MICROBIOLOGICAL QUALITY OF ORGANIC WHEAT GRAINS AND SPROUTS

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Abstract

Vegetable seed sprouts have become popular in many countries as a nutrient supplement due to their high content of proteins, minerals and vitamins. During sprout germination, complex compounds comprised of lipids, carbohydrates, and storage proteins are broken down into simple and digestible nutrients. Besides their nutritional value to people, sprouts can be a good food source of pathogenic and spoilage microorganisms.

Bacterial species on the surface of grown sprouts are often originated from the soil. They are members of pseudomonades, enterobacteria, lactic acid bacteria in addition to yeast species.

There is only limited information available on the growth of contaminating and spoilage microorganisms during the germination of sprouts in Hungary.

The purpose of this study was investigating the changes in the microbiota and microbiological load of germinated grains during the process of sprouting (from grain to ready-to-eat sprout).

The organic wheat grains were bought from herbalist shops for the study. The sprouts were grown under hydroponic conditions at 20 ^{o}C in sterile germinating dishes. We examine the microbiological quality (total aerobic plate count, coliform count, yeast and mould count) of grains before soaking, and after soaking in sterile water for 12 hours, and of the one-, two- and three-day sprouts. Enumeration of total aerobic plate count was performed using Plate count agar, coliform count using Violet Red Bile Lactose agar, furthermore yeast and mould count using Chloramphenicol Glucose agar.

Analysing the microbiological changes of the organic wheat grain samples before soaking we found that mean total aerobic plate counts, coliform count and yeast and mould count were 4.9 log_{10} cfu/g, 3.9 log_{10} cfu/g, 3.5 log_{10} cfu/g, respectively. After soaking, total aerobic plate count of grains increased to 6.2 log_{10} cfu/g, coliform count to 6.0 log_{10} cfu/g, yeast and mould count to 4.0 log_{10} cfu/g. In the first three days of sprouting, microbial populations on grains increased approximately 2-3 logs. During sprouting process, the total aerobic plate count of ready-to-eat (three-day) sprouts increased to 7.9 log_{10} cfu/g, coliform count to 7.4 log_{10} cfu/g, yeast and mould count to 6.6 log_{10} cfu/g.

These high microbial levels per se can reduce the shelf-life and safety of sprouts. Initial microbial load should be controlled, for minimizing the microbial contamination in the sprouts prior to consumption. On the basis of our results, we recommend the decontamination of seeds before sprouting.

Key words: wheat grain, wheat sprout, microbiological quality, microorganisms count

INTRODUCTION

The use of germinated seeds as food originated in Far East countries and has recently spread to the western world, where they are considered fashionable and healthy ingredients (Kuo et al., 2004). Green sprouts have been part of the human diet since old times especially in Japan where they

are widely consumed. Recently the interest in consuming fresh green sprouts has extended all over the world because they are considered to provide health benefits due to their high content of proteins, minerals and vitamins. Sprouts are grown from seeds placed in environmentally controlled, hydroponic conditions and incubated in warm, moist, nutrient-rich conditions, which are ideal environments for microbial growth. The common sprouting conditions (2–7 days of sprouting, temperatures of 20–40 ^{o}C and optimum water activity, pH and nutrients availability) are also favourable for bacterial growth (Taormina et al., 1999).

Seeds commonly contain high microbial loads, ranging between 10^3 and 10^6 cfu/g which are constituted mainly of pseudomonads, enterobacteria, lactic acid bacteria and yeast (Prokopowich & Blank, 1991; Randazzo et al., 2009; Robertson et al., 2002). These levels can increase during sprouting, reaching populations as high as 10^8–10^{11} cfu/g (Ghandi & Matthews, 2003; Peńas et al., 2008).

In case of seed, various surveys have revealed aerobic plate counts of 3–6x10^4 cfu/g (Andrews et al., 1979), 9x10^5 cfu/g (Andrews et al., 1982), and 5–400x10^3 cfu/g (Prokopowich & Blank, 1991) on alfalfa seeds; 1–20x10^4 cfu/g on mung beans (Andrews et al., 1982); 1x10^5 cfu/g on onion seeds (Prokopowich & Blank, 1991); and 3x10^7 cfu/g on rice seeds (Piernas & Guiraud, 1997).

In case of sprouts, several authors have reported aerobic plate counts between 10^8 and 10^{11} cfu/g in alfalfa, mung bean or onion sprouts (Ghandi & Matthews, 2003; Lang et al. 2000; Patterson & Woodburn, 1980; Peńas et al., 2008; Prokopowich & Blank, 1991).

These high microbial counts are the main reason for the short shelf-life of sprouts and increase the likelihood of infections as described by National Advisory Committee on Microbial Criteria for Foods (NACMCF) (1999) and Taormina et al. (1999), if seeds are contaminated with pathogens.

Therefore, to minimize microbial contamination of sprouts prior to consumption, initial microbial load should be controlled (Akbas & Olmez, 2007).

There is only limited information available on the growth of contaminating and spoilage microorganisms during the germination of sprouts in Hungary.

The purpose of this study to investigate the changes in the microbiota and microbiological status of germinated grains during the process of sprouting (from grain to ready-to-eat sprout).

MATERIAL AND METHOD

Germination conditions and samples

The organic wheat grains were bought from herbalist shops. The sprouts were grown under hydroponic conditions at 20 ^{o}C in sterile germinating dishes. We examine the microbiological quality (total aerobic plate count, coliform count, yeast and mould count) of grains before soaking, and after soaking in sterile water for 12 hours, and of the one-, two- and three-day sprouts.

Microbial analysis

According to the requirements of the EN ISO 4833:2003 standard to examine the total plate count of seed and sprout samples Plate count agar and aerobic incubation at 30^{o}C for 72±3 hours were used.

For the examination of coliform bacteria count of samples, Violet Red Bile Lactose agar (VRBL-agar) media were used under aerobic incubation conditions at 30^{o}C for 24±2 hours, according to the instruction of the ISO 4832:2006 standard.

According to the instruction of the ISO 7954:1999 standard to examine the yeast and molds count of samples, we used Chloramphenicol Glucose agar and aerobic incubation at 25^{o}C for 3-5 days.

Statistical analysis

To perform the statistical calculations, the cfu/g results were converted to decimal logarithmic values ($\log_{10}$). We calculated mean, standard deviation; and minimum and maximum values from the data.

For the statistical analysis of the results analysis of variance (ANOVA) and Tukey-test or nonparametric Kruskal-Wallis test and comparative Dunn's test were used. Differences were considered statistically significant at the 95% confidence level ($P<0.05$). All statistical analyses were performed using SPSS v.13.0 and GraphPad Prism 3.02 statistical programs.

RESULTS AND DISSCUSIONS

Changes of total plate count during germination period

The mean and standard deviation of initial total plate count of wheat grains were 4.9±0.3 $\log_{10}$ cfu/g (Fig. 1). After the 12 hours soaking procedure the total plate count of grains increased more than 1 log unit

(6.2±0.4 $\log_{10}$ cfu/g). During the sprouting process the total plate count continuously increased day after day. The largest increase was observed on the first day of sprouting. On the second and third day we noticed moderate increase. Total plate counts of one-day sprouts were 7.4±0.4, second-day sprouts were 7.8±0.3 and third-day sprouts were 7.9±0.4 $\log_{10}$ cfu/g.

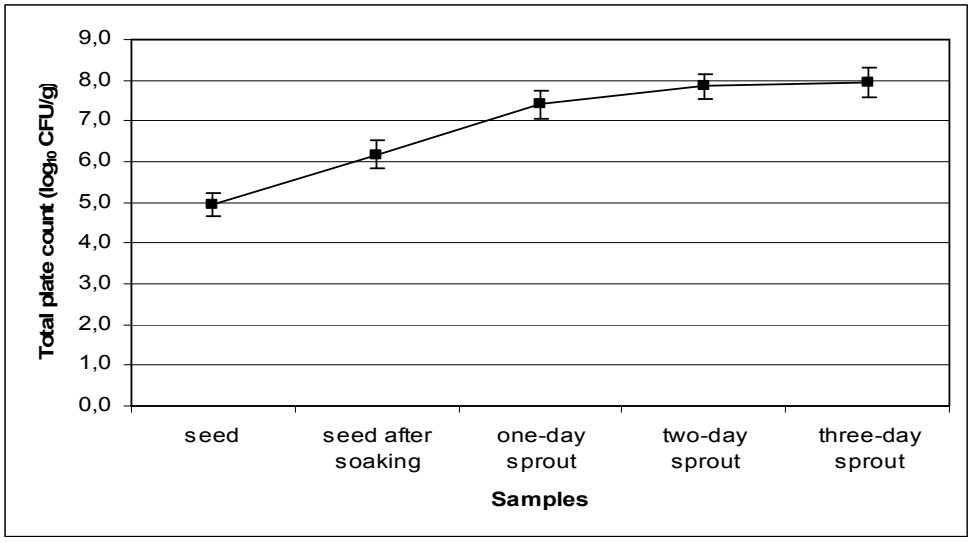

Fig. 1 Changes of total plate count during germination period

Total aerobic plate count increased gradually up to 3 log cycles after three days of germination.

Rises in bacterial number observed in wheat sprouts are similar to others described in the literature. Thus, during germination period, 2 log cycle increase in microbial populations was found in the case of rice (Piernas & Guiraud, 1997), 2.3 log cycles in wheat (Weiss et al., 2007), lupin and fenugreek (Martínez-Villaluenga et al., 2006), 3 log cycles in alfalfa and mung bean (Andrews et al., 1982; Splittstoesser et al., 1983) and up to 4 log cycles in kidney bean (Kimanya et al., 2003).

Fresh sprouts typically have total plate counts as high as 10^8–10^9 cfu/g (Viswanathan & Kaur, 2001; Gabriel et al., 2007) due to the intrinsic microflora of the seeds and the favorable environment in which they are grown.

Changes of coliform bacteria count during germination period

Changes of coliform bacteria count of grains and sprouts during the sprouting process are shown in Fig. 2. The number of coliform bacteria in

wheat grains was 3.9±0.4 $\log_{10}$ cfu/g. It was 1 log unit lower than the mean total plate count of grains.
Coliform bacteria count, similarly to the total plate count, successively increased during the germination period. Coliform counts of one-day sprouts were 6.9±0.7, second-day sprouts were 7.3±0.4 and third-day sprouts were 7.4±0.5 $\log_{10}$ cfu/g. Coliform bacteria counts increased gradually up to 3.5 log cycles after three days of germination.
Results obtained for wheat grains and sprouts on coliform count agree with those found in the literature for different kinds of seeds and sprouts (Prokopowich & Blank, 1991; Piernas & Guiraud, 1997; Soylemez et al., 2001; Martínez-Villaluenga et al., 2006; Weiss et al., 2007).

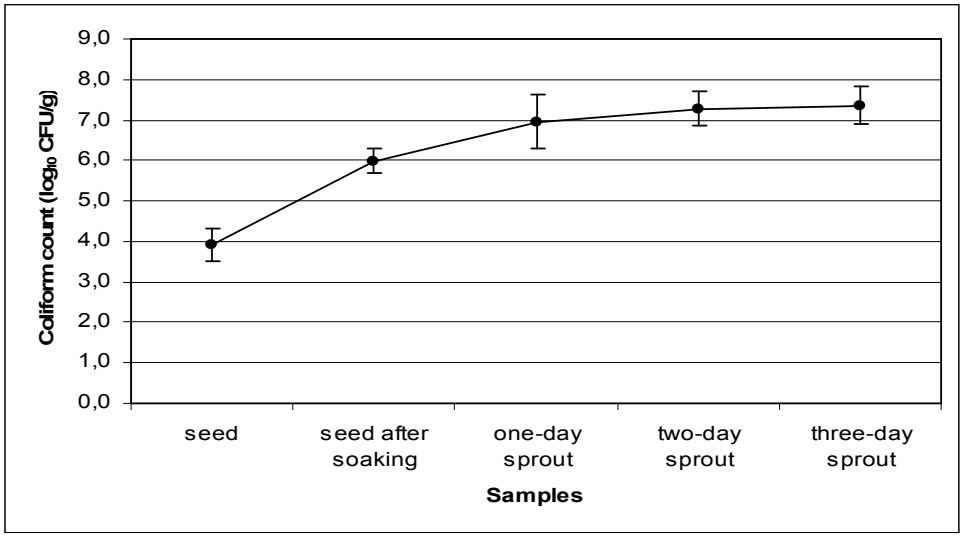

Fig. 2 Changes of coliform count during germination period

Changes of yeast and mould count during germination period

Changes of yeast and mould count of grains and sprouts during the sprouting period are shown in Fig. 3.
The number of yeasts and moulds in wheat grains was 3.5±0.6 $\log_{10}$ cfu/g and this microbial population increased 3 log cycles in three-day sprouts.
Yeast and mould count, similarly to the total plate and coliform count, continually increased during the germination period.
Yeast and mould counts of one-day sprouts were 5.7±0.3, second-day sprouts were 6.5±0.2 and third-day sprouts were 6.6±0.7 $\log_{10}$ cfu/g.

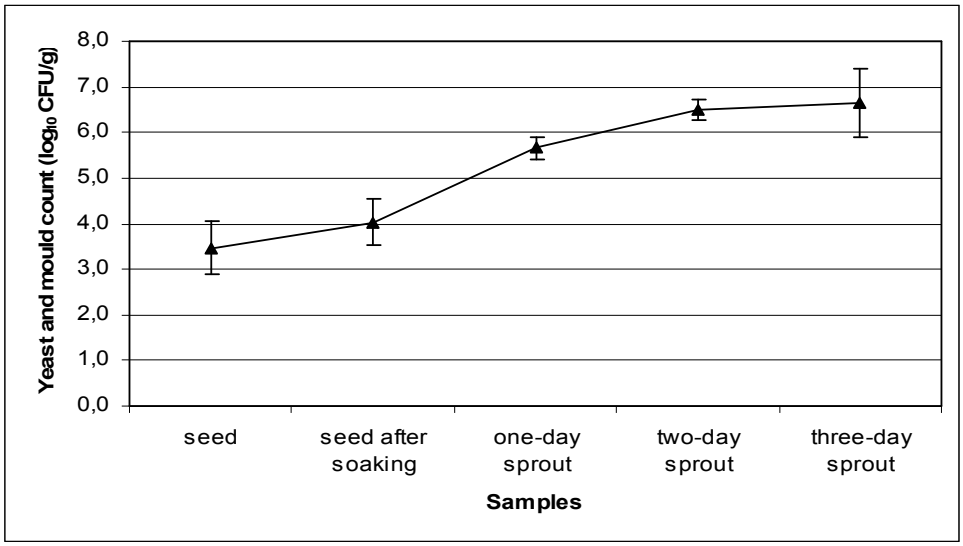

Fig. 3 Changes of yeast and mould count during germination period

CONCLUSIONS

This survey provides information on the microbiological status of Hungarian organic wheat grains and sprouted seeds. During the analysis of the microbiological quality of organic wheat grain samples before soaking we found that the mean total aerobic plate counts were 4.9 $\log_{10}$ cfu/g, coliform count 3.9 $\log_{10}$ cfu/g, yeast and mould count 3.5 $\log_{10}$ cfu/g. After soaking, total aerobic plate count, coliform count and yeast and mould count of grains increased to 6.2 $\log_{10}$ cfu/g, 6.0 $\log_{10}$ cfu/g, and to 4.0 $\log_{10}$ cfu/g, respectively. In the first three day of sprouting, microbial populations on grains increased approximately 2-3 logs. During sprouting process, the total aerobic plate count of ready-to-eat (three-day) sprouts increased to 7.9 $\log_{10}$ cfu/g, coliform count to 7.4 $\log_{10}$ cfu/g, yeast and mould count to 6.6 $\log_{10}$ cfu/g.

These high microbial levels *per se* reduce the shelf-life and safety of sprouts. Initial microbial load should be controlled, for minimizing the microbial contamination in the sprouts prior to consumption. On the basis of our results, we recommend the decontamination of seeds before sprouting.

Our data indicate the requirement for further research in the area of seed and sprout decontamination, pathogen occurrence and risk of infection.

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