POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2006, Vol. 15/56, No 4, pp. 453–459

EFFECT OF GERMINATION TIME ON THE CONTENT OF PHENOLIC COMPOUNDS AND SENSORY QUALITY OF MUNG BEAN (*Vigma radiata* L.) SPROUTS

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Key words: mung bean, sprouts germination, sensory quality, phenolic compounds

Seeds of mung bean (*Vigma radiata* L.) were germinated for 7 days in dark, and the sensory quality of their sprouts was compared using a descriptive sensory analysis and consumer testing. In the descriptive analysis a trained panel (n=9) rated the sprouts for appearance, odour, taste and texture. In the affective tests the panelists rated the sprouts for overall quality. Changes in the contents of total phenolics and tannins were monitored for 7 days of germination using spectrophotometric methods. The results proved that the time of germination had a significant effect on the sensory profiles of the samples. All analysed attributes differentiated statistically significantly the sensory profiles of the sprouts. The overall sensory quality of 3-day-old sprouts was essentially better than that of the other samples. A statistically significant correlation was found between the total phenolics and the overall quality (p=0.05), the total phenolics and bitterness (p=0.01), the total phenolic and astringency (p=0.05), proanthocyanidins and bitterness (p=0.01), astringency and the overall quality (p=0.05), as well as bitterness and the overall quality (p=0.05).

INTRODUCTION

Legumes contain a high concentration of protein, polysaccharides (dietary fibre, starch oligosaccharides) and micronutrients (vitamins, macro- and microelements), hence are recommended as constituents of a daily diet [Messina, 1999]. They are also a rich source of a variety of polyphenolic compounds, including: simple phenols, flavonoids and tannins, which are considered to be natural antioxidants [Prior & Gu, 2005; Santos-Buelga & Scalbert, 2000; Amarowicz *et al*., 2004; Troszyńska & Ciska, 2002; Troszyńska *et al*., 2002]. In the past decade, plant phenolic compounds have been often discussed in the context of their positive effects in the prevention of development of many diseases such as cancer [Han, 1997; Yang *et al*., 1998; Clifford & Scalbert, 2000; Harbone & Williams, 2000] and atherosclerosis [Luo *et al*., 1997; Pearson *et al*., 1998; Troszyńska & Bałasińska, 2002], which are one of the major public health problems in the Western countries.

In contrast to these beneficial effects, the consumption of polyphenols-rich food is associated with the sensation described as astringency [Lee & Lawless, 1991; Bennick, 2002; Lesschaeve & Noble, 2005; Peleg *et al.,* 1999; Troszyńska *et al.,* 2006]. Besides, they often leave bitter aftertaste and contribute to off-notes that some consumers find unpleasant [Drewnowski & Gomez-Carneros, 2000]. Thus the increase of polyphenols in the diet may have an incompatible association with the consumer's acceptance. The consumers and marketing studies indicated that food-choice process is mainly based on the sensory quality of the foodstuff. The consumers would not be interested in consuming the functional products, if the ingredients caused noticeable off-flavours that the consumers found unpleasant, despite the added health advantages [Tepper & Trail, 1998; Tuorila & Cardello, 2002; Luckow & Delahunty, 2004]. Food manufactures are faced with the dilemma of how to increase the phenolics content of foods without losing their palatability.

The legume seeds can be consumed as cooked or after the germination process. The germination is technologically simple, inexpensive and known for centuries especially in the oriental culture. Many studies have reported higher levels of nutrients (amino acids, vitamins, minerals) and lower values of non nutrients (trypsin inhibitors, galactosides, saponins, tannins) in the germinated seeds in comparison to the ungerminated originals [Frias *et al.,* 2002; Urbano *et al.,* 2005; Savelkoul *et al.,* 1992; Bau *et al.,* 1997; Ibrahim *et al.,* 2002]. Nowadays, the germinated seeds have gained popularity in Western countries, and a wide variety of fresh sprouts is sold as new healthy food. At present, no information is available about the relation between their sensory quality and the content of polyphenols. For this reason, the objective of this work was to study the changes of polyphenolic compounds during germination of mung bean and their influence on the sensory profiles of sprouts.

MATERIALS AND METHODS

Seeds and germination. The seeds of mung bean were purchased from a local market in Olsztyn, Poland. A portion of 500g of mung bean seeds was soaked in 2000 mL for 3.5 h. Imbibed seeds were located in "Bio-natura" (Poland) trays and germinated in a seed germinator (Economic Delux EC00-065, Snijders, The Netherlands) for 7 days (168 h), in dark at 20°C and 99% relative humidity. The seeds were

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rinsed every day with distilled water during the germination. After each day of germination, fresh sprouts were subjected to the sensory evaluation. For the chemical analyses of seeds and sprouts the lyophilised samples were used.

Sensory evaluation

Sensory panel. An 8-member trained panel experienced in discrimination and descriptive tests on different food products performed the assessment of sprouts. Prior to their participation in the experiments, the panelists were trained to rate the perceived intensity of the following different sensations: sweetness, saltiness, sourness, bitterness and astringency using aqueous solutions of different concentration of sucrose, NaCl, citric acid, quinine sulphate, caffeine and tannic acid. The training sessions included also a brain-storming activity to identify descriptive terms for raw and germinated lentil seeds. Subsequently panelists tasted the mung bean sprouts and other legumes and rated their intensity for beaniness, bitterness, astringency and flourness as the four attributes that best differentiated legumes samples.

Sensory methods. A sensory profile was created by descriptive analysis for samples [Stone & Sidel, 1993; Lawless & Heymann, 1999] and compared to that of the 1-day-old sprouts that were used as reference. Prior to the analysis, the vocabularies of the sensory attributes were developed by the panel in a round-table session, using a standardised procedure [ISO/DIS 13299:1998]. Twelve attributes (descriptors) were selected and thoroughly defined for profiling (Table 1). The attribute intensities were rated on continuous unstructured, graphical scales. The scales were 10 cm in length and verbally anchored at each end, the left side of the scale corresponding to the lowest intensity (value 0) and the right side to the highest intensity (value 10) of the attribute. The same samples of sprouts were also examined rating the overall quality. For each sample, panelists scored the overall quality of samples using the same type of scale as above anchored on both ends: unlinking (0) – extremely linking (10).

TABLE 1. Attributes, their definitions and anchors used in the descriptive analysis.

Attribute	Definition and anchors
Appearence:	
colour	Visual impression of the sprouts colour (from white to light creamy)
Odour:	
grassy	The intensity of the odour typical of mowed grass (none – very intensive)
off-odour	Typical odour of old wet plaster (none – very intensive)
Taste:	
green	The intensity of the taste typical of fresh green pea (none – very intensive)
bitter	The intensity of the bitter taste (none – very intensive)
sweet	The intensity of the sweet taste (none – very intensive)
starch	The intensity of the taste typical of cereals mix (Muesli) (none – very intensive)
astringent	The intensity of dryness, roughness and puckerness in the mouth (none – very intensive)
aftertaste	The sensation of green pea staying after the removal of sample (low – high)
Texture:	
juiciness	Degree of juiciness perceived while chewing the sample 10 times (not juicy – juicy)
fibrousness	Degree of fibrousness perceived while chewing the sample 10 times (not fibrous – fibrous)
flourness	Degree of flourness perceived while chewing the sample 10 times (not floury – floury)
Overall quality:	Overall sensation in the therms like and dislike product (dislike-like)

Preparation of samples and evaluation conditions. Samples were taken out at least 1 h prior to evaluation in order to equilibrate to room temperature, and placed in transparent plastic boxes and presented to the panelists. With the samples the panelist received a cup of spring water (room temperature) for cleaning their palates. The assessments were carried out at the sensory laboratory room fulfilling the requirements of the ISO standards [ISO 8589:1998]. Scores were recorded and collected using a computerised system ANALSENS (IRZiBZ PAN, Olsztyn, Poland). Each sample was tested in two replications.

Chemical analyses

Extraction. Phenolic compounds were extracted from lyophilised sprouts with 75% aqueous ethanol for 30 min at a solid to solvent ratio of 1:7 (w/v) [Amarowicz *et al*., 1995]. The extraction was repeated twice more, supernatants combined and ethanol evaporated under vacuum at 40°C in a rotary evaporator. The remaining water solution was lyophilised and then evaluated by chemical analyses.

Total phenolics. The content of total phenolic compounds in each sample was estimated using Folin and Ciocalteau's reagent [Naczk & Shahidi, 1989]. The results were reported as (+) catechin equivalent per gram of sample.

Condensed tannins. Two spectrophotometric methods were used in this study. In the first one, tannins were evaluated by the protein precipitate assay according to Hagerman & Butler [1978]. The extracts were dissolved in 1 mL of methanol and added to protein dissolved in 2 mL of buffer (0.2 mol/L acetate, pH 5, containing 0.17 mol/L NaCl). The mixtures were vortexed, incubated for 30 min, and centrifuged at 5000 × g for 15 min. The supernatants were removed and the precipitates were rinsed with acetate buffer and centrifuged again. The protein-tannin/phenols complex was dissolved in a detergent system consisting of 1% sodium dodecyl sulfate (SDS) and 5% (v/v) triethanolamine. The tannins/phenolics present in the dissolved complex were measured at 510 nm after reaction with 1 mL of 0.01 mol/L $FeCl_3$. The results were expressed as absorbance values read at 510 nm per gram of extracts (A_{510}/g).

In the second method, tannins were determined by the acid butyl assay. Proanthocyanidins present in sprouts were hydrolyzed according to the method described by Porter *et al.* [1986]. To a 10 mL screw cap tube 6 mL of the acid butanol reagent (950 mL of *n*-butanol with 50 mL concentrated HCl), 1.0 mL aliquot containing 1 mg of the extract, and 0.2 mL of the iron reagent (2% ferric ammonium sulfate in 2 mol/L HCl) were added and vortexed. The tube was capped loosely,

and put into a boiling water bath for 50 min. Then the tube was cooled and, solution transferred to a volumetric flask and adjusted to 25 mL with acid butanol, and absorbance value was read at 550 nm. The results were expressed as absorbance values read at 550 nm per gram of extracts (A_{550}/g).

Statistical analysis. The sensory results and chemical analysis (factors: germination time / sensory attributes; germination time / phenolic contents) were analysed by ANOVA. Statistically significant differences in the results were tested by Fisher's protected least significant difference (LSD) test ($p \leq 0.05$). Principal component analysis (PCA) was applied for general assessment of similarity-dissimilarity of the evaluated samples and describing their sensory attributes. Statistical analyses were gained by Statgraphics Plus 5.1 (Statistical Graphics corp., USA, 2001). The correlation analysis between sensory data and phenolic contents was also performed using Microsoft Excel software.

RESULTS AND DISCUSSION

The sensory evaluation of sprouts was performed to obtain the results concerning the characteristics of the samples and to compare the results with the chemical analysis. Descriptive analysis was carried out based on twelve attributes for appearance, two for odour, six for taste and three for texture (Table 1). The mean sensory ratings for the sprouts are presented in Table 2. ANOVA showed that there were highly significant ($p<0.001$) differences in the intensity of attributes such as: colour ($F=38.9$), grassy odour ($F=59.5$), off odour ($F=12.7$), green ($F=20.0$), flour ($F=122.2$), sweet ($F=7.3$), astringent ($F=5.9$), bitter ($F=48.3$), juiciness ($F=4.5$), fibrousness ($F=35.7$), flourness ($F=109.3$) as well as overall quality ($F=5.0$) caused by the germination time. The average overall quality of scores for the sprouts ranged from 1.4 (7-day-old sprouts) to 6.3 (3-day-old sprouts) on a 0–10 non-structured scale (Figure 1). In order to observe the above differences in the analysed samples more clearly, the sensory profiles of 3-day-old sprouts (with the highest scores of overall quality) and 7-day-old sprouts (with the lowest scores of overall quality) were displayed as the spider diagrams in Figure 2. It can be seen that the sensory profiles of these samples were significantly different in the intensity of all analysed attributes for appearance, odour, taste, and texture.

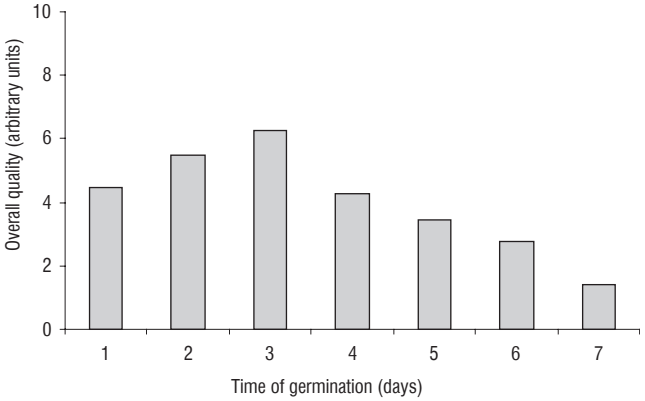


FIGURE 1. Overall quality for sprouts as affected by germination time.

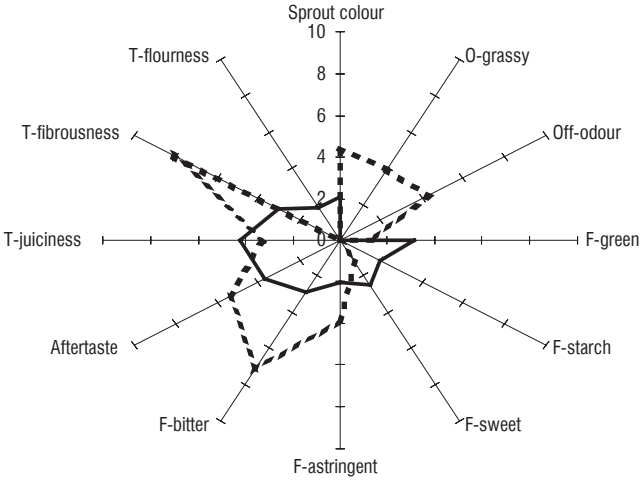


FIGURE 2. Spider diagrams of sensory profiling of sprouts: —3-day-old (the highes scores of overall quality) --- 7-day-old (the lowest scores of overall quality); O=odour, F=taste and T=texture.

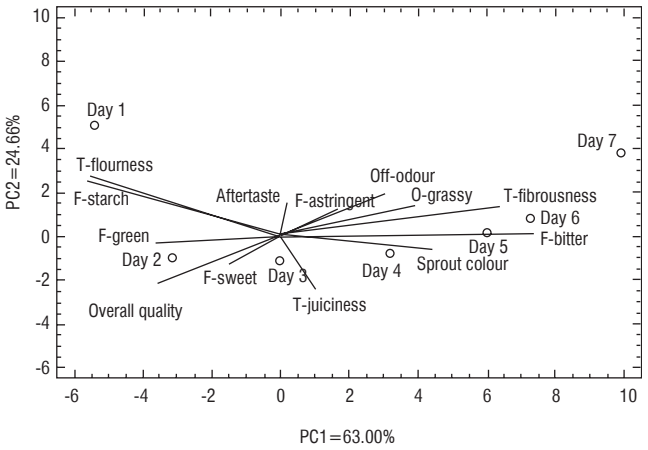
The principal component analysis (PCA) was conducted in order to explain the contribution of all sensory attributes to the overall quality of sprouts. This statistical method allows us to see a graphic representation of the data so that the variations between the samples can be more easily interpreted. The first two principal components were extracted which

TABLE 2. Effect of germination time on sensory profiles of sprouts.*,**

Sensory attributes***	Days of germination						
	1	2	3	4	5	6	7
Sprout colour	0.67^{a}	1.18^{ab}	2.10^{b}	3.91^{c}	4.79^{c}	4.38c	4.39^{c}
O-grassy	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	2.53^{b}	2.40^{b}	3.92^{c}
Off-odour	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	1.49^{b}	4.28^{c}
F-green	3.93^{b}	5.11^{c}	3.15^{b}	1.91^{a}	1.51^{a}	1.43^{a}	1.35^{a}
F-starch	6.43^{e}	2.71^{d}	1.88^{c}	0.98^{b}	0.20^{a}	0.00^{a}	0.00^{a}
F-sweet	1.61^{ab}	3.93^{c}	2.48^{b}	1.87^{ab}	1.30^{a}	1.67^{ab}	1.23^{a}
F-astringent	1.88^{b}	0.73^{a}	2.04^{b}	2.06^{b}	1.38^{ab}	2.33^{b}	3.85^{c}
F-bitter	0.11^{a}	0.18^{a}	2.89^{b}	4.49^{c}	5.25^{cd}	5.91^{d}	7.15^{e}
Aftertaste	5.28^{b}	4.23^{ab}	3.70^{a}	3.89^{a}	4.71^{ab}	4.19^{ab}	5.34^{b}
T-juiciness	1.36^{a}	4.19^{b}	4.20^{b}	4.41^{b}	3.72^{b}	3.82^{b}	3.24^{b}
T-fibrousness	2.16^{ab}	1.84^{a}	2.99^{b}	4.16^{c}	5.95^{d}	6.84^{d}	8.09^{e}
T-flourness	6.66^{d}	2.35^{c}	1.79^{bc}	1.18^{b}	0.28^{a}	0.01^{a}	0.01^{a}

* Mean descriptive analysis ratings of sprouts (0–10 scale). ** Values followed by the same letter in the same row are not significantly different ($p<0.05$). *** O=odour, F=taste, and T=texture.

together explained 87.7% of the variation among the samples (Figure 3). The first factor (PC1) and the second factor (PC2) explained 63% and 24.7% of the variation, respectively. The overall quality of the samples indicated a positive relationship with some sensory attributes, such as green, sweet and juicy according to PCA. It can be seen that these notes were located close to the overall quality and were characteristic for 2 and 3-day-old sprouts. However, the attributes such as bitter, grassy, astringent, fibrous and off odour (characteristic for 5-7-day-old sprouts) were situated on the opposite side of the chart and their vectors were opposite to the vector of the overall quality. It indicates that there was a negative correlation between these parameters. On the basis of the results obtained, it can be stated that prolonging the germination time over four days negatively affected the sensory quality of the sprouts.



O=odour, F=taste and T=texture.

FIGURE 3. PCA plot of sensory results of sprouts.

It is common knowledge that phenolic compounds may contribute to food odour and taste and may affect the acceptability of a number of products. Due to the fact that these compounds may be closely associated with the sensory quality of legume seeds [Troszyńska *et al*., 2006] their amounts in the samples were determined. The contents of total phenolics (expressed in catechin equivalents) in the extracts of seeds and sprouts are given in Table 3. The amount of the total phenolics in the ungerminated seeds of mung bean was 9.76 mg/g extract. The germination had a highly significant effect on the contribution of these compounds. The level of total phenolics increased together with the prolonging of the germination time and the highest amounts of these compounds contained the 7-day-old sprouts (20.53 mg/g extract). In general, the differences between the successive days of germination were statistically significant ($p<0.05$). The content of total phenols increased rapidly to 47.7% of the initial value in the seeds during the first day of germination. In the extreme germination time the increase in these compounds content was 110.4%.

TABLE 3. Content of total phenolics and tannins in seeds and sprouts.*,**

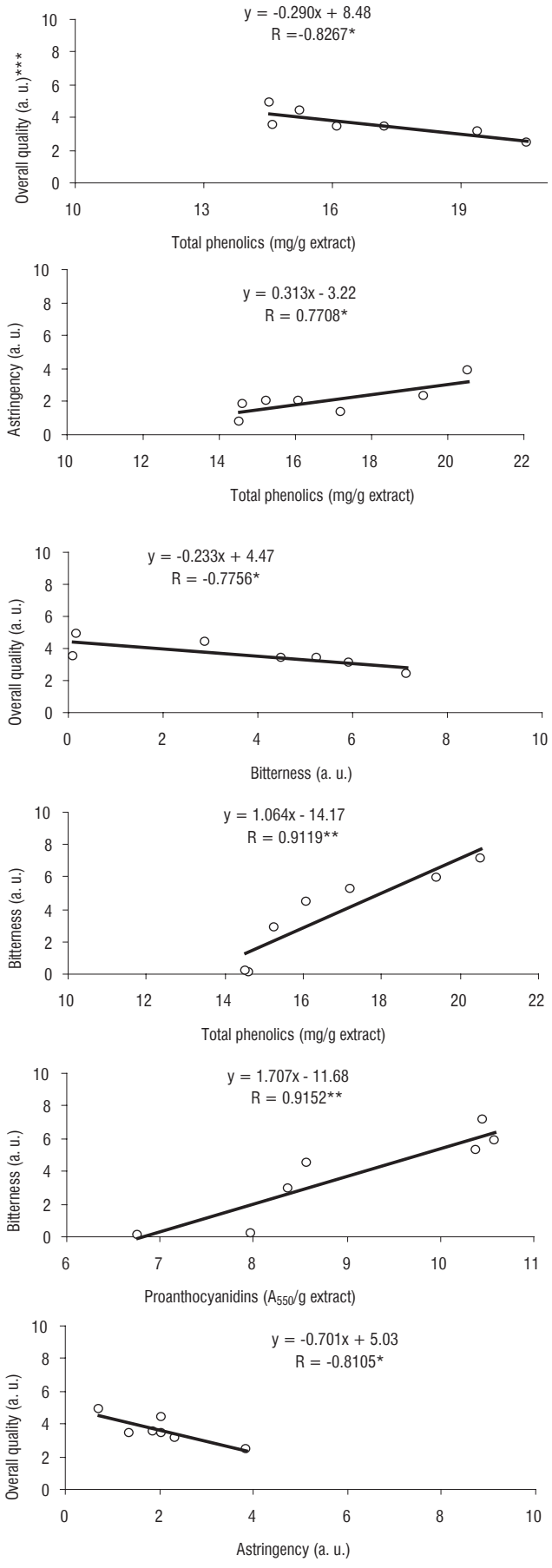
Days of germination	Total phenolics (mg/g extract)****	Tannins	
		Proanthocyanidins assay (A_{550}/g extract)	BSA assay (A_{510}/g extract)
0	9.76 ± 0.09[a]	6.27 ± 0.01[a]	nd***
1	14.61 ± 0.03[b]	6.76 ± 0.01[b]	nd
2	14.54 ± 0.09[b]	7.98 ± 0.01[c]	nd
3	15.26 ± 0.05[c]	8.38 ± 0.01[d]	nd
4	16.10 ± 0.03[d]	8.57 ± 0.02[e]	nd
5	17.21 ± 0.21[e]	10.38 ± 0.02[f]	nd
6	19.40 ± 0.35[f]	10.58 ± 0.04[g]	nd
7	20.53 ± 0.42[g]	10.46 ± 0.02[h]	nd

* Mean chemical analysis ratings of sprouts and their standard deviations, four replicates. ** Values followed by the same letter in the same row are not significantly different ($p<0.05$). *** nd = not detected. **** As catechin equivalents

For the determination of tannins in the samples we used *n*-butanol-HCl assay for proanthocyanidins. Similarly to the total phenolics, the progressively increasing tendency was observed in the accumulation of proanthocyanidins during the seed germination (Table 3). The lowest content of proanthocyanidins was reported for 1-day-old sprouts (6.27 A_{550}/g), while the highest one was for 6-day-old sprouts (10.58 A_{510}/g). The content of these compounds increased to 7.8% of the initial value in the seeds during the first day of germination. In the extreme germination time the increase in proanthocyanidins content was 66.83%.

For the determination of tannins in the samples we also used the method based on the biological property of these compounds, because the astringency has been identified to be associated with the tannin-protein interaction in the mouth and saliva [Hagerman & Butler, 1978; Wróblewski *et al*., 2001; Bennick, 2002]. The results indicated that the extracts of ungerminated seeds and the sprouts did not precipitate the bovine serum albumin (Table 3). It suggests that the astringency of the sprouts might be evoked by low-molecular-weight phenolics which were incapable of protein precipitation. It is well known that the sensory activity of tannins depends not only on their relative concentration, but that it is also strictly connected with the chemical structure. According to literature, 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]. In addition, a small difference in the conformation can produce significant differences in the sensory properties. The comparison of equal weights of catechin and epicatechin, which are chiral isomers, indicated that the epicatechin was characterised by a higher intensity of astringency [Kielhorn & Thorngate, 1999]. It can be emphasised that the astringent sensation can also be elicited by the phenolic acids. According to Peleg & Noble [1995], benzoic acid derivatives in equimolar concentrations elicited astringency but the intensity of this persistent attribute was significantly different. Salicylic and gentisic acids were the highest in astringency. The question whether the phenolic acids could cause the astringency and bitterness of sprouts is a challenge for further research.

The results of the correlation analysis between the phenolic compounds and the sensory attributes as well as the overall quality of the sprouts are presented in Figure 4. The statistically significant correlation was found between the total phenolics and the overall quality ($p=0.05$), the total phenolics and the bitterness ($p=0.01$), the total phenolic and astringency ($p=0.05$), the proanthocyanidins and bitterness ($p=0.01$), the astringency and the overall quality ($p=0.05$),



* significant at α 0.05; ** significant at α 0.01; *** a.u. = arbitrary units

FIGURE 4. Correlation between sensory and instrumental results.

the bitterness and the overall quality (p=0.05). It indicates that the phenolic compounds significantly affected the sensory profiles and the overall quality of the samples analysed. Thus, more detailed studies are requested on the individual phenolic compounds present in the sprouts.

CONCLUSION

The results obtained from this study reveal the importance of germination time for the phenolic contents as well as the sensory quality of mung bean sprouts. The influence of the phenolic compounds was significant for the bitterness and astringency as well as the overall quality of samples. The germination for 72 h would be the most suitable time for the highest overall sensory quality of sprouts. The incorporation of such materials into the industry would enhance the acceptability of mung bean sprouts. Still, their healthy properties concerning polyphenols should be taken into consideration.

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Received May 2006. Revision received and accepted September 2006.

WPŁYW CZASU KIEŁKOWANIA NA JAKOŚĆ SENSORYCZNĄ KIEŁKÓW FASOLI MUNG (*Vigma radiata* L.) I ZAWARTOŚĆ W NICH ZWIĄZKÓW FENOLOWYCH

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W pracy zbadano wpływ czasu kiełkowania na sensoryczną jakość kiełków fasoli Mung w aspekcie zawartości związków fenolowych. Kiełkowanie nasion prowadzono w szafie klimatyzacyjnej przez 7 dni w temperaturze 20°C, bez dostępu światła. Świeże kiełki po każdym dniu kiełkowania oceniono sensorycznie metodą profilową oraz w kategoriach hedonicznych (jakość ogólna kiełków). W próbach zliofilizowanych, metodami spektrofotometrycznymi oznaczono zawartość fenoli ogółem, proantocyjanidyn i tanin precypitujących BSA. Analiza statystyczna wyników wykazała istotne zróżnicowanie profili sensorycznych badanych produktów ($p<0.001$) oraz istotną korelację pomiędzy: (1) zawartością fenoli ogółem a ogólną jakością sensoryczną kiełków ($p=0.05$); (2) zawartością fenoli ogółem a goryczą ($p=0.01$); (3) zawartością fenoli ogółem a cierpkością ($p=0.05$); (4) zawartością proantocyjanidyn a goryczą ($p=0.01$); (5) cierpkością a ogólną jakością sensoryczną kiełków ($p=0.05$); oraz (6) goryczą a ogólną jakością sensoryczną kiełków ($p=0.05$) (tab. 2, rys. 4). Analiza PCA wykazała, że jakość produktów w kategoriach hedonicznych była pozytywnie skorelowana z takimi wyróżnikami jak: słodycz, soczystość i smak „zielonego groszku" (typowymi dla 2 i 3 dniowych kiełków), (rys. 3).