon, xanthocillin
Aspergillus flsvus	Aflatoxins, aflatrem, aflavinin, aspergillic acids
Aspergillus niger	Maltformins, nigragilin
Aspergillus sydowii	Nidulotoxin, sterigmatocystin, griseofulvin
Aspergillus versicolor	Nidulotoxin, sterigmatocystin
Penicillium chrysogenum	Roquefortine C, PR-toxin, xantocillin, penicillin

All of this species are widely distributed (K l i c h , 2002).

Eurotium species are world-wide but predominantly in tropical and subtropical areas and they were reported from dried food products, spices, peas, milled rice, nuts (D o m s c h et al., 1980).

Aspergillus flsvus is the most widely reported food-borne fungus and from many other substrates in indoor and outdoor environments (S a m s o n et al., 2001).

Aspergillus niger has been reported from soils, plant litter, dried fruits, nuts and indoor environments (P i t t and H o c k i n g , 1997).

Habitat of *Aspergillus sydowii* is primary soil, but it has been reported from many other substrates in indoor and outdoor environments. *A. sydowii* is much less present in food than *Aspergillus versicolor* which occurs especially in spices, dried cereals and nuts, and is common in indoor environments (D o m s c h et al., 1980).

Penicillium chrysogenum has been reported from indoor environments, deserts, dried foods, cheese, salterns (S a m s o n and F r i s v a d , 2004).

CONCLUSIONS

Different kinds of moulds were isolated from all samples of tea. Total mould count was the highest in teas in bulk (1.84-4.55 $\log_{10}$cfu/g). The most frequent moulds were species from *Aspergillus* and *Eurotium* genera.

After treatment with hot, sterile, distilled water total mould counts in all samples were reduced (in dry residues after thermal treating number of colonies was in range from 1.00 cfu/g to 2.02 cfu/g and in tea filtrates from 0.70 cfu/g to 1.84 cfu/g).

Aspergillus awamori was the most resistant and appeared in six samples of filtrates of tea, *Aspergillus niger* in one sample and *Penicillium chrysogenum* in one sample.

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КСЕРОФИЛНЕ МИКОПОПУЛАЦИЈЕ ЧАЈЕВА У РИНФУЗИ

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Резиме

После воде, данас је чај најпопуларније пиће на свету. Конзумира се већ дуги низ година, у почетку више као освежавајуће пиће, а у последње време више због својих лековитих својстава. Показало се да чајеви поседују антиоксидативне, антиканцерогене и антимикробне особине. Имајући у виду да чајеви током производње не пролазе кроз технолошке операције које подразумевају дејство високих температура, постоји велика могућност контаминације различитим врстама микроорганизама, посебно плеснима. Плесни су широко распрострањене у природи, а њихове споре се могу наћи у атмосфери чак и на веома великим висинама. Под повољним условима температуре и влажности, плесни расту на многим намирницама, као што су житарице, уљарице, језгровито воће, лековито биље и зачини. Многе врсте плесни су потенцијални произвођачи микотоксина, што представља велик ризик по људско здравље.

Циљ овог рада је био да се испита укупан број плесни и да се идентификују плесни изоловане из чајева у ринфузи, из чајева третираних врелом, стерилном дестилованом водом и из филтрата. Испитивани су чајеви: нана, жалфија, хајдучка трава, црни чај, увин чај, матичњак и мешавина чаја са Златибора. У чајевима у ринфузи је установљена висока контаминација различитим врстама плесни (1,84-4,55 цфу/г), као што су *Aspergillus awamori*, *A. lovaniensin*, *A. niger*, *A. phoenicus*, *A. repens*, *A. restrictus*, *A. sydowii*, *A. versicolor*, *Eurotium amstelodami*, *E. chevalieri*, *E. hermariorum*, *Penicillium chrysogenum* и *Scopulariopsus brevucaulus*. Најфреквентније су биле врсте родова *Aspergillus* и *Eurotium*. Термички третман врелом стерилном дестилованом водом је редуковао број плесни. *Aspergillus awamori* је био најотпорнија врста и појавио се у шест узорака филтрата чаја, *Apergillus niger* у једном и *Penicillium chrysogenum* такође у једном узорку филтрата.