Separate and combined influence of *Origanum vulgare* and *Thymus serpyllum* phytoextracts at the rye grain bioactivation

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Abstract. The *Thymus* and *Origanum* extracts were obtained at different temperature, separate and combined biological effects of them were investigated. There were shown the influence of this substances at the rye grain bioactivation taking into account their antioxidant and membrane-protective properties. The antioxidant activity of *Thymus* extracts, but not *Origanum*, directly depended on the temperature of extraction (30 or 80°C). When "hot" *Thymus* extract (80°C) used the reduction degree of chemiluminescence was 3...6 times higher than in the control and 2...3 times higher than the oregano extracts used. The results are in good agreement with the data on the assessment of extracts membrane-protective activity. Under the influence of individual preparations of *Thymus*, as well as the combined preparation of *Thymus*+*Origanum* (1: 1), the osmotic resistance of the liposomal suspension increased. Under using combined phytoextracts of oregano and thyme (1: 2; 2: 1; 1: 1), the dynamics and energy of germination exceeded the control level significantly. The best result was observed at a 1:1 ratio, when the number of germinated seeds was more then in control by 4 times by the 12th hour of exposure, and the optimal length of germinated seeds was formed by the 10th hour. Thus, using of the combined preparation of thyme + oregano (1: 1) allows to halve the longest stage of plant raw materials preparation for obtaining a multi-grain flourless bakery product.

1. Introduction

Whole (not peeled) grain of various cereals is a source of essential amino acids, minerals, B-vitamins, dietary fiber. Such kind of bread is more useful and healthier than ones which made from refined flour [1]. New varieties appear on the bakery market, for example grain, whole grain, multi-grain products, although their nomenclature is still absent in the current regulatory and technical documentation. This distance is gradually decreasing. The project of standard "Bakery products with the addition of grain and its processing products" has been developed and is being tested now [2]. In this document, multi-grain bread is defined as a bakery product without a filling with a moisture content of more than 19%, containing three or more types of grain and its processed products in the recipe, except for wheat and rye flour. One of the new areas is the development of multi-grain products based on bioactivated rye grain [3]. Bioactivation means a process of grain germination when enzymatic depolymerization is under the influence of moisture saturation, heat and air oxygen. Bioactivated grain is an environmentally friendly product [4]. Usually germination time takes 20-36 hours. The first 20 hours are considered the optimal interval. During this time, enzymes have time to be activated, while organic substrates are not consumed yet, and the amount of pathogen microflora is minimal [5]. The indicator is the size of the seedling (1 mm). At the same time, 20 hours is quite long and unprofitable for the production process. Therefore, it is necessary to find a way to make this period shorter.

Using of bioactivated grain allows you to obtain a product without yeast and refined flour. Many new opportunities appear to vary the composition by adding other grain nutrients to the base (buckwheat, oats, etc.), as well as extruded mixtures and phytocomplexes based on garden crops and spicy wild plants. Fortification of food products with biologically active nutrients based on local plant materials is especially important for the inhabitants of Siberia. This allows solving the problems of balanced nutrition and health preservation without rise in price by saving money on transportation and storage of raw materials [6].
Analysis of the literature showed that among 85 patents for bakery, grain, whole grain and multi-grain products, only 15 patents were in the bioactivated grain area. Among them, in turn, only 4 described using of Siberian wild plants as oregano *Origanum vulgare* and *Thymus serpyllum* (Fig. 1).

Figure 1. Using phytoextracts to grain bioactivation as a new direction in the technology of bread products

These spicy wild plants contain optimal amounts of selenium, calcium, copper, manganese, iron and lipophilic vitamins [7, 8]. In most methods, phytoextracts are added to the dough to enrich the taste. However, their properties as potential bioregulators have not been studied enough. There is no information about the biological activity of thymus and origanum which are extracted under the different conditions. The available information is scattered and contradictory, since it was obtained under the conditions of various experimental models. It is necessary to have information about antioxidant properties of phyto-extracts in the composition of a multi-grain product. Antioxidant activity indicates the ability to inhibit the processes of auto-oxidation. Organic compounds take part in this processes during fermentation and subsequent mechanical grinding. In addition, the degree of influence of these extracts on the stability of cell membranes, which are treated at the different technological stages, has not been studied. Finally, there is no data on the ability of phytoextracts to accelerate the process of grain bioactivation.

The aim of this work was to obtain the extracts of thymus and origanum under different temperature and estimate their separate and combined influence at the free radicals production, biomembrane stability and the rye grain bioactivation rate.

### 2. Materials and methods

We used dry phytomass of thymus and origanum. Phytomass was collected in August and September 2020 near the city of Krasnoyarsk. Raw material (25 g) was mixed to 100 ml of hot (80°C) or warm (30°C) water and exposure under 1 hour on a water bath. To determine the antioxidant activity, we used chemiluminescent luminol-dependent analysis using the Fenton model [9].

Chemiluminescent (CL) analysis was performed using the automated software complex «Biochemiluminometer 3607». The device operates in the mode of light quanta counting. The number of light quanta correlate with the reactive oxygen forms (ROF) production rate; ROF were produced during the Fe^{2+} induced decomposition of 0,1 mM hydroperoxide. The Fenton reaction mixture contained 100 μl H_{2}O_{2} (0,01%), 100 μl probe (3%) in distilled water and 50 μl FeSO_{4} (5 mM). To enhance the signal, 150 μl luminol (10^{-4} M) was used. All reagents were taken from «Sigma», Novosibirsk. The method of analysis is described in detail [10]. The recording and storage of results, graphic and statistical processing of the kinetic curves (kinetograms) were performed using the BLM 07 software package (instrument operation control using a PC) and BLM 07PR software (processing of measurement results). The antioxidant activity of the compounds was evaluated by the change in the peak of the CL (I, impulse/s) and light sum (S, thousand imp.) regarding control. The measurement time in the chemical model was from 5 to 10 minutes. The kinetic parameters were automatically recorded and archived as a database. Each sample was analyzed three times at least.

Membrane-protective activity was investigated using a liposomal suspension. It was prepared by dispersing of the egg phospholipids in 0,85% NaCl solution [11]. The optical density of the liposome suspension was measured under osmotic stress using a photocolorimetric method. Osmotic
stress was formed by adding HCl (25 μl 0.4%). The stability of liposomes was assessed in the control (without extracts) and under the influence of an aqueous extracts (0.5 ml) of thymus or origanum. Phytoextracts influence at bioactivation of rye grain was studied by dynamics and energy of germination. For this, the time during seedlings got a length of 1 mm was measured, as well as the general length of the seedlings for a observation time was fixed [12]. Statitical processing was carried out using Student's criterion (the distribution of sample data was normal, the variances were comparable) at a confidence level of 0.95.

3. Results and discussion

3.1. Antioxidant activity of thymus and origanum extracts

Under the influence of both extracts, the production of free radicals were decreases significantly (Fig. 2, a). As the level of light sum (S) shows (Fig. 2, b), the antioxidant activity of oregano weakly depended on the temperature of the extract.

Contrary, the antioxidant activity of the “hot” thyme extract was twice as high as “warm” one, and 2 ... 3.6 times higher than oregano extracts. Thus, the antioxidant activity of the "hot" extracts of thyme and oregano was higher than that of the "warm" ones. The antioxidant activity of thymus extracts is at least 3 times higher than the antioxidant activity of origanum.

3.2. Membrane-protective properties of thymus and origanum extracts

The optical density of the suspension was different under the influence of two extracts (Fig.3).
Liposomal suspension was destroyed in control, but remained stable in presence of extracts (fig. 3). The positive effect of the thymus was higher. This fact correlate with data about antioxidant effect of the thymus extract. Thus, it was suggested that the positive effect of thyme on the bioactivation of rye would also be higher.

3.3. Separate and combined effects of extracts on the bioactivation of rye grain

Under the influence of extracts, the bioactivation process proceeded more efficiently than in pure water, where 1 mm-length seedlings were formed no earlier than after 20 h of exposure (Fig. 4).

When the grain was moistened using separate preparations of oregano and thyme, the germination time of seeds was reduced by 6 hours. Individual effects of separate samples were similar and exceeded control values by 30%. The maximum difference from the control (p <0.001) was recorded under the influence of the combination of origanum: thymus (1:1). In this case, the proportion of germinated seeds exceeded the control level by 4 times by the 12th hour of exposure (Fig. 4, a), and the optimal length of the germination (1 mm) was formed by the 10th hour, not by the 20th hour, i.e. ... 2 times faster (Fig. 4, b).

4. Conclusion

1. The temperature of extraction has a greater effect on the antioxidant properties of thymus than origanum. The antioxidant activity of thymus extract is at least 3 times higher than origanum extract.

2. Membrane-protective activity of individual extracts of thymus is higher than that of origanum, while the effect of the combined preparation thymus+origanum is comparable to the effect of an individual extract of thymus.

3. Using of a combined extract origanum+thymus (1:1) makes it twice reduce the longest preparation stage of plant raw materials to obtain a multi-grain flourless bakery product.

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