LIETUVOS ŽEMĖS ŪKIO UNIVERSITETAS

Skaidra Kordušienė

MAISTUI DAIGINTŲ SĖKLŲ DŽIOVINIMO IR ŠALDYMO BŪDAI BEI MIKROBIOLOGINĖS TARŠOS MAŽINIMAS

Daktaro disertacijos santrauka
Biomedicinos mokslai, Agronomija (06 B)

Akademija, 2010

Disertacija rengta 2005-2009 metais Lietuvos žemės ūkio universitete.
Mokslinė vadovė
prof. dr. Honorata Danilčenko (Lietuvos žemės ūkio universitetas, biomedicinos mokslai, agronomija, 06B).
Mokslinė konsultantė:
dr. Audronė Mankevičienė (Lietuvos agrarinių ir miškų mokslų centro filialas Žemdirbystės institutas, biomedicinos mokslai, zootechnika, 13B).

Disertacija ginama Lietuvos žemės ūkio universiteto Agronomijos mokslo krypties taryboje:

Pirmininkas:
prof. habil. dr. Rimantas Velička (Lietuvos žemės ūkio universitetas, biomedicinos mokslai, agronomija, 06B).
Nariai:
doc. dr. Aurimas Krasauskas (Lietuvos žemės ūkio universitetas, biomedicinos mokslai, agronomija, 06B).
prof. habil. dr. Algirdas Sliesaravičius (Lietuvos žemės ūkio universitetas, biomedicinos mokslai, agronomija, 06B).
prof. habil. dr. Vida Stravinskienė (Vytauto Didžiojo universitetas, biomedicinos mokslai, ekologija ir aplinkotyra, 03B).
dr. Nobertas Uselis (Lietuvos agrarinių ir miškų mokslų centro filialas Sodininkystės ir daržininkystės institutas, biomedicinos mokslai, agronomija, 06B).

Oficialieji oponentai
doc. dr. Natalija Burbulis (Lietuvos žemės ūkio universitetas, biomedicinos mokslai, agronomija, 06B).
prof. habil. dr. Romas Gružauskas (Lietuvos veterinarijos akademija, biomedicinos mokslai, zootechnika, 13B).

Disertacija bus ginama viešame Agronomijos mokslo krypties tarybos posėdyje 2010 m. birželio 22 d. 11 val. Lietuvos žemės ūkio universiteto centrinių rūmų 261 auditorijoje.

Adresas: Lietuvos žemės ūkio universitetas, Studentų g. 11, Akademija, LT-53361 Kauno raj., Lietuva

Disertacijos santrauka išsiuntinėta 2010 m. gegužės 21 dieną.
Disertaciją galima peržiūrėti Lietuvos žemės ūkio universiteto ir Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės instituto filialo bibliotekose.

INTRODUCTION

One of the major tasks of the latter period is to improve human nutrition, to make it healthy, full-value, rational and safe. With rising standard of living in various countries, consumers are increasingly focused on a healthy lifestyle and food safety. An interest in sprouted grain and seed intended for human consumption has been increasing during the last decade, since they supplement and diversify diets. Sprouted cereal, legume, oil and other seeds are used for human consumption. The seed of potato (*Solanum*) family plants is an exception because of the high glycoside levels.

To germinate, seeds require particular environmental conditions. However, they promote not only the process of germination but also the occurrence and activity of various micro-organisms. Each year, one third of industrialised countries' population suffer health disorders resulting from food-borne bacteria, viruses, or fungi and mycotoxins produced by them, which ultimately incur economic losses. As a result, safeguarding of food safety is deemed to be a globally relevant issue.

Various additives and preservatives are used seeking to protect food raw materials and products from microbial deterioration. However, the dominating use of synthetic food additives in food industry creates a rising consumer discontent. Consequently, it is essential to look for new safe food production and processing methods that would preserve nutritive and taste qualities and biological activity.

Although numerous research studies have analysed chemical composition and its variation in sprouted seed intended for human consumption, preprocessing of perishable products using natural substances isolated from plants, which could partly replace synthetic food additives, and their industrial application is still relevant.

Research hypothesis

The expiry date of fresh sprouted seed intended for human consumption is relatively short. We believe that the expiry period could be prolonged by choosing a more optimal processing method and by reducing microbiological seed contamination using inhibitory substances during the sprouting period. In this way it would be possible to preserve a high quality of the product and would enable its wide usage as food.

Research objective

To choose and assess methods designed for processing of sprouted seed intended for human consumption, to investigate the variation of chemical composition and biological activity and sensory acceptability of the product, and to evaluate the effects of various disinfectants on seed microbiological stability.

Research tasks

✓ To estimate and compare pre-processing (pre-treatment) methods of sprouted seed intended for human consumption and to estimate the variation of chemical composition.

✓ To carry out sensory evaluation of processed sprouted seed intended for human consumption.

✓ To assess the efficacy of hydrogen peroxide and grapefruit seed extract for the reduction of microbiological contamination of sprouted seed.

✓ To identify the effect of disinfectants on the ratio of amino acids.

Propositions to be defended

✓ Minimal processing of sprouted seeds prolongs their expiry period; however, their sensory appeal is not always satisfactory.

✓ Biocides applied during the seed sprouting period to reduce microbiological contamination influence the variation of the content of bioactive substances. Hydrogen peroxide and grapefruit seed extract are not always effective for the reduction of microbiological contamination of sprouted seed intended for human consumption.

Scientific novelty of the work

This is the first time the pre-processing methods of sprouted seed intended for human consumption have been investigated and the variation of chemical composition, and biologically –active compounds characterised by antioxidant properties has been evaluated. It has also been found that disinfectants in sprouted seed affect the total micro-organism count as well as the contents of bacteria and microfungi and mycotoxins produced by them.

Practical importance

By minimally processing sprouted seed intended for human consumption it is possible prolong its expiry period, widen the range of plant products in food industry and to get processors interested in the product. The use of inhibitory substances secures a more effective reduction of seed microbiological contamination.

Approbation of work results

Research results presented at the scientific conferences:

1. Kraujutienė I., **Kordušienė S.**, Savickienė J., Svirskis A. Alternative plant additives in bread. // International scientific – practical conference "Innovation development trends of food products", Jelgava. 2004.

2. Kordušienė S., Kraujutienė I. Pavojingų sveikatai teršalų tyrimas daigintose maistui sėklose. // KMU Respublikinė visuomenės sveikatos mokslų studentų konferencija. 2006.

3. Kraujutienė I, Jarienė E., Danilčenko H., Šlapakauskas V., Venskutonienė E., **Kordušienė S.** An influence of growing technology to

enzyme activity in germinated for food wheat grains. // 3rd international conference on quality and safety in food production chain. Wroclaw. 2007.

4. **Kordušienė S.,** Kraujutienė I. Zearalenono kiekis maistui daigintose sėklose ir jo detoksikavimo galimybės. // Tarptautinė mokslinė doktorantų konferencija „Jaunimas siekia pažangos“. LŽŪU. 2007.

5. **Kordušienė S**. Zearalenono ir ochratoksino kiekių studija daigintose maistui sėklose // 13-oji tarptautinė mokslinė – praktinė konferencija „Žmogaus ir gamtos sauga“, LŽŪU. 2007.

6. **Kordušienė S.,** Kraujutienė I. Pavojingiausių (T-2 ir zearalenono) mikotoksinų kiekis maistui daigintose sėklose ir jų detoksikavimo galimybės. // Tarptautinė mokslinė-praktinė konferencija “Žmogaus ir gamtos sauga”. LŽŪU. 2009.

7. **Kordušienė S.,** Kraujutienė I. Maistui skirtų daigintų sėklų sauga. // Tarptautinė mokslinė doktorantų konferencija „Jaunimas siekia pažangos“. LŽŪU. 2009.

Volume of the work

The doctoral dissertation is written in the Lithuanian language. It consists of the introduction, literature review, materials and methods, results and discussion, conclusions and list of scientific publications on the dissertation theme and references.

.

INVESTIGATION METHODS

The research object was the seed of broccoli (*Brassica olearacea* L. *convar. otrytis* L), amaranth (*Amaranthus cruentus*), lucerne (*Medicago sativa* L.), and radish (*Raphanus sativus* L.).

Research was done during the period 2005-2009. Seed sprouting and disinfection were carried out at LUA Department of Horticulture's SC "Kauno grūdai" KPC laboratory. Mycotoxin and dry matter contents were determined at SC "Kauno grūdai" laboratory. Microbiological analyses were done in the microbiological laboratory of SC "Kaišiadorių paukštynas". Colour coordinates, antioxidant activity (RSA), respiration intensity and dry matter losses, content of phenolic compounds were identified at the Lithuanian Institute of Horticulture's Laboratory of Biochemistry and Technology. Amino acids composition in sprouted seed was determined at Hohenheim University's Laboratory of Animal Nutrition Institute (Germany).

High-quality, untreated seeds of broccoli cv. 'Cesar', amaranth cv. 'Geltonukai', lucerne 'Europa', radish cv. 'Warta' were used for the study.

The seeds were germinated (replicated three times) in Polish 'Bio-Natura' sprouters (diameter – 20 cm, plate capacity – 1L,) (Fig. 1). Prior to sprouting, the sprouters were disinfected with 70% ethyl spirit solution. The seeds were sprouted for 120 hours in the dark at a temperature of 23 - 25°C in an aerated room. For sprouting, 250 g of seeds were taken and thoroughly sorted out, litter admixtures were removed, and the seeds were soaked for 12 hours in distilled water at a ratio 1:4.

Non-sprouted seeds were disinfected with 6% hydrogen peroxide solution and with 1% grapefruit seed solution. Exposure time was 15 minutes. The sprouting seeds were periodically, every 24 hours, irrigated with distilled water.

The viability (germination power) of the seeds tested was 95 – 99 %, except for amaranth seed, whose germination power was 50-55%.

Two experiments were conducted:

I – minimal processing of seed (freeze-drying, lyophilization) and evaluation of chemical composition variation, colour co-ordinates, and sensory assessment.

I. 1) Freezing tests were done in compliance with the recommended international code of practice for the processing and handling of quick frozen foods (CAC/RCP 8-1976) (Codex Alimentarius, FAO, 1994). The seeds were frozen in a deep freezer "Derby-Danfoss" at 40 ± 1°C, stored at 30 ± 1°C. The sprouted seeds frozen in plastic boxes were de-frosted by high frequency waves in a microwave oven AFMW-290. Capacity 100 – 1000 W, frequency – 2450 Mhz.

I. 2) The sprouted seeds were dried in:

a) vacuum dryer;

b) convection dryer;

c) by active ventilation.

I. 3) For seed lyophilization, we used an ilShin Lab Co. Ltd. (Korea) freeze-dryer (lyophilizer). The seeds were lyophilized for 48 hours at condenser's temperature –80°C, and pressure 5mTorr (millitorr).

Sprouted seeds were evaluated for sensory qualities in compliance with the descriptive qualitative analysis (profile) method according to LST ISO 11036:2003 and LST ISO 6564:2003. A panel of 7 judges took part in the study. A descriptor list was worked out for sprouted seed colour, odour, flavour, exterior appearance. A general acceptance of the sprouted seed was also estimated.

II – Reduction of microbiological contamination and mycotoxin content through seed disinfection with 6% hydrogen peroxide and 1% grapefruit seed extract and the effect of these biocides on the amino acids content.

While doing data analysis, a relative digital expression was attributed to the intensity of a sensory property sensed and perceived by the panel judges. This digital expression was further used for the statistical analysis of results.

Analytical methods. The seeds germinated for 120 hours were analysed for:

dry matter content – gravimetrically, by drying the seed at +105°C to the constant weight (Food analysis, 1986; LST EN ISO 665:2001; LST 1530:2004; LST ISO 712:2000);

soluble dry matter – by digital refractometer ATAGO;

respiration rate (intensity) - by measuring the variation of carbon dioxide content by a gas analyser Anagas 95 (Viškelis, 2002);

nitrates – potentiometrically with an ion-selective nitrate electrode (Ермаков, 1987);

ascorbic acid (vitamin C) – by titrating with 2,6-Dichloropheno-lindophenol Sodium Salt Solution, using chloroform (for intensively coloured extracts);

ascorbic index was determined by dividing the average vitamin C content by the average nitrate content;

inverted saccharides, sucrose – by Bertrand method;

photosynthetic pigments (a, b chlorophylls) content was determined in 100% acetone extract by a spectrophotometric Wettstein method (Beadle, 1987) using a spectrophotometer "Genesys 6" (ThermoSpectronic, JAV);

protein content was determined by the Kjeldahl method;

amino acids were separated by ion exchange chromatography and were determined photometrically by ninhydrin reaction at a wavelength of 570 nm (IEC Commission directive 98/64/EB, 1998);

total phenolic acids content was measured by a spectrophotometric Folin method (Ragee et al., 2006);

colour co-ordinates in the space of colours of identical contrast were measured by a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). Light reflection regime was used to determine L*, a* and b* parameters (correspondingly, lightness, indices of redness and yellowness according to CIELab scale) and colour purity (chroma) ($C = (a^{*2} + b^{*2})^{1/2}$) and hue tone ($h° = \arctan(b^*/a^*)$) were calculated (McGuiere, 1992). The values L*, C, a*, and b* were measured in NBS units, hue tone h° - in degrees from 0 to 360º. Before each series of measurements, the spectrophotometer was calibrated with a light trap and standard white colour, whose coordinates XYZ in the colour space are: X = 81.3, Y = 86.2, Z = 92.7. Value L* indicates the ratio of white to black colour, value a* – the ratio of red to green colour, value b* – the ratio of yellow to blue colour. Averaged data of three measurements are presented.

Antioxidant activity was established spectrophotometrically using a stable free radical 2,2-Diphenyl-1-Picrylhydrazyl hydrate (DPPH) after Brand-Williams et al. The 6x10-5 M solution of DPPH in methanol was prepared for the assay daily before analysing with a Genesys 10 UV spectrophotometer

(Thermo Spectronic, Rochester USA). To carry out the measurements, 20 µl of known-concentration methanol seed extract was mixed with 2 ml of prepared DPPH reagent's methanol solution in a 1 cm quartz cuvette. Absorption variation was measured at 515 nm wavelength for 30 minutes.

Microbiological research on non-sprouted, sprouted, and sprouted disinfected seeds was done observing the standards: LST EN ISO 4833:2003, LST ISO 4832:2006, LST EN ISO 7937:2004, LST ISO 16649-2:2002, LST ISO 21527-2:2008.

The content of mycotoxins (zearalenone, aflatoxins, ochratoxins, deoxynivalenol, T-2 toxin) was determined by immunoenzyme ELISA method.

Statistical data processing. Statistical data processing was done using Microsoft software "Excel" and estimated by the analysis of variance method using *Anova* statistical programme. Means and standard deviations were calculated. In column graphs, the limits of significant difference were marked by dashes. Duncan test was used for data comparison. Significant differences were determined at 5% probability level.

Statistical relationship between separate parameters was estimated by correlation coefficient and regression equations (linear and logarithmic).

RESULTS AND DISCUSSION

The variation of chemical composition of sprouted seed as affected by freezing. Cell membranes are damaged during the sprouted seed freezing process, and during the de-frosting process juices leak from the product, which reduces its output, deteriorates its appearance and consistence. Dry matter losses resulting from freezing and de-frosting were recorded for all seeds tested. The highest content of dry matter (Fig. 1) was established in the sprouted seed of amaranth and radish - 16.03 and 21.19 per cent, respectively and that of soluble dry matter (Fig. 2) also in the sprouted seed of radish (8.17%) and lucerne (8.2%). Dry matter content significantly differed having de-frosted the seed after freezing only for broccoli and radish seed. Soluble dry matter content having de-frosted all frozen sprouted seeds tested significantly differed from that in fresh sprouted seed.

The highest nitrate content was identified in radish and broccoli sprouted seed. After freezing, having de-frosted broccoli and radish seed the remaining nitrate content was found to be by 33 and 67% lower. The lowest nitrate content was found in fresh amaranth and lucerne seed, and the variation of nitrate content having de-frosted the seed was insignificant.

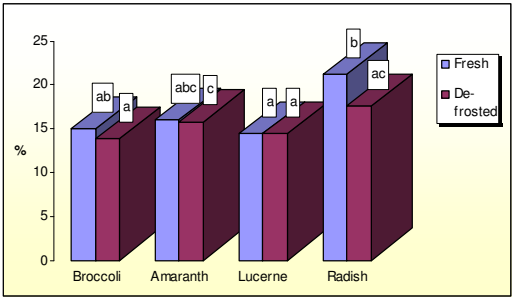


Fig 1. Dry matter content in sprouted fresh and de-frosted seed
a-c mean that averages with different letters differ statistically significantly (P<0.05)

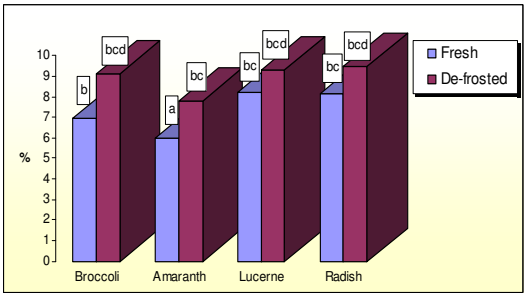


Fig. 2. Soluble dry matter content in sprouted fresh and de-frosted seed
a-c mean that averages with different letters differ statistically significantly (P<0.05)

Sprouted radish seed was found to contain the highest ascorbic acid amount, however, having de-frosted the seed, the ascorbic acid content declined (Table 1). For broccoli vitamin C content declined by 37.67%. For radish and broccoli, in which the highest nitrate contents were established, ascorbic index was not high; for frozen and de-frosted seed, the index did not change or changed inappreciably. Having compared fresh and de-frosted sprouted seed, a significant reduction in total chlorophyll content was established when the seed had been frozen.

Table 1. The content of ascorbic acid in sprouted and de-frosted seed

Seeds	Ascorbic acid, mg kg^{-1}		Index of ascorbic acid	
	Fresh	De-frosted	Fresh	De-frosted
Broccoli	286,7a±9,2	108,0a±0,9	0,06	0.06
Amaranth	86,7a±4,6	100,0a±2,1	1,22	1,41
Lucerne	141,3a±1,2	137,0a±7,1	2,47	1,05
Radish	496,7a±11,5	292,0a±11,3	0,21	0,18

The effect of drying methods on the quality of sprouted seed intended for human consumption. Some literary sources indicate that the content of biologically active substances in plant raw material depends on different drying methods (Dambrauskienė, 2008). Our research evidence on ascorbic acid content in sprouted seed dried using various methods agrees with the above mentioned findings only partly. For all tested seed, except for radish, the highest ascorbic acid content was identified when drying the seed by active ventilation at 23°C. Radish seed dried by active ventilation, like in the case of vacuum drying, was found to contain the lowest vitamin C levels - 154mg kg^{-1} and 165mg kg^{-1}, respectively, which is by 25 % less, compared with the contents in lyophilized and dried in convection oven seed, and this difference is significant.

In all the seed tested, except for radish, the highest ascorbic acid content was identified in the seed dried with active ventilation (Fig. 3). The highest ascorbic acid content was found in broccoli seed dried by active ventilation and in a convection oven.

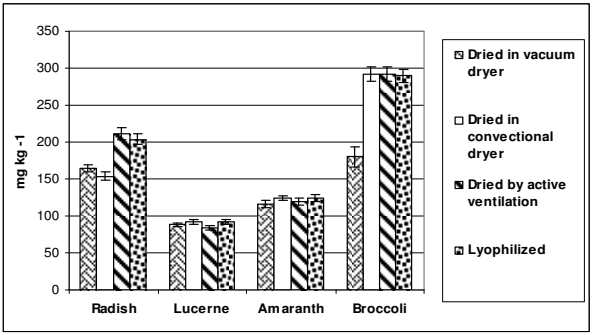


Fig. 3. The effect of drying method on ascorbic acid content in the sprouted seed intended for human consumption

Plant products containing phenolic compounds are characterised by antioxidant, antimicrobial and antiradical properties. Phenolic acids, tannins and flavonoids are major phenolic compounds found in seed. The highest content of phenolic compounds was identified in sprouted radish and broccoli seed. Amaranth cv. 'Geltonukai' was noted for an especially low phenolic compounds content. We think that red-coloured amaranth seed can contain higher phenolic compounds content. In sprouted broccoli, lucerne, amaranth and radish seed DPPH* free radical scavenging activity was similar and ranged from 0.853% for lucerne and radish to 0.877% for amaranth.

Table 2. Phenolic compounds content in sprouted seed intended for human consumption

Sprouted seeds	Phenolic compounds, mg $100g^{-1}$	DPPH* ,%
Broccoli	286,80bc±5,28	0,860
Amaranth	69,87a±12,15	0,877
Lucerne	254,30b±13,66	0,853
Radish	291,70bc±6,81	0,853

Polyphenolic compounds are characterised by antioxidant activity due to their ability to inactivate free radicals. The highest antioxidant activity was determined in sprouted broccoli seed dried in vacuum, while the lowest antioxidant activity was identified in lucerne seed dried by active ventilation. Free radical scavenging indicator was more dependent on seed species rather than on drying method.

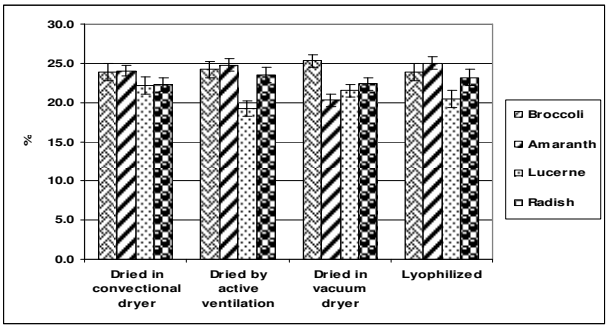


Fig 4. The effect of drying methods on sprouted seed antioxidant activity (according to the DPPH* test)

The highest content of phenols was measured in lyophilized broccoli seed – 1851,5mg $100g^{-1}$ and radish seed dried in a vacuum drier and in a convection oven - 1765,9mg $100g^{-1}$ and 1768,5mg $100g^{-1}$, respectively. When drying lucerne seed, the highest content of phenols was identified in lyophilized seed – 1310,9mg $100g^{-1}$, while the lowest content in the seed dried in a convection oven – 1063,7mg $100g^{-1}$, that is by 20% less. Comparison of the effects of drying on the content of phenols in sprouted seed intended for human consumption showed that the difference for lucerne and radish seed was significant.

It has been confirmed that DPPH* radical's inactivation process balance is achieved in 15 – 16 minutes (Kasperavičienė, Briedis, 2003). In our research, during this time, methanolic concentrate inactivated only 16.4% of DPPH* radicals for vacuum-dried amaranth seed and for lyophilized seed – 19.5%. Since the 20^{th} minute, the activity of vacuum-dried amaranth seed stabilised and within the

next 8 minutes increased by as little as 2% and statistically significantly differed from that of seed dried by other methods, whose DPPH* activity on the 30th minute varied from 24.05% in a convection drier to 25% when lyophilized.

The colour intensity of fresh sprouted seed was mostly in the yellow b* value region, the highest content of reddish tint a* was measured for amaranth and lucerne sprouted seed intended for human consumption (Table 3). When the seed was vacuum dried, the amount of reddish hue a* increased, however, the values of other indicators declined. Having lyophilized the seed, it was found that although the co-ordinates of yellowness b* prevailed, colour lightness L* indicator was high, and colour purity (chroma) C declined. Having dried sprouted seed in a convection oven, high colour values were noted for radish seed, and having dried by active ventilation the colour of seed prevailed within the yellow colour b* region, lucerne and radish seed assumed a reddish tint.

Table 3. Colour co-ordinates of sprouted seed intended for human consumption

Sprouted seeds	L*	a*	b*	C	h°
Broccoli	60,29±2,80	2,13±1,07	19,59±0,79	19,72±0,86	83,86±2,97
Amaranth	53,38±0,77	4,06±0,20	15,50±1,39	16,02±1,39	75,28±0,61
Lucerne	52,40±2,89	4,52±0,72	13,53±0,64	14,29±0,39	71,45±3,53
Radish	57,45±1,40	2,01±0,24	24,00±4,83	24,09±4,83	85,14±0,70

The sensory analyses data of sprouted seed dried using different methods proved lyophilization to be the best method. The sprouted seed of radish, both lyophilized and dried in a convection drier, scored the highest points for all indicators assessed. Although the minimally processed sprouted amaranth seed scored the lowest points, lyophilization process exerted a positive effect on seed flavour, odour, external appearance, and texture qualities.

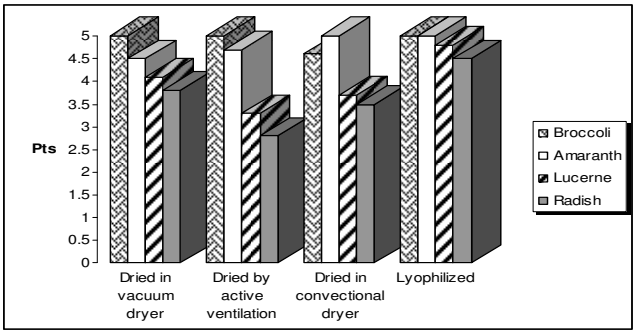


Fig. 5. Sensory assessment of sprouted seed dried by various methods (in points (Pts))

Microbiological contamination of sprouted seed intended for human consumption. Disinfection is reduction and extermination of micro-organisms (especially of those capable of causing infection process) to such a level which would no longer be harmful for human health. Often, spores and relatively resistant micro-organisms can remain viable for a long period of time. Sprouting in a warm and humid medium increased the general microbiological contamination for all seeds tested. Sprouted seed intended for human consumption is a finished food product, i.e. food which is designed for direct consumption and which, most often, does not need boiling or any other processing, in order to eliminate, or minimize to an acceptable level, micro-organism content. As a result, it is vital to safeguard this product's safety. According to the EU Commission regulation No. 2073/2005, the presence of micro-organisms, their toxins or metabolites is not allowed in food products, so that they become unacceptable risk factor for human health. Disinfection with hydrogen peroxide reduced the total microbial contamination only for sprouted broccoli and amaranth seed. Grapefruit seed extract solution did not have any positive effect on the reduction of contamination, and having disinfected amaranth seed with this solution, the total microbiological contamination was found to be twice as high as that on non-disinfected seed (Table 4).

Table 4. The effect of biocides on the total microbiological contamination in sprouted seed intended for human consumption, cfu g^{-1}

Seeds	Raw	Sprouted		
		Not disinfected	Disinfected with	
			GSE*	H_2O_2**
Broccoli	$(4,5\pm2,5)x10^3$	$(5,4\pm0,0)x10^6$	$(2,0\pm2,1)x10^6$	$(2,6\pm0,0)x10^6$
Amaranth	$(1,3\pm2,5)x10^3$	$(3,1\pm2,5)x10^5$	$(8,6\pm3,5)x10^5$	$(2,7\pm3,4)x10^5$
Lucerne	$(2,4\pm2,5)x10^3$	$(1,3\pm2,1)x10^6$	$(2,3\pm0,0)x10^6$	$(1,5\pm1,3)x10^6$
Radish	$(4,5\pm2,1)x10^3$	$(5,4\pm6,5)x10^5$	$(1,5\pm3,5)x10^6$	$(3,2\pm4,0)x10^6$

*- grapefruit seed extract; ** - hydrogen peroxide

No coliformic bacteria were identified on non-sprouted seed, except for radish. After emergence, coliformic bacteria were found on the seed of all plant species tested –the highest coliformic bacteria count was identified on sprouted radish and lucerne seed (Table 5). Disinfection of the tested seed with grapefruit seed extract and hydrogen peroxide did not reduce the content of coliformic bacteria. No *Escherichia coli* were found in the tested broccoli, amaranth, lucerne and radish seed. Having disinfected the seed with grapefruit seed extract, the content of micromycetes and yeast fungi increased, except for sprouted lucerne seed.

In the tested seed, the fungi of 5 genera, the presence of which is undesirable, since they can produce mycotoxins, as well as *Candida* yeast fungi were isolated.

Table 5. The effect of biocides on bacteria count in lucerne seed, cfu g^{-1}

Seeds		Coliformic bacteria	*Escherichia coli*	*Clostridium perfringens*
Raw		No identified	No identified	No identified
Sprouted	Not disinfected	$(3,5\pm4,0)x10^4$	No identified	No identified
	Disinfected with GSE*	$(6,3\pm3,4)x10^5$	No identified	No identified
	Disinfected with H_2O_2**	$(1,4\pm2,5)x10^5$	No identified	No identified

*- grapefruit seed extract; ** - hydrogen peroxide

Disinfection with hydrogen peroxide did not have any positive effect against micromycete (fungi) and yeast growth, and grapefruit seed extract solution, reduced, though inappreciably, the contamination with micromycete propagules and yeast fungi in lucerne seed. However, what is of vital importance here is not the amount of mould fungi, but the fact that fungi genera capable of synthesizing mycotoxins compete with other mould fungi and can be superseded by them. Niyo et al. (1989) suggest that if no fungi are identified in food products, this does not mean that they do not contain mycotoxins. The latter can persist (survive) even after fungi have become non-viable (Table 6).

Table 6. Micromycete and yeast genera identified in lucerne seed

Micromycete and yeast genera	Raw seeds	Sprouted seeds		
		Not disinfected	Disinfected with	
			GSE*	H_2O_2**
Aspergillus spp.	*Flavus sp. Niger sp.*	-	-	
Cephalosporium spp.	-	-	-	-
Cladosporium spp.	+	+	+	+
Mucor spp.	+	+	+	+
Penicillum spp.	+	-	+	+
Candida	-	-	-	-

*- grapefruit seed extract; ** - hydrogen peroxide

Although deoxynivalenol and zearalenone, synthesised by the fungi of *Fusarium* genus, are found in seed, we did not succeed in isolating them in our study. Small amounts of aflatoxin in the tested seed were identified only in the dried lucerne seed disinfected with grapefruit seed extract, while fresh seed disinfected with grapefruit seed extract was found to contain the highest zearalenone levels. The highest total ochratoxin content was identified for radish. Disinfection did not have any significant effect on mycotoxin content. The highest DON concentration was identified in sprouted radish seed disinfected with hydrogen peroxide solution and dried. T-2 toxin was not found in

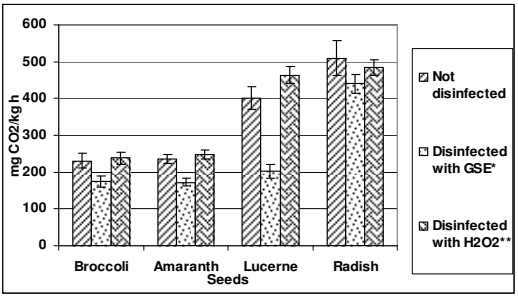
sprouted seed for human consumption, but after seed drying, small amounts of this mycotoxin were identified (Table 7).

Table 7. The content of T-2 toxin in sprouted and disinfected seed, ppb

Seeds	Sprouted seeds			Sprouted dried seeds		
	Not disinfected	Disinfected with		Not disinfected	Disinfected with	
		GSE*	H_2O_2**		GSE*	H_2O_2**
Broccoli	0	0	0	3,70±0,51	1,50±0,08	1,70±0,14
Amaranth	0	0	0	0,20±0,02	2,10±0,15	0,10±0,0
Lucerne	0	0	0	2,30±0,15	2,80±0,13	2,30±0,01
Radish	0	0	0	1,70±0,10	6,40±0,22	3,40±0,08

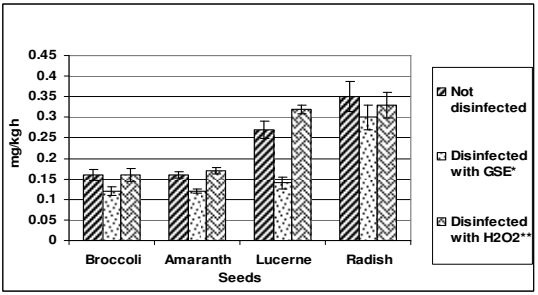
*- grapefruit seed extract; ** - hydrogen peroxide

The highest respiration rate (intensity) was determined in lucerne and radish sprouted seed which was not treated with disinfectants and in the seed which was disinfected with 6% hydrogen peroxide solution. Having disinfected the seed with grapefruit extract solution both the respiration rate and dry matter losses declined (Fig. 6, 7).



*- grapefruit seed extract; ** - hydrogen peroxide

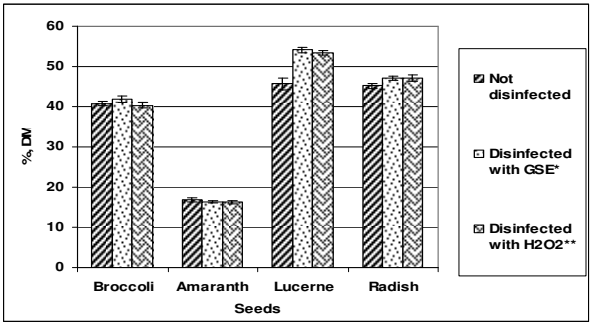
Fig. 6. Respiration rate in disinfected sprouted seed intended for human consumption.



*- grapefruit seed extract; ** - hydrogen peroxide

Fig. 7. Dry matter losses due to respiration rate (intensity)

Disinfection was found to have influenced seed protein content. Having treated the seed with grapefruit seed extract, protein content increased for all sprouted seeds, except for sprouted amaranth (Fig 8).



*- grapefruit seed extract; ** - hydrogen peroxide

Fig. 8. Protein content in sprouted seed intended for human consumption, % DM

Of all the seeds tested, radish and broccoli seeds were found to contain the highest amounts of valine and leucine, while broccoli and amaranth seeds contained the highest levels of lysine. In sprouted seed intended for human consumption, glutamine was the prevalent non-essential amino acid. Disinfection had effect on the ratio of amino acids depending on the biocide. Disinfection with grapefruit seed extract reduced the content of essential amino acids not only in broccoli but also amaranth sprouted seed intended for human consumption.

When using hydrogen peroxide, the content of essential amino acids inappreciably increased and that of lysine declined, and this difference was significant. Like in sprouted lucerne seed, significantly higher valine content was determined in sprouted and disinfected radish seed, and the difference of other essential amino acids content was inappreciable.

CONCLUSIONS

1. The highest free radical scavenging activity was identified in sprouted amaranth seed dried by active ventilation and in vacuum – dried sprouted broccoli seed. All lyophilized sprouted seed intended for human consumption was also characterised by a high free radical scavenging activity.
2. The sprouted broccoli seed was found to contain the highest amount of saccharides, while only their traces were identified in sprouted amaranth seed. High nitrate levels were found in sprouted radish and broccoli seed, which declined having de-frosted the seed.

3. Irrespective of the processing method, the sprouted seeds of broccoli and radish intended for human consumption were found to receive the highest acceptance. The highest sensory evaluation score was given to lyophilized and vacuum-dried sprouted seed intended for human consumption.
4. Disinfection influenced seed protein content. Having treated the seed with grapefruit seed extract, protein content increased for all seeds tested, except for sprouted amaranth seed. The use of inhibitors exerted some effect on the amino acids ratio, depending on the biocide. Radish and broccoli seeds were found to contain the highest concentrations of valine, leucine, while broccoli and amaranth seeds had the highest contents of lysine.
5. Irrespective of the species of the tested seed, a one-time disinfection with grapefruit seed extract and hydrogen peroxide did not have any positive effect on the reduction of the total microbial and bacteriological contamination.
6. No *Escherichia coli* and *Clostridium perfringens* bacteria were identified in sprouted seed. The *Aspergillus, Cephalosporium, Cladosporium, Mucor, Penicillium* fungi genera and *Candida* yeast fungi were found to be prevalent.
7. Grapefruit seed extract reduced the growth of *Aspergillus* fungi in radish seed. Hydrogen peroxide and grapefruit seed extract did not have any biocidal effect on other microfungi genera.
8. The highest zearalenone content was identified in fresh lucerne seed disinfected with grapefruit seed extract.

APPROBATION OF WORK RESULT

Scientific articles in ISI Master list

1. Kraujutienė I., Jarienė E., Danilčenko H., Šlapakauskas V., Tarasevičienė V., **Kordušienė S**. An influence of growing technology to enzyme activity in germinated for food wheat grains. // Polish Journal of Food and Nutrition Sciences. 2007. 57 (4, a).
2. **Kordusiene S.,** Danilcenko H., Taraseviciene Z., Jariene E., Jeznach M. Disinfection on sprouted seeds for food. // International Journal of Food, Agriculture & Environment. 2010. Nr. 8 (2).

Scientific articles in reviewed publications

1. Danilčenko H., Tarasevičienė Ž., Jarienė E., Pranaitis P., **Kordušienė S.** Mikotoksinų kiekis ir jų detoksikavimo galimybės maistui skirtose daigintose sėklose. // Vagos. LŽŪU mokslo darbai. 2006. Nr. 73 (26), p. 7-12
2. Kraujutienė I., Jarienė E., Danilčenko H, Aleknavičienė P., Steponavičius D., **Kordušienė S**. Fermentų aktyvumas daigintuose ir nedaigintuose kviečių grūduose. // Vagos. LŽŪU mokslo darbai. 2006. Nr. 72 (25), p. 13 – 18.
3. Kraujutienė I, Jarienė E., Danilčenko H., Šlapakauskas V., Venskutonienė E., **Kordušienė S.** An influence of growing technology to enzyme activity in germinated

for food wheat grains. // 3rd international conference on quality and safety in food production chain/ Wroclaw university. 2007. P. 280.
4. **Kordušienė S.,** Kraujutienė I. Zearalenono kiekis maistui daigintose sėklose ir jo detoksikavimo galimybės. // Tarptautinė mokslinė doktorantų konferencija „Jaunimas siekia pažangos“. LŽŪU. 2007. P. 36-41.
5. **Kordušienė S.,** Kraujutienė I. Pavojingiausių (T-2 ir zearalenono) mikotoksinų kiekis maistui daigintose sėklose ir jų detoksikavimo galimybės // Tarptautinė mokslinė-praktinė konferencija “Žmogaus ir gamtos sauga”. LŽŪU. 2009. (2). P.95-98.
6. **Kordušienė S.,** Kraujutienė I. Maistui skirtų daigintų sėklų sauga. // Tarptautinė mokslinė doktorantų konferencija „Jaunimas siekia pažangos“. LŽŪU. 2009. P. 115-118.

ACKNOWLEDGEMENT

I would like to express my special thanks to scientific advisor prof. dr. Honorata Danilčenko for suggested interesting thematic and warm collaboration. I wish to thank assoc. prof. dr. E. Jariene for critical and friendly advices, assoc. prof. dr. Zivile Tarasevičienė and full team of Horticulture Department for valuable comments. I am grateful to the SC ‘Kauno grūdai’ team for the opportunity to carry out research for the understanding and support, Mr. Prof. Dr. Rainer Mosenthin for assistance in investigations.

I want to express special thanks to assoc. prof. dr. Pranas Viskelis for new ideas and significant help in the investigations.

I thank my friend and colleague Laimute Šimienė for assistance in investigations and considerable moral.

I am grateful to my family for patience and understanding of doctoral studies.

SANTRAUKA

Tyrimų tikslas - parinkti ir įvertinti daigintų maistui sėklų perdirbimo būdus, cheminės sudėties ir biologinio aktyvumo kitimą, juslinį priimtinumą. Įvertinti įvairių dezinfekantų panaudojimo sėklų mikrobiologiniam stabilizavimui poveikį.

Tyrimų uždaviniai

- ✓ Įvertinti ir palyginti daigintų maistui sėklų pirminio perdirbimo būdus bei įvertinti cheminės sudėties kitimą.
- ✓ Jusliškai įvertinti perdirbtas daigintas maistui sėklas.
- ✓ Įvertinti vandenilio peroksido ir greipfrutų sėklų ekstrakto efektyvumą daigintų maistų sėklų mikrobiologinės taršos mažinimui.
- ✓ Nustatyti dezinfekantų įtaką aminorūgščių santykiui.

Ginamieji disertacijos teiginiai

- ✓ Minimalus daigintų maistui sėklų perdirbimas prailgina jų galiojimo terminą, tačiau juslinis patrauklumas ne visada yra patenkinamas.
- ✓ Naudojami biocidai mikrobiologinės taršos mažinimui sėklų daiginimo metu įtakoja bioaktyvių medžiagų kiekio kitimą.
- ✓ Vandenilio peroksidas ir greipfrutų sėklų ekstraktas ne visada yra efektyvus daigintų maistui–sėklų mikrobiologinės taršos mažinimui.

Mokslinis darbo naujumas. Pirmą kartą ištirti maistui daigintų sėklų pirminio perdirbimo būdai bei įvertintas cheminės sudėties, biologiškai aktyvių ir antioksidacinėmis savybėmis pasižyminčių junginių kitimas. Taip pat nustatyta, kad dezinfekantai maistui daigintose sėklose daro įtaką bendram mikroorganizmų skaičiui, bakterijų ir mikroskopinių grybų bei jų produkuojamų mikotoksinų kiekiui.

Praktinė darbo reikšmė. Minimaliai perdirbus daigintas maistui sėklas prailginamas jų vartojimo terminas, galima praplėsti augalinių produktų asortimentą maisto pramonėje, sudominti perdirbėjus. Panaudojus inhibitorinės medžiagas efektyviau sumažinama sėklų mikrobiologinė tarša.

Išvados. Ištyrus maistui daigintas burnočio (*Amaranthus cruentus*), brokolio (*Brassica olearacea* L. *convar. otrytis* (L)), mėlynžiedės liucernos (*Medicago sativa* L.) ir ridikėlio (*Raphanus sativus* L.) sėklas, padarytos tokios išvados:

1. Džiovintuose aktyviąja ventiliacija burnočiuose ir džiovintuose vakuume brokoliuose buvo aukščiausias radikalų sujungimo aktyvumas. Visos tirtos liofilizuotos maistui daigintos sėklos pasižymėjo aukštu radikalų sujungimo aktyvumu.
2. Didžiausias sacharidų kiekis nustatytas daigintose brokolių sėklose, o burnočiuose aptikti tik jų pėdsakai. Nustatytas didelis nitratų kiekis daigintuose ridikėliuose ir brokoliuose, sėklas defrostavus sumažėjo.
3. Nepriklausomai nuo perdirbimo būdo priimtiniausios buvo maistui daigintos brokolių ir ridikėlių sėklos. Geriausiai jusliškai įvertintos liofilizuotos ir džiovintos vakuuminėje džiovykloje visos tirtos sėklos.
4. Apdorojus greipfrutų sėklų ekstraktu, visose tirtose daigintose maistui sėklose, išskyrus burnočius, padidėjo baltymingumas. Daugiausiai valino ir leucino nustatyta ridikėlių ir brokolių, o lizino – brokolių ir burnočių sėklose. Naudoti biocidai turėjo poveikį aminorūgščių kiekio santykiui.
5. Nepriklausomai nuo daigintų sėklų rūšies, vienkartinis dezinfekavimas greipfrutų sėklų ekstraktu ir vandenilio peroksidu bendrai mikrobiologinei ir bakteriologinei taršai teigiamos įtakos neturėjo.
6. Daigintose maistui sėklose neaptikta *Escherichia coli* ir *Clostridium perfringens* bakterijų. Iš grybų genčių vyravo *Aspergillus, Cephalosporium, Cladosporium, Mucor, Penicillium* bei *Candida* mieliagrybiai.
7. Greipfrutų sėklų ekstraktas mažino *Aspergillus* genties mikromicetų augimą ridikėlių sėklose. Tačiau, kaip ir vandenilio peroksidas, kitoms mikroskopinių grybų gentims biocidinio poveikio neturėjo.
8. Daigintose maistui šviežiose sėklose, išskyrus ridikėlius, aflatoksinų, T-2 toksino ir zearalenono neaptikta. Aflatoksinai identifikuoti tik dezinfekuotose greipfrutų sėklų ekstraktu džiovintose liucernose. Vienkartinė dezinfekcija mikotoksinų kiekio mažinimui daigintose džiovintose sėklose esminės įtakos neturėjo.