# Application of β-D-Glucans Isolated from Mushrooms *Pleurotus ostreatus* (Pleuran) and *Lentinus edodes* (Lentinan) for Increasing the Bioactivity of Yoghurts

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**Abstract**

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The objective of the model experiment was to examine the microbiological (yeasts, moulds, coliform bacteria) and sensory (appearance, colour, consistency, taste) qualities as well as the lifetime of white and fruit yoghurts enriched with different additions of two types of hydrogels of β-D-glucan, namely pleuran (from *Pleurotus ostreatus*) and lentinan (from *Lentinus edodes*). The yoghurts were stored for 30 days under the refrigerator conditions (5°C ± 2°C), the sampling being done on the 1st, 15th and 30th day of the storage. The results obtained by the analyses of yoghurts with the addition of pleuran and lentinan showed that the fermentation ability of white and fruit yoghurts was not inhibited by the addition of hydrogels before this process; the acid equivalent (°SH) and pH of samples showed values typical for this kind of product during a month-lasting storage; the groups of microorganisms followed (coliform bacteria, yeasts and moulds) did not appear during the whole storage period (< 1 CFU/g); the application of both hydrogels added to yoghurts had no negative influence on the sensory acceptability of the products; all samples maintained a very good quality during the whole storage period and did not differ significantly from one another in the individual parameters evaluated.

**Keywords**: β-D-glucan; yoghurt; microbiology; sensory evaluation; pH; °SH; storage

β-D-glucans are indigestible polysaccharides occurring naturally in various organic sources such as corn grains, yeasts, bacteria, algae, and mushrooms. They are important components of the cereal fibrous material containing unbranched polysaccharides consisting of β-D-glucopyranose units bound through (1→4) and (1→3) glycosidic bonds. Out of all corn grains, barley and oat contain the highest amounts of β-glucans: the former 3–11% and the latter 3–7% plus 1% of solids. In the case of wheat, they are usually concentrated in the internal aleurone and subaleurone cell walls of the endosperm (Charalampopoulos *et al*. 2002). β-D-Glucan from yeasts is sold as an alimentary supplement (Zymosan). It is a polyglucose polysaccharide obtained from the cell walls of *Saccharomyces cerevisiae* or from the baker's yeast.

Microbial glucans have a similar structure like manans, except that their basic unit is glucose. They can be found generally inside or on the surface of microbial cells or be excreted by microbial cells into the neighbouring environment extracelularly. Surface glucans are carriers of the immunochemical specificity. Some of them, such as, for example, (1→3)-β-D-glucans, can stimulate the immune mechanisms of the host and have also antitumor and antimicrobial effects (Škárka & Ferenčík 1992).

The most frequently studied β-glucans gained from mushrooms include lentinan from *Lentinus edodes,* grifolan (called also GRN and grifolan LE) from *Grifola frondosa,* schizophyllan (called also SPG, sonifilan, sizofiran and sizofilan) from *Schizophyllum commune,* SSG from *Sclerotinia sclerotiorum,* PSK (called also crestin) and PSP (polysaccharide peptide) from *Coriolus versicolor* (Chang 1996), and β-glucan isolated from *Pleurotus ostreatus* called pleuran. The latter has a branched structure consisting of the backbone formed by β-D-glucopyranose residues bound to (1→3) glycosidic bonds, each fourth being substituted on 0–6 by one D-glucopyranose group. This alkali-insoluble polysaccharide contains a slight amount (7%) of internal residues bound to (1→6) and (1→4) glycosidic bonds (Karácsonyi & Kuniak 1994; Manzi & Pizzoferrato 2000).

According to the experimental and some clinical studies, glucans are characterised by antibacterial, antiviral and immunomodulating properties utilisable for prevention and therapy in human medicine (stimulation of macrophages through the β-glucan receptor on their surface) (Kubala *et al*. 2003; Tsukada *et al*. 2003). Further biological effects of β-glucans, which may be perspectively utilised in the clinical practice, can reside in the stimulative action on the haematopoiesis of the bone marrow, and also in the radioactive and antimutagenic effects (scavenger of free radicals) (Hirasawa *et al*. 1999).

The antitumoral activity of β-D-glucans from different mushrooms (lentinan, schizophyllan, scleroglucan) shows at doses of 1.0–0.2 kg (Wasser 2002). Of great importance are also: antidiabetic (Cavallero *et al*. 2002) and antiallergic actions, the reduction of the concentration of LDL cholesterol (Meier *et al*. 1995), and the total inhibition of the tissue ageing process as a result of the antioxidant effect (Zadák 2003).

Recently, the above-mentioned medical effects have stimulated the growing world-wide industrial application of β-glucans, gained from various sources, for the production of the so-called functional foods, for example, as supplements to soluble fibrous materials in the form of hydrocolloid concentrates, in the production of maize tortiles, puddings, granulated bars (Meier *et al*. 1995), low-fat cheeses of the Cheddar type (Konuklar *et al*. 2004) or in the production of brine-salting cheese products (Volikalis *et al*. 2004), yoghurts with the addition of müsli (Primaliv 2002), etc. For this application, an ideal medium from the aspect of consistency is mainly yoghurts in the form of hydrogels. On the basis of the afore-said information excerpted by an extensive literary search in the field of medicine and food-stuffs research, a model experiment was undertaken in our department. The experiment was accomplished within a research project in which our attention was aimed particularly at the preparation of yoghurts with the addition of two types of hydrogels of β-glucans gained from the fresh mushroom *Pleurotus ostreatus* (pleuran) and from the dried mushroom *Lentinus edodes* (lentinan), after which the individual applications were compared. Our aim was to define the influence of different additions before the fermentation process (2.5; 5.0; 10.0 mg/150 ml); to observe the fermentation ability of yoghurts prepared in this way, the microbiological stability, and the sensory acceptability during 30 days of the storage in refrigerator (5°C ± 2°C). The samples of white and fruit yoghurts were taken for analysis after the preparation (1$^{st}$day), and after 15 and 30 days of storage, and the yoghurts were also pursued under the refrigerator conditions from the aspect of their durability. Both pleuran and lentinan belong to the most explored β-D-glucans coming from mushrooms. Considering their well-known properties, it can be expected that regular consumption of yoghurts prepared in the above-mentioned way will have the assumed positive influence on the human organism.

## MATERIALS AND METHODS

***Raw materials, formula of yoghurt***. Whole milk Babička, dried milk – semi-fat dried milk Laktino, frozen yoghurt culture – YO-MIX™ Yoghurt Cultures, peach and strawberry jams ANDROS, pasteurised, free of preservation substances, hydrogels of β-glucan from the fresh mushroom *Pleutorus ostreatus* and the dried mushroom *Lentinus edodes* – concentration: 10 mg of β-glucan/ml. Yoghurts were prepared under the pilot plant conditions according to the modified procedure of Rašić and Kurmann (1978). The technological scheme of the samples preparation is shown in Figure 1.

***Methods***. Aerobic mesophilic bacteria were determined by the plate count method, on the tryptone-glucose-yeast extract agar (Šarišské Michaľany, Slovak Republic) (STN ISO 4833 – Anonymous 1997c).

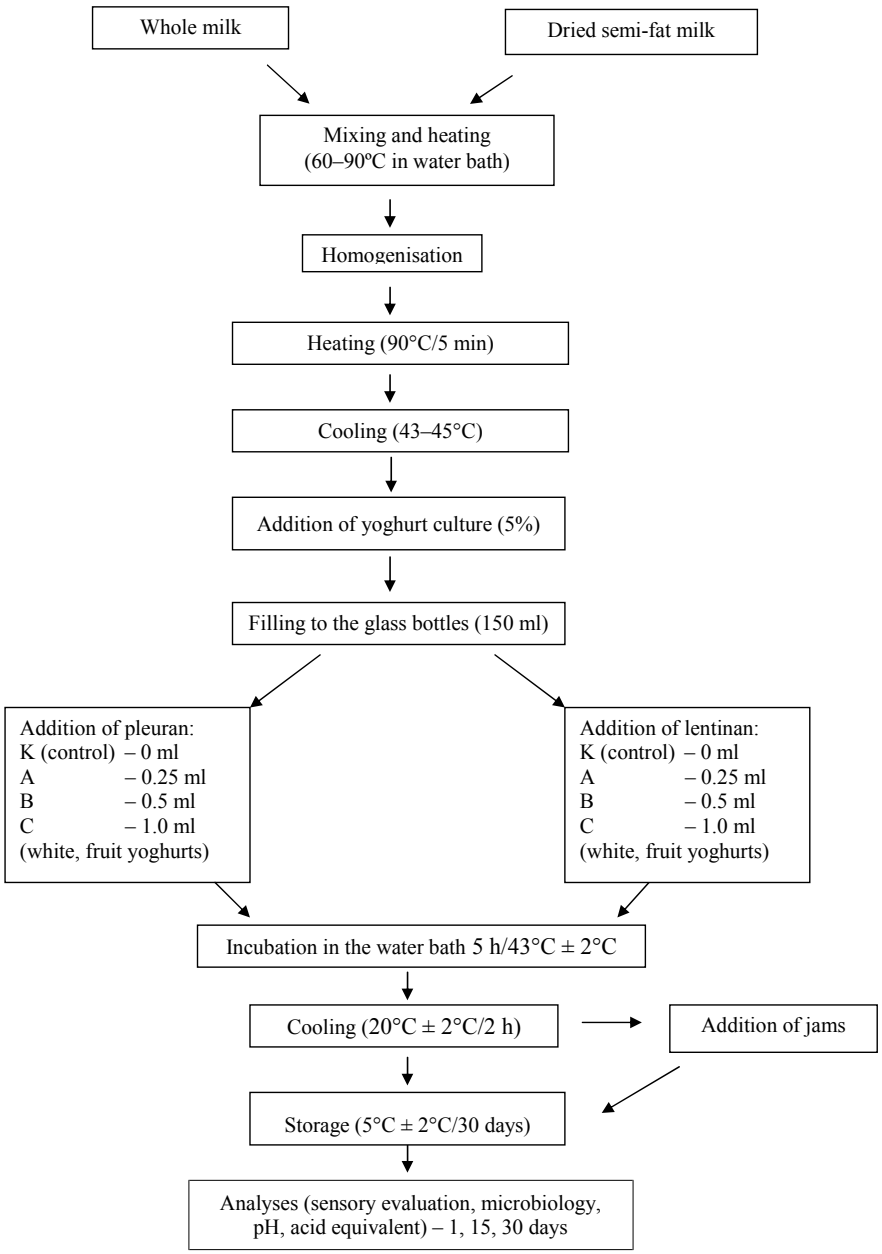


Figure 1. Technological scheme of the samples preparation

1. Yeasts and moulds were determined by the plate count method on the chloramphenicol-glucose-yeast extract agar (Šarišské Michaľany, Slovak Republic) (STN ISO 7954 – Anonymous 1997a).

2. Coliform bacteria were determined by the plate count method, on the VRB agar (Šarišské Michaľany, Slovak Republic) (STN ISO 4832 – Anonymous 1997b).

3. Sensory evaluation of the final products was accomplished by means of the five point hedonic scale determination (by six panel assessors) in such a way that the highest degree of the evaluation corresponded to the fulfillment of all claims for organoleptic properties, and the lowest degree of the evaluation marked 1 implied major qualitative deficiencies; the total tastiness (%) of yoghurts was evaluated graphically by means of the 100 mm unstructured line segment (Pokorný 1997; Ingr *et al*. 1997).

4. pH values were measured with a pH meter PORTAMESS® 911 (KNICK, Germany) containing a measuring element Sensoface®. Before measure-

ments, the instrument was calibrated with buffer at pH = 7.00 and 4.00, respectively (documentation of KNICK, GmbH Co., Germany).

5. Determination of the acid equivalent (°SH) (Palo 1987) was performed in ml of the volumetric solution of NaOH (0.25 mol/l) necessary for the titration of 100 ml sample using phenolphtalein indicator.

***Statistical evaluation***. means ($\bar{x}$), standard deviations (SD) (Eckschlager *et al*. 1980).

## RESULTS AND DISCUSSION

### Microbiological evaluation

The microbiological examination of the total number of microorganisms, coliform bacteria, yeasts and moulds, also involving the hydrogels of β-D-glucan (pleuran, lentinan), was performed before each application ($n$ = 2). Hydrogels were stored

Table 1. Scale of the sensory scoring evaluation of white and fruit yoghurts

| Attribute | Characteristics of white yoghurts | Characteristics of fruit yoghurts |
|---|---|---|
| **Appearance and colour** | | |
| 5 | cream-white, typical of the white yoghurt | fresh, typical of the fruit yoghurt |
| 4 | creamy | fresher, less typical of the fruit yoghurt |
| 3 | creamy with a light-yellowish tint | less agreeable, unattractive |
| 2 | light yellow | disagreeable, non-typical of the fruit yoghurt |
| 1 | yellow | strange, undesirable |
| **Odour** | | |
| 5 | clear, expressive, after the yoghurt culture | clear, expressive, after the yoghurt culture, fruit-like |
| 4 | clear, soft, after the yoghurt culture | clear, soft, after the yoghurt culture, fruit-like |
| 3 | after the yoghurt culture with a soft strange odour | after the yoghurt culture with a soft strange odour |
| 2 | medium-expressive, strange | medium-expressive, strange |
| 1 | very expressive, strange | very expressive, strange |
| **Consistency** | | |
| 5 | soft, creamy, characteristic of the white yoghurt | soft, creamy, characteristic of the fruit yoghurt |
| 4 | adequately thick or smooth | adequately thick or smooth |
| 3 | thinner, flocculent | thinner, flocculent |
| 2 | thin, liquid, with syneresis | thin, liquid, with syneresis |
| 1 | undesirable, thin, watery | undesirable, thin, watery |
| **Taste** | | |
| 5 | typical yoghurt-like, clear, from slightly to moderately sour | typical yoghurt-like, clear, from slightly to moderately sour, typical of the fruit yoghurt |
| 4 | clear, yoghurt-like, with a more expressive sour flavour, with a slight starch flavour | clear, yoghurt-like, taste after the presence of fruit, with a more pronounced sour flavour, with a slight starch flavour |
| 3 | yoghurt-like, expressively sour, with a slight starch flavour | yoghurt-like, taste after the presence of fruit, expressively sour, with a medium starch flavour |
| 2 | strange, without the yoghurt taste, with a strong starch flavour | strange, without the yoghurt taste, with a strong starch flavour |
| 1 | untypical, disagreeable | untypical, disagreeable |
| **Total tastiness** | | |
| unacceptable | very tasty, excellent | |

at laboratory temperature (20 ± 2°C). The results of the microbiological evaluation were negative during each analysis (< 1 CFU/ml), which proved the microbiological faultlessness of the gels.

The microbiological examination of white and fruit yoghurts with the addition of pleuran and lentinan proceeded during 30-day refrigerator storage (on the 1st, 15th and 30th day). From each type of the sample, 2 products were analysed in 2 parallel determinations ($n$ = 4).

The results obtained by the microbiological determinations showed that the investigated groups of microorganisms were not found in any of the analysed yoghurt samples during the whole duration of the experiment and therefore no tabular and graphically demonstrated results are presented in this contribution.

### Yeasts and moulds

The presence of yeasts and moulds was not confirmed in any sample analysed (< 1 CFU/g) during the 30-day refrigerator storage.

The Food Codex (Sour-milk products: yoghurt, yoghurt drinks, yoghurt desserts, sour buttermilk, drinks based on buttermilk, kefir, sour cream, etc.), supplement No. 3 to the fourth Chapter of the second Section for yoghurts, indicates that the maximum acceptable number of yeasts is $10^2$ CFU/g in 5 tested samples, two of them being allowed to contain maximum number of $5 \times 10^2$ CFU/g; the highest permitted number of moulds, except for *Geotrichum candidum*, is 50/g in 5 samples, two of them being allowed to contain the highest number of $2 \times 10^2$ CFU/g. Consequently, it is possible to conclude that during the whole storage time all the samples analysed remained in the limits given for the permitted number of yeasts and moulds in accordance with the Food Codex of the Slovak Republic (Anonymous 1998).

### Coliform bacteria

Coliform bacteria were not found in any of the sample analysed during the 30-day refrigerator storage (< 1 CFU/g).

The Food Codex indicates that the maximum admissible number of coliform bacteria is $10^2$ CFU/g in 5 samples, two of them being allowed to contain the highest number of $10^3$ CFU/g.

Therefore it is possible to claim that during the whole storage period all samples analysed remained within the limits for the admissible number of coliform bacteria as defined by the Food Codex of the Slovak Republic (Anonymous 1998).

### Sensory evaluation

Changes in the organoleptic properties (scoring evaluation) were evaluated at fifteen-day intervals during 30-day storage. The following sensory parameters were examined: appearance and colour, odour, consistency, and the taste of the products (Table 1). The average results of the sensory scoring evaluation of white and fruit yoghurts with and without (control) the addition of pleuran and lentinan as well as those obtained by the statistical evaluation are demonstrated later in the paragraph.

### I. Yoghurts with different additions of pleuran

(a) ***White yoghurt***. During the scoring evaluation of the appearance and colour, odour, consistency, and taste of the samples containing various additions of pleuran no remarkable differences were observed in the majority of samples on individual storage days. During the storage, the most remarkable change was registered in the case of the sample with the addition of 5.0 mg pleuran/150 ml yoghurt when evaluating the qualitative property – "consistency" (a decrease of points by 1.33 on the 30th day of storage). The overall scoring evaluation of the samples with different additions of pleuran showed that they differed from one another only a little on individual storage days (Table 2).

The total tastiness of white yoghurts with different additions of pleuran displayed a slight decline in all samples stored for the time period of 30 days. A considerable decrease representing as much as 11% was recorded in the sample with the addition of 2.5 mg pleuran/150 ml yoghurt (a decrease from 87.5% to 76.5%); on the other hand, the best evaluation on the 15th and 30th storage days obtained the sample with the addition of 10.0 mg pleuran/150 ml yoghurt.

(b) ***Peach yoghurt***. As for the scoring evaluation of the appearance and colour, odour, consistency, and taste of the samples with the various additions of pleuran, no distinctive differences were monitored in the majority of samples on the individual storage days. On the contrary, the evaluation demonstrated maximum decrease of values in the odour of all samples, and on the 30th day of storage somewhat

Table 2. Sensory scoring evaluation ($\bar{x}$ ± SD, $n$ = 6) of the white and fruit yoghurts with different amounts of pleuran

| Sensory parameter/samples | Storage (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 15 | | | | 30 | | | |
| | K | A | B | C | K | A | B | C | K | A | B | C |
| **White yoghurts** | | | | | | | | | | | | |
| Appearance and colour | 4.33 ± 0.821 | 4.50 ± 0.55 | 4.33 ± 0.82 | 4.17 ± 0.75 | 4.50 ± 0.84 | 4.67 ± 0.52 | 4.67 ± 0.84 | 4.33 ± 0.82 | 4.83 ± 0.41 | 4.33 ± 0.82 | 4.67 ± 0.52 | 4.83 ± 0.41 |
| Odour | 4.17 ± 0.75 | 4.17 ± 0.75 | 4.33 ± 0.52 | 4.17 ± 0.41 | 4.17 ± 0.75 | 4.00 ± 0.63 | 4.33 ± 0.52 | 4.00 ± 0.00 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.33 ± 0.82 | 4.50 ± 0.55 |
| Consistency | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.83 ± 4.41 | 4.50 ± 0.55 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.33 ± 0.52 | 4.67 ± 0.82 | 4.33 ± 0.82 | 3.50 ± 1.05 | 4.50 ± 0.55 |
| Taste | 4.33 ± 0.82 | 4.33 ± 0.82 | 4.33 ± 0.82 | 4.67 ± 0.52 | 4.50 ± 0.84 | 4.33 ± 0.82 | 4.17 ± 0.75 | 4.50 ± 0.55 | 4.33 ± 0.52 | 4.17 ± 0.41 | 4.00 ± 0.63 | 4.17 ± 0.41 |
| Σ | 17.50 | 17.67 | 17.82 | 17.51 | 17.84 | 17.50 | 18.00 | 17.16 | 18.16 | 17.16 | 16.50 | 18.00 |
| Total tastiness (%) | 82.3 ± 12.4 | 87.5 ± 12.4 | 80.2 ± 16.5 | 84.8 ± 10.2 | 82.3 ± 16.6 | 81.0 ± 17.3 | 77.2 ± 16.4 | 85.2 ± 17.2 | 81.5 ± 25.5 | 76.5 ± 21.7 | 70.8 ± 27.8 | 81.8 ± 15.9 |
| **Peach yoghurts** | | | | | | | | | | | | |
| Appearance and colour | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 4.67 ± 0.52 | 5.00 ± 0.00 | 5.00 ± 0.00 |
| Odour | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.50 ± 0.55 | 3.67 ± 0.52 | 4.17 ± 0.41 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.17 ± 0.41 |
| Consistency | 4.83 ± 0.41 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.33 ± 0.82 | 4.50 ± 0.55 | 4.17 ± 0.75 | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.00 ± 0.55 | 4.67 ± 0.52 | 4.83 ± 0.41 |
| Taste | 4.83 ± 0.41 | 4.50 ± 0.84 | 4.50 ± 0.84 | 4.83 ± 0.41 | 4.50 ± 0.55 | 4.17 ± 0.41 | 4.67 ± 0.52 | 3.50 ± 0.55 | 4.50 ± 0.55 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.50 ± 0.55 |
| Σ | 19.32 | 18.84 | 18.99 | 18.67 | 17.33 | 17.18 | 18.00 | 17.16 | 18.66 | 17.67 | 18.50 | 18.50 |
| Total tastiness (%) | 88.7 ± 7.9 | 89.5 ± 6.7 | 90.0 ± 8.4 | 93.3 ± 8.1 | 74.8 ± 23.3 | 79.5 ± 18.9 | 76.7 ± 19.5 | 68.2 ± 27.2 | 84.5 ± 11.5 | 87.2 ± 12.3 | 85.2 ± 8.6 | 89.5 ± 8.8 |
| **Strawberry yoghurts** | | | | | | | | | | | | |
| Appearance and colour | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.67 ± 0.52 | 4.67 ± 0.52 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.50 ± 0.84 | 4.50 ± 0.84 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.83 ± 0.41 | 5.00 ± 0.00 |
| Odour | 4.33 ± 0.52 | 4.33 ± 0.82 | 4.50 ± 0.55 | 4.33 ± 0.52 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.67 ± 0.52 | 4.33 ± 0.52 | 5.00 ± 0.00 |
| Consistency | 4.83 ± 4.41 | 4.83 ± 0.41 | 4.50 ± 0.55 | 4.33 ± 0.82 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.50 ± 0.55 | 4.83 ± 0.41 |
| Taste | 4.67 ± 0.52 | 4.83 ± 0.41 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.67 ± 0.52 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.67 ± 0.52 |
| Σ | 18.50 | 18.49 | 18.67 | 18.33 | 19.67 | 19.50 | 18.84 | 19.17 | 19.00 | 18.84 | 18.16 | 19.50 |
| Total tastiness (%) | 89.2 ± 7.7 | 91.8 ± 6.8 | 89.8 ± 5.6 | 89.0 ± 9.0 | 94.0 ± 5.6 | 94,4 ± 5.6 | 94.5 ± 4.2 | 95.5 ± 4.5 | 91.2 ± 7.9 | 86.2 ± 5.5 | 89.2 ± 9.1 | 95.2 ± 3.8 |

K – control (without pleuran); A – pleuran 2.5 mg/150 ml of yoghurt; B – pleuran 5.0 mg/150 ml of yoghurt; C – pleuran 10.0 mg/150 ml of yoghurt

better values in the appearance and colour of yoghurts as it is presented in the table. On the basis of the total scoring evaluation, the maximum number of points was determined for the control sample (19.32 points) on the first day, for a sample with the addition of 5.0 mg pleuran/150 ml yoghurt (18.00 points) on the 15th day, and again for the control sample (18.66 points) on the 30th day of the storage. During the storage, all samples exhibited a slight decrease in the total scoring evaluation.

On the first day of storage, the values of tastiness were very similar in all samples and increased with the growing addition of pleuran. On the fifteenth day of storage, the sample with the highest addition of pleuran was evaluated as the worst; on the 30th day of the storage, the differences in values fluctuated but, despite this fact, they were higher in all samples in comparison with the 15th day of storage.

(c) ***Strawberry yoghurt***. During the scoring evaluation of the appearance and colour, odour, consistency, and taste of the samples with different additions of pleuran, no distinctive differences were registered on individual storage days. The total scoring evaluation of samples with different additions of pleuran achieved maximum limit after 15 days of storage where upon it gradually decreased till the 30th day of the storage.

The total tastiness of the samples with different additions of pleuran displayed only minimum differences on individual storage days which means that no distinctive changes occurred and the yoghurts maintained their high standard during the whole storage period.

As can be seen in Table 2 comparing the sensory indicators of the quality of peach and strawberry yoghurts, the assessors considered the strawberry yoghurts tasteful and acceptable, which they expressed in most cases by five-point evaluation.

**II. Yoghurts with the different additions of lentinan**

(a) ***White yoghurt***. The evaluation of the colour, odour, consistency, and taste of the samples with different additions of lentinan confirmed only small differences between the majority of samples on individual storage days. Table 3 shows that the samples were best evaluated in most of their parameters on the 30th day of storage; as for the quality attribute – consistency, all samples were given even the best, 5-point evaluation. The total evaluation reveals that the lowest values were assigned to samples on the 15th day and the highest values, paradoxically, on the 30th day of the storage.

At the end of the storage period, the total tastiness was higher than that on the first day of storage. The smallest change was observed in the sample with the addition of 5.0 mg lentinan/150 ml yoghurt (increase from 84.7% to 85.3%), and the most remarkable improvement in the total tastiness was noticed in the sample with the addition of 10.0 mg lentinan/150 ml yoghurt (increase from 78.3% to 91.7%). The evaluation of the same kind of samples containing the addition of lentinan showed almost no difference as far as the total tastiness is concerned.

(b) ***Peach yoghurt***. The evaluation of the appearance and colour, consistency, and taste of the samples with different additions of lentinan demonstrated only minimum differences between individual storage days. Significant differences were not detected even in the total scoring evaluation which, however, indicated a moderate decrease in all samples during 30 days of storage.

The total tastiness of all samples deteriorated markedly during the storage. The results summarised in Table 3 reveal that yoghurts, after their preparation, were considered most tasty and represented about 97%, and that after 15 days yoghurts were still tasteful and represented ca 95% (more than the control 90.2%). The maximum drop (by 17%) was monitored after 30 days in the sample with the addition of 5.0 mg lentinan/150 ml yoghurt. As concerns the same kind of samples with different additions of lentinan, no distinct differences were observed within individual storage days.

(b) ***Strawberry yoghurt***. The evaluation of the appearance and colour, odour, consistency, and taste of samples with different additions of lentinan showed no observable differences on individual storage days. It also demonstrated that, in the case of almost all samples, the values on the 30th day of storage were higher than on the first day (control sample and the sample with the addition of 5.0 mg lentinan/150 ml yoghurt achieved the 5-point evaluation in all parameters except the taste on the 30th day of storage), which is also in agreement with the higher total scoring evaluation of the samples on the 30th day of the storage.

The greatest differences in the total tastiness of the samples with different additions of lentinan

Table 3. Sensory scoring evaluation ($\bar{x}$ ± SD, $n$ = 6) of the white and fruit yoghurts with different amounts of lentinan

| Sensory parameter/samples | Storage (days) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | | 15 | | | | 30 | | | |
| | K | A | B | C | K | A | B | C | K | A | B | C |
| **White yoghurts** | | | | | | | | | | | | |
| Appearance and colour | 4.50 ± 0.841 | 4.67 ± 0.82 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.50 ± 0.84 | 4.00 ± 0.89 | 4.17 ± 0.75 | 4.00 ± 0.89 | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.83 ± 0.41 |
| Odour | 4.50 ± 0.55 | 4.67 ± 0.52 | 4.33 ± 0.52 | 4.50 ± 0.55 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.50 ± 0.55 | 4.50 ± 0.55 | 4.67 ± 0.52 |
| Consistency | 4.50 ± 0.55 | 4.50 ± 0.84 | 4.33 ± 0.82 | 4.50 ± 0.84 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.50 ± 0.55 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 |
| Taste | 4.50 ± 0.55 | 4.00 ± 0.63 | 4.83 ± 0.41 | 4.33 ± 0.82 | 3.83 ± 0.75 | 3.83 ± 0.75 | 4.00 ± 0.63 | 4.17 ± 0.50 | 4.83 ± 0.41 | 4.67 ± 0.52 | 4.17 ± 0.75 | 5.00 ± 0.00 |
| Σ | 18.00 | 17.84 | 18.16 | 18.00 | 17.33 | 16.83 | 17.33 | 17.17 | 19.49 | 18.84 | 18.34 | 19.50 |
| Total tastiness (%) | 78.7 ± 21.5 | 78.3 ± 12.7 | 84.7 ± 9.8 | 78.3 ± 19.2 | 80 ± 16.8 | 77.8 ± 18.8 | 82 ± 15.2 | 85.5 ± 14.1 | 88.7 ± 10.5 | 89.2 ± 7.28 | 85.3 ± 12.1 | 91.7 ± 5.9 |
| **Peach yoghurts** | | | | | | | | | | | | |
| Appearance and colour | 4.67 ± 0,52 | 4.50 ± 0.84 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.67 ± 0.52 | 4.83 ± 0.41 |
| Odour | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.83 ± 4.81 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.50 ± 0.55 | 4.50 ± 0.55 | 4.50 ± 0.55 | 4.33 ± 0.52 | 4.33 ± 0.52 | 4.17 ± 0.41 |
| Consistency | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 4.67 ± 0.52 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 4.67 ± 0.52 | 4.50 ± 0.55 |
| Taste | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.50 ± 0.55 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.50 ± 0.84 | 4.50 ± 0.55 | 4.50 ± 0.55 | 4.50 ± 0.55 |
| Σ | 19.00 | 18.99 | 19.66 | 19.00 | 18.66 | 19.49 | 19.16 | 19.16 | 18.83 | 18.83 | 18.17 | 18.00 |
| Total tastiness (%) | 96.3 ± 4.1 | 98.8 ± 1.3 | 97.3 ± 3.0 | 95.8 ± 3.7 | 90.2 ± 4.5 | 94.3 ± 4.0 | 95.5 ± 3.6 | 95.0 ± 2.0 | 82.8 ± 12.7 | 83.5 ± 9.7 | 80.0 ± 9.9 | 86.5 ± 7.2 |
| **Strawberry yoghurts** | | | | | | | | | | | | |
| Appearance and colour | 4.33 ± 0.52 | 4.50 ± 0.55 | 4.33 ± 0.52 | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0,41 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 |
| Odour | 4.33 ± 0.82 | 4.33 ± 0.82 | 4.67 ± 0.52 | 4.50 ± 0.55 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.67 ± 0.52 | 5.00 ± 0.00 | 4.67 ± 0.52 | 5.00 ± 0.00 | 4.50 ± 0.55 |
| Consistency | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.50 ± 0.55 | 4.83 ± 0.41 | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 5.00 ± 0.00 | 5.00 ± 0.00 | 4.83 ± 0.41 | 5.00 ± 0.00 | 5.00 ± 0.00 |
| Taste | 4.67 ± 0.52 | 4.67 ± 0.52 | 4.50 ± 0.55 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.83 ± 0.41 | 5.00 ± 0.00 | 4.83 ± 0.41 | 4.50 ± 0.55 | 4.33 ± 0.52 | 4.67 ± 0.52 | 4.67 ± 0.52 |
| Σ | 17.83 | 18.33 | 18.00 | 18.83 | 19.16 | 19.16 | 19.50 | 19.50 | 19.50 | 18.83 | 19.67 | 19.17 |
| Total tastiness (%) | 86.0 ± 12.3 | 74.7 ± 19.6 | 74.4 ± 17.4 | 88.5 ± 8.2 | 96.7 ± 2.34 | 96.3 ± 1.9 | 96.2 ± 1.5 | 96.2 ± 2.6 | 83.5 ± 12.0 | 83.7 ± 11.6 | 85.3 ± 11.2 | 85.3 ± 13.1 |

K – control (without lentinan); A – lentinan 2.5 mg/150 ml of yoghurt; B – lentinan 5.0 mg/150 ml of yoghurt; C – lentinan 10.0 mg/150 ml of yoghurt

were recorded on the first day of storage. As can be seen from Table 3, the highest evaluation was assigned to the sample with 10.0 mg/150 ml yoghurt (88.5%), and the lowest to the sample with the addition of 5.0 mg lentinan/150 ml yoghurt (74.4%). The samples were best evaluated on the 15th day of storage when their total tastiness ranged from 96.2 to 96.7%.

Comparing the evaluation of the sensory indicators of quality for white and fruit yoghurts, it may be stated that the assessors regarded peach yoghurts generally better and taste-acceptable on the first day of storage, strawberry yoghurts – on the 15th day of storage, and white yoghurts on the 30th day of storage.

The results of the sensory evaluation lead to the statement that:

– additions of both hydrogels of β-glucan did not manifest themselves negatively either in the appearance and colour, odour, consistency, and taste, or in the total tastiness of yoghurts. On the contrary, the samples with the addition of hydrogels were evaluated in most cases to be better than control samples;
– no relation was confirmed between the amounts of hydrogels added and the sensory evaluation of yoghurts;
– a negative impact of the addition of hydrogels to yoghurts did not show even during the 30-day refrigerator storage in any of the investigated samples; all samples retained very good quality during the whole storage period and did not differ from one another significantly;
– in terms of the parameters pursued, the samples with the addition of β-glucans did not display any syneresis even after 30 days of storage in the refrigerator (hydration properties of pleuran and lentinan) and did not acquire any "starch flavour" from the polysaccharide added; it has been proved that yoghurts can be preserved for a longer period without using stabilisers if the correct production technology is used and suitable storage conditions are chosen.

### pH

The pH values of white and fruit yoghurts with different additions of pleuran and lentinan were determined on the 1st, 15th and 30th days of the refrigerator storage. The resulting values in white yoghurts were about 4.33 (on the 1st day) and those in fruit yoghurts were somewhat lower (4.30). During storage, most of the samples revealed a moderate decline (4.20), and after 30 days of storage another growth (4.30 and in the case of fruit yoghurts up to 4.25). Different additions of the pleuran and lentinan hydrogels did not have principally any influence on the measured pH values which varied in the range characteristic for this kind of products (Rašić & Kurmann 1978).

### °SH

In agreement with the course of pH values, the acid equivalent was measured as follows: on the 1st day after the preparation it was 46–48 °SH, after 15 days – about 52 °SH, and at the end of storage a moderate decrease was recorded from 49 °SH to 50 °SH. On applying different additions of pleuran and lentinan, no remarkable differences appeared and no inhibition in the course of the sour-milk fermentation was observed.

## CONCLUSIONS

On the basis of the experimental results, it is possible to conclude that both investigated hydrogels of β-D-glucan are equally suitable as natural alimentary supplements to yoghurts. Furthermore, after a successful *in vitro* and *in vivo* testing on a wide statistical set of the selected population groups, they can be recommended as innovated sour-milk products for the milk production. The regular daily consumption of the innovated milk products with the application of an average dose – i.e. 5 mg per 150 ml yoghurt package, which are efficient from the medical and acceptable from the economic aspects, would certainly contribute to the reduction of the occurrence of relapsing or chronic infectious as well as autoimmune and oncological, diseases, especially in more risky age groups (children, older generation).

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## Súhrn

Hozová B., Kuniak Ľ., Kelemenová B. (2004): **Aplikácia β-D-glukánov izolovaných z húb** *Pleorotus ostreatus* **(pleuran) a** *Lentinus edodes* **(lentinan) na zvýšenie bioaktivity jogurtov.** Czech J. Food Sci., **22:** 204–214.

V modelovom experimente sme sledovali mikrobiologickú (kvasinky, plesne, koliformné baktérie) a senzorickú (vzhľad, farba, konzistencia, chuť) akosť a trvanlivosť bielych a ovocných jogurtov obohatených rôznym prídavkom 2 typov hydrogélov β-D-glukánu, a to pleuranu (z *Pleurotus ostreatus*) a lentinanu (z *Lentinus edodes*). Jogurty boli skladované 30 dní v chladiarenských podmienkach (5 °C ± 2 °C), odber vzoriek sa vykonal 1., 15. a 30. deň. Výsledky analýz jogurtov s prídavkom pleuranu a lentinanu ukázali, že prekysávacia schopnosť bielych a ovoc-

ných jogurtov nebola inhibovaná prídavkom hydrogélov aplikovaných pred fermentáciou; titračná kyslosť a pH vzoriek vykazovali počas mesačného skladovania hodnoty typické pre tento druh tovaru; sledované skupiny mikroorganizmov (koliformné baktérie, kvasinky a plesne) sa nevyskytli počas celého skladovacieho obdobia (< 1 KTJ/g); aplikácia oboch hydrogélov do jogurtov nemala negatívny vplyv na senzorickú akceptovateľnosť produktov; všetky vzorky si zachovali veľmi dobrú akosť počas celého skladovacieho obdobia a nelíšili sa významne v jednotlivých posudzovaných parametroch.

**Kľúčové slová**: β-D-glukan; jogurt; mikrobiologická akosť; senzorické vyhodnotenie; pH; °SH; skladovanie

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