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IMMUNOREACTIVE PROPERTIES AND SENSORY QUALITY OF LENTIL (*LENS CULINARIS*) AND MUNG BEAN (*VIGMA RADIATA* L.) SPROUTS

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Key words: lentil, mung bean, germination, immunoreactivity, sensory quality

The objective of the study was to compare parallel immunoreactive properties and sensory characteristics of lentil (*Lens culinaris*) and mung bean (*Vigma radiata* L.) sprouts. The seeds were germinated with and without light at 20°C up to 3 days. The changes in immunoreactivity of the sprouts were determined by competitive ELISA method using rabbit polyclonal antisera to lentil and mung bean proteins. The sensory quality of samples was evaluated by quantitative descriptive analysis (QDA). In QDA a trained panel (n=9) rated the sprouts for odour, taste and texture. The results indicated that the effects of germination on the immunoreactive properties of sprouts with cotyledons depended on the type of legumes. The presence or absence of light during the germination process did not affect the results achieved. The removal of the cotyledons almost completely reduced immunoreactivity of samples (97% for lentil and 99% for mung bean). The effects of germination on the sensory profile of sprouts were found to depend on the type of legumes but as far as the sprouts of mung bean are concerned also on the processing conditions. The QDA demonstrated significant differences between the sprouts for the following attributes: bitterness ($p<0.001$), "pea-pod" taste ($p<0.01$), flourness ($p<0.001$) and juiciness ($p<0.01$).

INTRODUCTION

Germination of legume seeds for human consumption is a well-known process which has been widely used throughout the centuries in the oriental culture. Nowadays, the sprouted seeds have gained popularity in the Western countries and they are offered to consumers as traditional biotechnological healthy food. Several nutritive factors such as amino acids, vitamins, and minerals are reported to increase during germination [Kuo *et al.,* 2004; Frías *et al.,* 2002; Bau *et al.,* 2000]. At the same time there are indications that germination is effective in reducing trypsin inhibitors, galactosides, tannins, lectins and in increasing protein digestibility [Ibrahim *et al.,* 2002; Bau *et al.,* 1997; Savelkoul *et al.,* 1992; Urbano *et al.,* 2005]. Changes in the polyphenolic compounds of different legumes were also observed during germination [Bartolomé *et al.,* 1997; Troszyńska *et al.,* 2006b]. With regard to the important changes of nutrient and non-nutrient components, germination could be a valuable processing technique for modifying the immunoreactive properties [Besler *et al.,* 2001; Sathe *et al.,* 2005] and sensory perception [Bau *et al.,* 2000] however, information concerning these areas is scant.

The purpose of this study was to evaluate the effect of germination on the immunoreactive properties of lentil and mung bean sprouts and to investigate how this process influenced the sensory perception of seeds.

MATERIAL AND METHODS

Seeds and germination. Dry seeds of lentil and mung bean were purchased in a local market in Poland. They were soaked in distilled water for 3.5 h and imbibed seeds were located in 3 tier salad sprouters with reservoir trays (Raszyn-Rybie "Bio-natura," Poland). The germination process was carried out in a seed germinator (Economic Delux EC00-065, Snijders, The Netherlands) at 20°C for 3 days (72 h) with continuous light and in darkness. The seeds were rinsed every day with distilled water during the germination. The 3-day-old sprouts with cotyledons, when they were ready for the consumption, were subjected to the sensory evaluation and determined for the immunoreactive properties (abbreviated as: LLC – lentil with light; MLC – mung bean with light; LDC – lentil in darkness; MDC – mung bean in darkness). Apart from the sprouts with cotyledons (as above) the immunoreactivity was also determined fora protein extract from the sprouts without cotyledons (abbreviated as: LL – lentil with light; ML – mung bean with light; LD lentil in darkness; MD – mung bean in darkness). The seeds and sprouts to be examined for the immunoreactive properties were frozen in liquid nitrogen and lyophilised.

Proteins extraction. The proteins were extracted from lentils or mung bean seeds, both raw and after germination for 2 h with 0.1 mol/L Tris-HCl buffer containing 0.1 mol/L

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NaCl (pH 8.5). The extract was centrifuged for 30 min (20,000 × g), dialysed against 0.05 mol/L NH_4HCO_3, frozen, lyophilised and stored at -20°C until use [Barnett *et al.*, 1987].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoretic separations of proteins were performed in 12.5% polyacrylamide gel (SDS--PAGE) according to Laemmli method [Laemmli, 1970]. Before electrophoresis, all protein samples (2 mg/mL) were boiled for 3 min in the presence of SDS (3% w/v) and 2-mercaptoethanol (0.1% v/v). Low molecular weight markers (Sigma) ranging from 6.5 to 66 kD were used as a standard. The gels were run in a Tris-glycine buffer, pH 8.3. Protein staining was carried out by Coomassie Brilliant Blue R-250.

Enzyme-linked immunoabsorbent assay (ELISA). Microplates were coated with the antigen (lentils or mung bean proteins) (10 μg/mL) in a 9 mmol/L carbonate buffer solution, pH 9.6 and incubated for 18 h at 4°C; then rinsed 4 times with PBS-T. This procedure was repeated after each step of this method. The sites of the microplates that were not saturated by the antigen were filled with a 1.5% gelatine solution of 150 μL/well and incubated for 30 min at 25°C. The wells were filled with both a sample (containing the antigen or analysed samples) and the polyclonal rabbit antibodies obtained for a given antigen (50 μL of each solution of an adequate concentration per well). In order to blend the process components, the microplates were placed in a shaker for 5 min and incubated for 1 h at 37°C. Next, the microplates were incubated with anti-rabbit IgG peroxidase conjugate. After rinsing the microplate, the substrate (TMB) was added and after 30 min the reaction was stopped by adding 2 mol/L H_2SO_4. Absorbance was measured at 450 nm using a Sunrise-Tecan automatic reader. The obtained results were processed with the Immunofit™ EIA/RIA software by Beckman.

Production of polyclonal antibodies. Polyclonal antibodies were obtained through subcutaneous and intramuscular quadruple rabbit immunisation at 2-week intervals. Each time, 1 mL solution containing 10 mg protein was used. The first immunisation was performed in the presence of complete Freund adjuvant, and the subsequent – in the presence of incomplete Freund adjuvant. Indirect ELISA method was used to determine the titre of the obtained antibodies.

Sensory panel. The sensory assessments of the samples were carried out by a panel consisting of 9 members selected and trained according to ISO guidelines [ISO 8586-1:1993]. All assessors have passed the basic taste test, the odour test and the colour vision test. Prior to their participation in the experiments, the subjects were trained on sensory descriptors for fresh sprouts of legume seeds.

Sensory method. The quantitative descriptive analysis (QDA) was used to determine the differences in the sensory characteristics of the samples [Stone & Sidel, 1993; Lawless & Heymann, 1999]. Prior to the analysis, vocabularies of the sensory attributes were developed by the panel in a round-table session, using standardised procedure [ISO/DIS 13299:1998]. Thirteen attributes for odour, taste and texture were selected and thoroughly defined for profiling (Table 1). The attributes' intensities were rated on continuous unstructured, graphical scales. The scales were 10 cm long and verbally anchored at each end and the results were converted to numerical values (from 0 to 10 units) by a computer.

TABLE 1. Attributes for sensory descriptive analysis of sprouts and their definitions.

Attribute	Definition of attributes
Odour	
beany	smell characteristic for boiled dry legume seeds
grassy	like aroma of freshly cut grass
Taste	
green	note characteristic for fresh green pea seeds
beany	note typical for boiled legume seeds
fresh	like fresh vegetables (versus processed products)
bitter	basic taste (reference sample: caffeine in water 0.5%)
astringent	like strong tea or unripe banana (reference sample: tannic acid 0.2%)
pungent	note associated with fresh cruciferous vegetables (reference sample: slice of radish)
pea-pod	specific note reminding of pea-pod without pea seeds
grassy	note characteristic for boiled spinach
Texture	
flourness	feeling during chewing the samples of legume flour
juiciness	degree releasing water from sprouts while chewing the sample
fibrousness	feeling of fibrousness perceived while chewing the sample

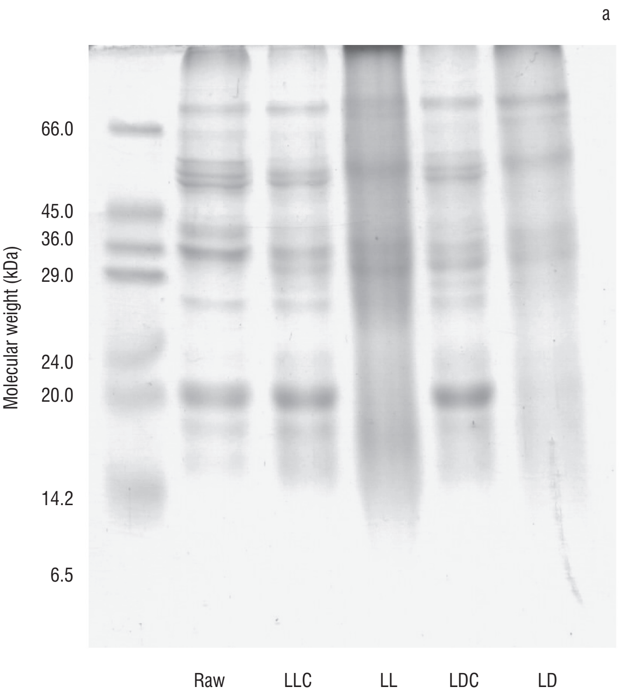
Anchoring points: Odour/Taste: none – very intensivE; Texture: low – high (while chewing the sample 10 times)

Samples preparation and evaluation conditions. The sprouts were taken out from a seed germinator at least 1 h prior to evaluation in order to equilibrate to room temperature. The samples of about 5 g of sprouts each, were presented in random order to the assessors from three-digital coded plastic containers (5 cm × 10 cm) covered by lids. With the samples the panellists received a cup of room temperature spring water (Aleksandria, Poland) for cleaning their palates. The assessments were carried out at the sensory laboratory room fulfilling requirements of the ISO standards [ISO 8589:1998] and results were collected using a computerised system. Testing sessions took place in the morning, between 10 and 12 h a.m. The analysis was performed in two independent sessions, in two replications.

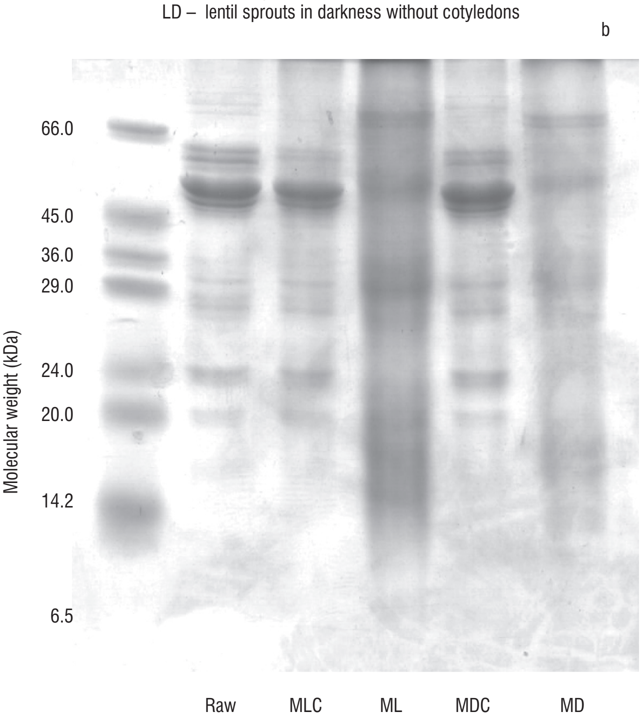
Statistical analysis. ANOVA was used to test statistical differences in sensory attributes between the samples of sprouts. Treatment means were compared using Fisher's protected least significant difference (LSD) test. Statistical significance was considered at $p<0.05$. Statistical analysis was performed using software package (statSoft Inc., v. 7.1, Tulsa, OK, USA).

RESULTS AND DISCUSSION

SDS-PAGE separations of proteins from lentils and mung bean seeds are shown in Figure 1 a and b. The results



LLC – lentil sprouts with light with cotyledons,
LL – lentil sprouts with light without cotyledons,
LDC – lentil sprouts in darkness with cotyledons,
LD – lentil sprouts in darkness without cotyledons



MLC – mung bean sprouts with light with cotyledons,
ML – mung bean sprouts with light without cotyledons,
MDC – mung bean sprouts in darkness with cotyledons,
MD – mung bean sprouts in darkness without cotyledons

FIGURE 1. SDS-PAGE separation of proteins: (a) lentil, (b) mung bean.

indicated that ungerminated lentils and mung bean contained 12 and 10 bands, respectively with molecular weights ranging from 6.5 to 70 kDa. The predominant lentil protein fractions are those characterised by molecular masses of 20, 36, 40 and 50 kDa (Figure 1a). The mung bean is characterised by a high content of 50 kDa (Figure 1b) protein fractions. Additionally, there are a few bands corresponding more to 60 kDa and lower molecular masses visible on the electrophoregram. Our results showed that germination of lentil and mung bean seeds for 3 days resulted in hydrolysis of proteins, which was reflected in the disappearance or loss of density from the main protein bands, together with the appearance of a smear of lower molecular weight polypeptides. SDS--PAGE patterns of proteins from germinated seeds with cotyledons indicated that lentil (LLC, LDC) contained 9 bands each and mung bean MLC, MDC) 8 bands for two germination conditions. The same changes in proteins electrophoretic profile were shown during germination of pea seeds [Urbano *et al.,* 2005]. The sprouts of lentils and mung bean contained 6 (LL), 5 (LD), and 8 (ML), 7 (MD) bands, respectively. The low and high-molecular-weight protein bands, which were observed in lentils and mung bean sprouts without cotyledons (LL, LD, ML, MD), were probably newly synthesised proteins. The results obtained are in accordance with the literature data. It was reported that proteins of different types of legumes were degraded at 72 h of germination [Ahmed *et al*., 1995]. For this reason, it can be anticipated that the hydrolysis of proteins during germination would result in a change in immunoreactive properties of seeds.

Some strong allergens have been isolated from lentil seeds [López-Torrejón *et al*., 2003; Pascual *et al*., 1999]. The main lentil seed allergen Lec c 1 retains its high allergenic activity even during the boiling process [Sánchez-Monge *et al*., 2000]. The germination process resulted in lowering immunoreactive properties by 40–35% depending on germination process conditions and the 50 kDa fraction was not visualised on the electrophoregram. The fraction can be supposed to be the vicilin subunits (7S) and Len c 1 allergen is the vicilin protein, as it has been reported in the literature [Barre *et* al., 2005]. The lower immunoreactivity can be contributed to lower density of 50 kDa lentil protein band. There were no 40 kDa protein bands observed on the electrophoregram, which can also result in lower immunoreactive properties. The 29 kDa protein band visualized can be the protein degraded during the germination process. Such changes in the protein electrophoretic profiles are not observed regarding the mung bean. The electrophoretic patterns of proteins either before and after the germination process were not significantly changed and thus the immunoreactive properties of seeds with cotyledons were insignificantly lower by 12–14% as compared to those of the ungerminated seeds.

Proteins from cotyledons are hydrolyzed during germination, which may result in eliminating some of epitopes within seeds proteins. There is no doubt that the content and kind of particular proteins with low molecular weight depends on species of seeds and time of germination [Ahmed *et al*., 1995]. A 72-h period of germination used in the study is not sufficient to achieve complete degradation of proteins with high molecular weight.

There is a lack of information in the literature about sprouts allergenicity. A review by Besler *et al*. [2001] describes studies on a protein extract from soybean sprouts which shows 70% inhibition of IgE-binding soybean allergic patients. When the sprouts without cotyledons (LL, LD, ML, MD) were subject-

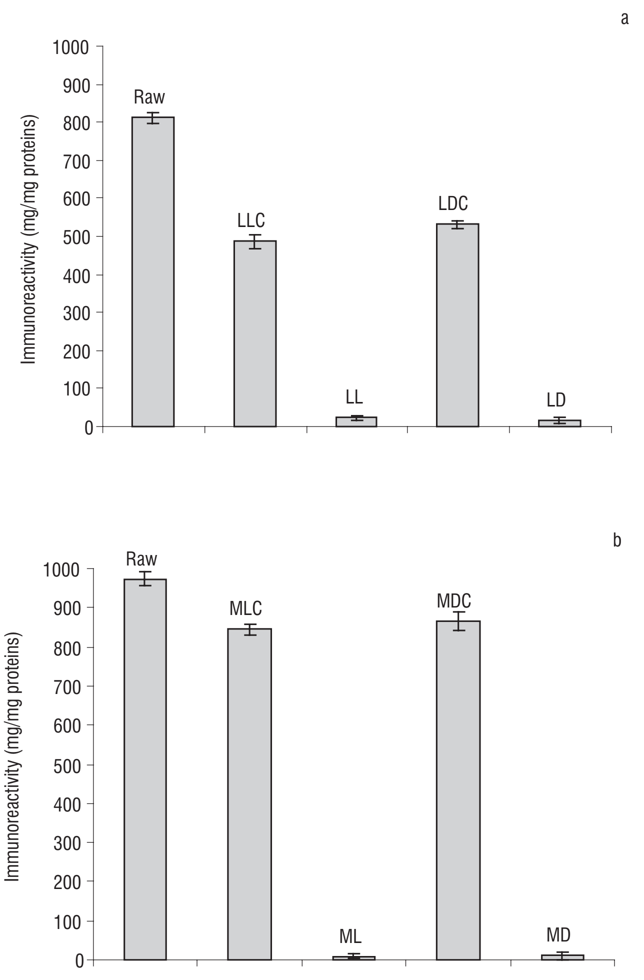


FIGURE 2. Residual immunoreactivity of proteins after germination: (a) lentil, (b) mung bean.

TABLE 2. Effect of germination on sensory attributes of lentil and mung bean sprouts [a,b].

Sensory attributes [c]	Sprouts			
	LDC	LLC	MDC	MLC
O-beany	2.5[a]	1.9[a]	1.6[a]	2.3[a]
O-grassy	3.3[a]	2.9[a]	3.6[a]	2.7[a]
F-green	2.0[a]	2.2[a]	1.8[a]	1.5[a]
F-beany	3.6[a]	2.6[a]	2.9[a]	2.4[a]
F-fresh	2.8[a]	3.0[a]	3.6[a]	2.2[a]
F-bitter	0.7[a]	0.9[a]	0.4[a]	2.2[b]
F-astringent	1.8[a]	1.6[a]	1.0[a]	1.6[a]
F-pungent	0.7[a]	0.8[a]	0.4[a]	0.8[a]
F-pea-pod	2.1[a]	2.4[ab]	5.2[c]	4.2[bc]
F-grassy	4.3[a]	3.6[a]	4.1[a]	3.7[a]
T-flourness	5.8[c]	5.3[c]	1.8[a]	3.4[b]
T-juiciness	3.9[a]	3.6[a]	6.1[b]	3.6[a]
T-fibrousness	3.5[a]	2.9[a]	3.7[a]	3.4[a]

[a] Mean descriptive analysis ratings of sprouts (0–10 scale); [b] Values followed by the same letters in the same row are not significantly different ($p<0.05$); [c] O=odour, F=taste, and T=texture

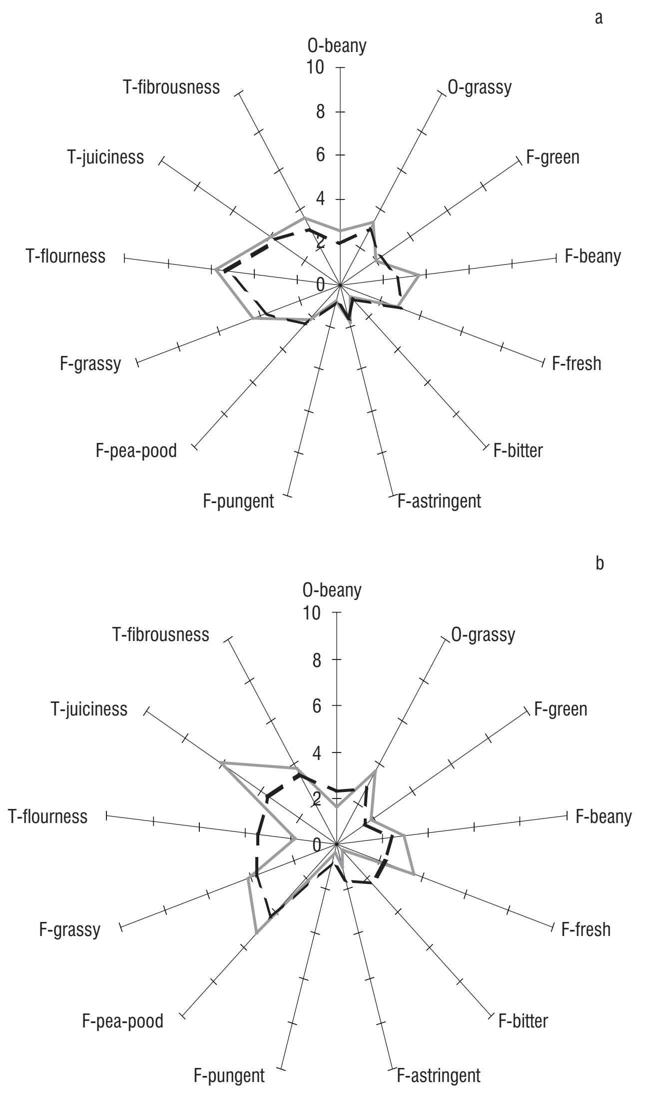


FIGURE 3. The sensory profiles of sprouts: (a) lentil — LDC, – – – LLC), (b) mung bean (— MDC, – – – MLC).

ed to the immunoreactivity analysis it was found that their immunoreactive properties were reduced by 97-99% (Figure 2a and 2b) as compared to those of the raw lentils and bean mung seeds, regardless of germination conditions. Sprouts separated from cotyledons were characterised by minimum immunoreactive properties, which may indicate that sprouts can be used as food supplements produced for people with food allergy problems.

To find attributes which influenced the sensory quality of sprouts quantitative descriptive analysis (QDA) was used in the study. QDA is the most sophisticated method in sensory evaluation and involves the discrimination and description of both the qualitative and quantitative sensory attributes of a product by trained panels. The mean sensory ratings for the samples and the analysis of variance are presented in Table 2. The results show that among 13 attributes, 4 of them were statistically significant. Significant differences were observed in the intensity of bitterness ($F=6.75$; $p<0.001$), "pea-pod" taste ($F=4.19$; $p<0.01$), flourness ($F=33.32$; $p<0.001$) and juiciness ($F=6.17$; $p<0.01$) caused by germination conditions. In order to observe the above differences in the sprouts more clearly, the sensory profiles of samples were displayed as spider diagrams in Figures 3a and 3b. It can be seen that profiles of both kinds of lentil sprouts (LDC and LLC) were similar although the LDC was a bit more intense in some of sensory attributes (the beany odour, "beany" taste, "grassy" taste and fibrousness). These results indicate that the sensory perception of lentil sprouts was not dependant on the germination conditions of seeds. On the other hand, the sensory profiles of the mung bean samples (MLC, MDC) were differentiated. The dominating attributes differentiating the samples with light from the samples without light were: bitter taste, "fresh" taste, "pea-pood" taste and juiciness as well as flourness. It should be emphasized that the MDC sprouts demonstrated more intensive bitterness than the other products. This sensory attribute is perceived as having a negative hedonic value and can be one of the most substantial reasons for the limited use of food. The bitter taste of plant-derived foods is attributed to the presence of polyphenols [Peleg & Noble, 1995; Lesschaeve & Noble, 2005; Troszyńska *et al*., 2006a]. The sensory activity of these compounds is strictly connected with the chemical structure. For example, lower--molecular-weight tannins are more bitter whereas the higher-molecular-weight polymers are more likely to be astringent [Peleg *et al*., 1999; Lesschaeve & Noble, 2005]. Our previous studies indicated that the polyphenols significantly affected the sensory profiles and the overall quality of the mung bean sprouts [Troszyńska *et al*., 2006b]. The bitter taste can also be elicited by the phenolic acids and peptides containing hydrophobic amino acid which can be released during the germination process.

According to literature, the major factor contributing to the reduction of consumer acceptability of legume products is associated with the beany flavour [Rackis *et al*., 1972]. Some studies have been performed on the possibility of eliminating this negative attribute in order to increase the consumption of legumes [Fujimaki *et al*., 1968; Chen & Snyder, 1983; Uwaegbute *et al.*, 2000; Braudo *et al*., 2001]. However, the methods of modifying the beany flavour are still not well known. It should be stressed that the 3-day-old sprouts of lentil and mung bean showed a mild beany flavour (odour and taste). The presence or absence of light during the germination process had no significant effects on these sensory attributes (Table 2).

CONCLUSIONS

In the conclusion, we found that immunoreactive properties of sprouts dependent on the fact whether they were with or without cotyledons. The removal of the cotyledons almost completely reduced the immunoreactivity of the samples. It suggests that lentil and mung bean sprouts without cotyledons could be used to elaborate special products for people who suffer from food allergic disorders. Further studies in this field should be continued to explain whether it is possible to reduce the total immunoreactivity of legume seeds by prolonging their time of germination. Still, their sensory quality should be taken into consideration.

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WŁAŚCIWOŚCI IMMUNOREAKTYWNE I JAKOŚĆ SENSORYCZNA KIEŁKÓW SOCZEWICY (*LENS CULINARIS*) I FASOLI MUNG (*VIGMA RADIATA* L.)

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W pracy porównano właściwości immunoreaktywne i jakość sensoryczną kiełków soczewicy i fasoli Mung. Kiełkowanie nasion prowadzono w szafie klimatyzacyjnej przez 3 dni w temperaturze 20°C z i bez światła. Analizę sensoryczną świeżych kiełków wykonano metodą profilowania sensorycznego (QDA), a właściwości immunoreaktywne metodą ELISA (współzawodniczącą). Wyniki wykazały, że warunki kiełkowania (w świetle, bez światła) nie miały wpływu na właściwości immunoreaktywne kiełków. Stwierdzono, że kiełki bez liścieni nie wykazywały właściwości immunoreaktywnych. Analiza QDA wykazała, że jakość sensoryczna kiełków była zróżnicowana i zależała od gatunku użytych nasion. Wykazano, że warunki kiełkowania nie różnicowały profili sensorycznych kiełków soczewicy, natomiast miały one wpływ na profile sensoryczne kiełków fasoli Mung.