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RELATION BETWEEN THE SENSORY QUALITY OF PEA SPROUTS (*PISUM SATIVUM* L.) AND CONTENTS OF THEIR PHENOLICS AND NITROGENOUS COMPOUNDS

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Key words: pea (*Pisum sativum* L.), sprouts, sensory quality, phenolic compounds, nitrogenous compounds

Seeds of pea were germinated for 7 days in dark, and the sensory quality of sprouts was compared using quantitative descriptive analysis (QDA) and hedonic tests. In QDA a trained panel rated the sprouts for colour, odour, taste and texture. In the affective tests the panelists evaluated the samples for overall quality. Changes in the contents of total phenolics and nitrogenous compounds were also monitored for 7 days of germination. The results proved that the time of germination had a significant effect on the sensory quality of sprouts. Attributes that differentiated the sensory profiles were: colour, green pea odour, off odour, sweet taste, "astringent" taste, bitter taste, "starch" taste, fibrousness and flourness. Principal component analysis (PCA) demonstrated that the first (PC1) and the second (PC2) component together explained 96.70% of the total variability of the sensory quality of the samples. The overall quality of 2-day-old sprouts was better that that of the other samples.

A statistically significant, negative correlation was observed between the overall quality of the sprouts and contents of: total phenolics, total nitrogen and non-protein nitrogen.

INTRODUCTION

In highly-developed countries, consumers are interested in wholesome food products, the so-called health-promoting food or functional food [Lahteenmaki, 2006]. This category of foodstuffs includes, among other things, seeds of legume which for thousands of years have been used in nutrition of the Far East populations [Garcia *et al*., 1998; Mazur *et al*., 1998; Messina, 1999; Duranti, 2006]. Nowadays, a great interest is observed in germinated legume seeds due to their health-promoting properties. They can be consumed as fresh sprouts or processed further for different products [Torres *et al*., 2007; Uwaegbute *et al*., 2000]. According to the current state of knowledge, germinated seeds are characterised by higher contents of nutrients (vitamins, amino acids, minerals) and lower concentrations of non-nutrients (trypsin inhibitors, galactosides, saponins, tannins) as compared to the ungerminated seeds [Frias *et al.,* 2002; Savelkoul *et al.,* 1992; Bau *et al.,* 1997; Ibrahim *et al.,* 2002; Vidal-Valverde *et al.,* 1992]. In addition, sprouts are a source of polyphenols [Fernandez--Orozco *et al*., 2003; López-Amóros *et al*., 2006; Zieliński *et al*., 2007], which due to their antioxidant properties may serve a prophylactic function in a number of civilisation diseases including heart disease and certain cancers [Diplock, 1994]. Health-promoting substances include also some non-protein nitrogen compounds generated during enzymatic hydrolysis of proteins. For example, bioactive peptides playing a role in the prevention of cardiovascular disease, cancer and obesity as well as 3,4-dihydroxyphenylalanine (L-DOPA) [Korhonen *et al*., 1998; De Mejia & De Lumen, 2006; Randhir *et al*., 2002]. L-DOPA is the precursor a neurotransmitter dopamine required by the brain to reduce the symptoms of Parkinson's disease.

Both groups of polyphenols and non-protein nitrogen compounds, apart from valuable biological properties, display negative sensory activity. They can incorporate to food such sensory attributes as bitterness and astringency that are likely to reduce the intake of food products to a significant extent [Lesschaeve & Noble, 2005; Gill *et al.,* 1996; Troszyńska, 2004; Drewnowski & Gomez-Carneros, 2000]. The studies indicated that food-choice process is mainly based on the sensory quality of the foodstuff [Tepper & Trail, 1998; Tuorila & Cardello, 2002; Luckow & Delahunty, 2004]. 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. Changes of both phenolics and nitrogenous compounds that occur during germination of pea seeds and their influence on sensory quality of sprouts are presented in this paper.

MATERIAL AND METHODS

Seeds and germination

Dry seeds of pea were purchased from 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

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process was carried out in a seed germinator (Economic Delux EC00-065, Snijders, The Netherlands) for 7 days in the dark at 20°C. After germination, fresh sprouts were subjected to sensory evaluation, whereas seeds and sprouts to be subjected to chemical analyses were frozen in liquid nitrogen and lyophilised.

Sensory evaluation

Sensory panel

Sensory assessments of the samples were carried out by a panel consisting of 8 members previously selected and trained according to ISO guidelines [ISO 8586-1:1993]. The panelists were familiarised with sensory evaluation of legume sprouts by participation in a previous related study [Troszyńska *et al*., 2006]. Prior to their participation in the experiments, the subjects 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 also included a brain-storming activity to identify descriptive terms for raw and germinated pea seeds.

Sensory methods and evaluation conditions

Quantitative descriptive analysis (QDA) was used to determine differences in the sensory characteristics of the sprouts [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 a standardised procedure [ISO/DIS 13299:1998]. Thirteen attributes related to the appearance (colour), odour (green pea, fermented, off odour), taste (green pea, sweet, astringent, bitter, starch, aftertaste), and texture (juiciness, fibrousness, flourness) of sprouts were selected and thoroughly defined for profiling (Table 1). The panelist rated the sensory attributes on continuous unstructured graphical scales. The scales were 10 cm long and verbally anchored at each end. The results were converted to numerical values (from 0 to 10 units) by a computer.

The overall quality (in hedonic aspect) of sprouts was assessed using the same type of scale as above anchored on both ends: disliked (0) – extremely liked (10). The samples were served in random order to the panelists. Mineral water was offered between the samples. The assessments were carried out at a sensory laboratory room which fulfils the requirements of the ISO standards [ISO 8589:1998]. The results were recorded and collected using a computerised system ANALSENS (IRZiBZ PAN, Olsztyn, Poland). The results are based on means from 8 individual issues of two replications.

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

Attribute	Definition of the attributes
Appearance	
colour	Visual impression of the sprouts colour
Odour	
green-pea	The intensity of the odour typical of fresh green pea
fermented	The intensity of the odour typical of fermented cabbage
off-odour	The intensity of the odour typical of raw potatoes
Taste	
green-pea	The intensity of the taste typical of fresh green pea
sweet	The intensity of the sweet taste
astringent	The intensity of dryness, roughness and puckerness in the mouth
bitter	The intensity of the bitter taste
starch	The intensity of the taste typical of cooked pea seeds
aftertaste	The sensation of green pea staying after the removal of sample
Texture	
juiciness	Degree releasing water from sprouts while chewing the sample
fibrousness	Feeling of fibrousness perceived while chewing the sample
flourness	Feeling during chewing the samples of legume flour
overall quality	Overall sensation in the terms dislike and like product
Anchoring points	
colour: from white to light creamy	
odour/taste: none – very intensive	
texture: low – high (while chewing the sample 10 times)	

Chemical analysis

Total phenolics

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 were combined and ethanol was evaporated under vacuum at 40°C in a rotary evaporator (BUCHI R-200V, Switzerland). The remaining water solution was lyophilised and then evaluated by means of chemical analyses. The content of total phenolics in each sample was estimated using Folin and Ciocalteau's reagent [Naczk & Shahidi, 1989]. The data were calculated on mg (+) catechin equivalent per gram of sample. All chemical experiments carried out in this study were replicated four times.

Total and non-protein nitrogen content

Total nitrogen content of the samples was determined with the Kjeldahl's method [AOAC, 1990]. For non-protein nitrogen (NPN) content, approximately 3 g of the sample were extracted with a 12% (w/v) solution of trichloroacetic acid using a shaker for 90 min at room temperature. The extracted nitrogenous compounds were quantified by means of Kjeldahl analysis [Periago *et al*., 1996]. The content of NPN of each sample was then calculated as the percentage ratio of the dissolved nitrogen in TCA to the total nitrogen content of the samples.

Statistical analysis

The sensory attributes and chemical data were analysed by ANOVA using Fisher's Least Significant Difference test (LSD). Principal component analysis (PCA) was performed in order to describe the variance among all sensory data obtained, whereas the correlation analysis was applied in order to explore the relationship between the sensory data and chemical results. Statistical analyses were performed using StatSoft Inc., v. 7.1software package (Tulsa, OK, USA).

RESULTS AND DISCUSSION

The quantitative descriptive analysis (QDA) was used to find attributes which influenced the sensory quality of sprouts. The mean sensory ratings for the samples are presented in Table 2. The results show that among 13 attributes only the intensity of two of them (odour fermented and juiciness) was not statistically significant. Highly significant differences ($p<0.001$) were observed in the intensity of attributes such as: colour, green pea odour, off odour, sweet taste, "astringent" taste, bitter taste, "starch" taste, fibrousness and flourness. The average overall quality of scores for the sprouts ranged from 0.6 (7-day old sprouts) to 4.3 (2-day old sprouts). In order to observe the above differences in the analysed samples more clearly, the sensory profiles of 2-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 spider diagrams in Figure 1. At the first glance one could see that the profiles of these samples were significantly different in the intensity of all analysed attributes for appearance, odour, taste, and texture. In the profile of 7-day old sprouts with the lowest scores of overall quality there dominated the sensory attributes desirable for the consumers, such as off-odour, "astringent" taste, bitter taste and fibrousness.

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

Sensory attributes	Days of germination					
	2	3	4	5	6	7
Appearance						
colour	2.4[a]	2.5[a]	3.7[ab]	3.9[ab]	5.3[b]	5.2[b]
Odour						
green pea	2.8[d]	2.1[cd]	2.0[bcd]	1.2[ab]	1.2[abc]	0.6[a]
fermented	3.4[a]	3.6[a]	3.5[a]	2.3[a]	3.1[a]	2.6[a]
off-odour	0.0[a]	0.7[a]	0.7[a]	2.6[b]	6.3[c]	8.7[d]
Taste						
green pea	3.5[bc]	3.8[c]	3.5[bc]	2.7[ab]	2.1[a]	2.3[a]
sweet	2.7[c]	2.8[c]	1.7[b]	1.3[ab]	0.7[a]	0.6[a]
astringent	3.0[ab]	2.7[a]	3.6[ab]	4.3[bc]	5.3[cd]	6.2[d]
bitter	3.1[a]	2.9[a]	4.2[a]	4.7[ab]	6.4[b]	6.3[b]
starch	3.7[d]	2.7[c]	2.3[c]	1.9[bc]	1.1[ab]	0.8[a]
aftertaste	5.5[a]	4.7[a]	5.1[a]	5.6[a]	7.5[b]	7.3[b]
Texture						
juiciness	4.0[b]	3.2[ab]	3.1[ab]	2.9[ab]	2.0[a]	2.0[a]
fibrousness	4.2[a]	3.9[a]	4.2[a]	4.8[ab]	5.9[bc]	7.5[c]
flourness	4.2[d]	2.9[c]	2.7[bc]	1.9[ab]	1.1[a]	1.1[a]
Overall quality	4.3[c]	3.7[c]	3.0[bc]	1.8[ab]	0.7[a]	0.6[a]

*- Mean descriptive analysis ratings of sprouts (0-10 scale). **- Values followed by the same letter in the same row are not significantly different ($p<0.05$).

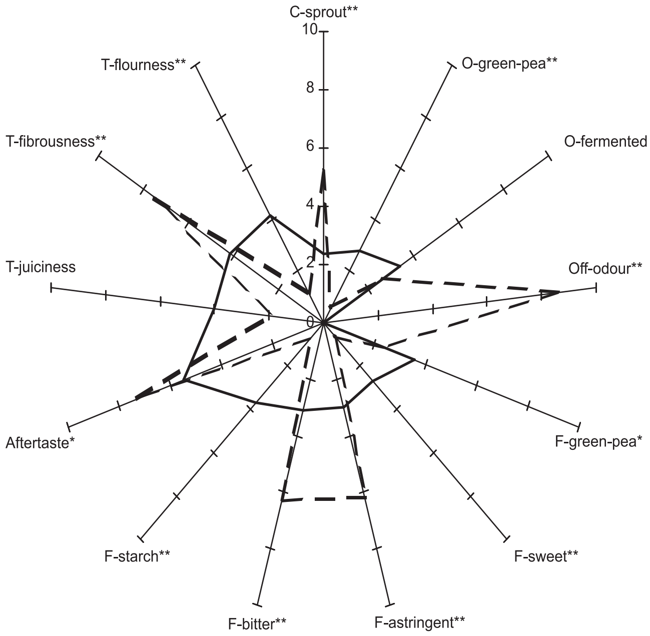

FIGURE 1. Spider diagrams of sensory profiling of sprouts: —— 2-day-old (the highest scores of overall quality); – – – 7-day-old (the lowest scores of overall quality);
* – significant at α 0.01; ** – significant at α 0.001.

The data obtained from the profile analysis (QDA) were subjected to principal component analysis (PCA). PCA was performed on the covariance matrix of the samples with no rotation. Five principal components (PC1 – PC5) were extracted among which the first principal component (PC1) had eigenvalue greater than one and accounted for 93.44% of the total variance. Eigenvalues accounted for by each principal component were 25.286, 0.883, 0.473, 0.289 and 0.131, respectively. The first two principal components (PC1 and PC2), which together explained 96.70% of the variation, were plotted in Figure 2a, b. It can be seen that PCA technique differentiated the samples by days of germination (Figure 2a). Table 3 shows the loadings of the PC1 and PC2. It indicates that the PC1 was differentiated by all attributes except "fermented" odour. It is a common knowledge that the PC1 contains the most important information and includes more important attributes. Therefore these attributes are the most significant for differentiating the samples of sprouts. Negatively related to the PC1 were such attributes as sprouts colour, off-odour, astringent and bitter taste, aftertaste and fibrousness.

It is well known that the phenolics and the non-protein nitrogen compounds may contribute to the food astringency and bitterness, which may affect the acceptability of products. For this reason their contents were determined in the sprouts. Changes in the contents of both total phenols and total nitrogen as well as non-protein nitrogen (NPN) in pea during 7 days of germination are summarised in Table 4. The amount of the total phenols (expressed in catechin equivalents) in the ungerminated seeds of pea was 5.73 mg/g. The content of these compounds in the sprouts ranged from 5.75 m/g extract to 11.00 m/g extract. After 2 days of germination, a progressively increasing tendency was observed in the accumulation of phenolics. In the ex-

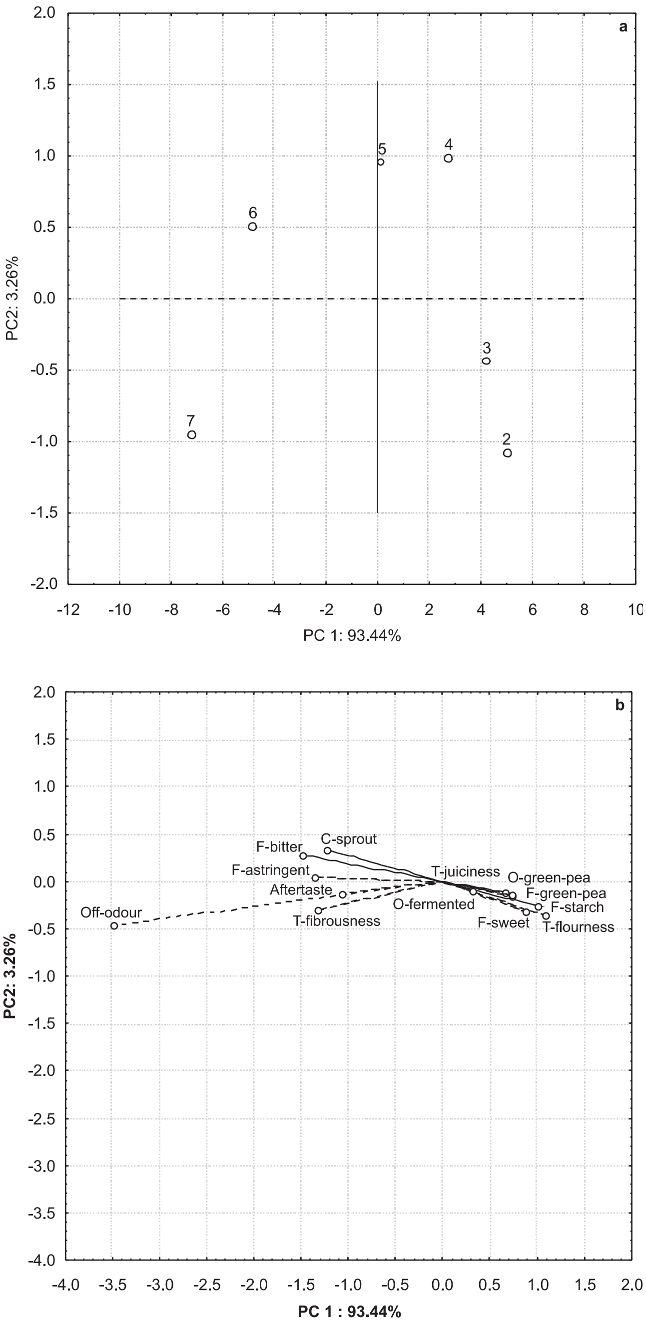

FIGURE 2. Principal component analysis (PCA) scores (a) and loadings (b) of sensory data for pea sprouts.

treme germination time (7th day) the increase in these compounds content was about 92%. Similarly to the phenols, the content of nitrogenous compounds in the ungerminated seeds was lower than in the sprouts. The highest contents of total nitrogen and NPN were reported for 7-day-old sprouts (42.30 g/kg DM and 13.70 g/kg DM, respectively). It should be emphasised that the level of NPN increased together with the extension of the germination time and the differences between the successive days of germination were statistically significant ($p<0.05$). In the extreme germination time the increase in these compounds content was approximately 292%. However the increase in the content of total nitrogen reached about 22%.

TABLE 3. Principal component factor loadings from PCA of sensory scores (QDA) from pea sprouts.

Attributes*	Principal components	
	PC1	PC2
C-sprout	-0.953119	0.259142
O-green-pea	0.919064	-0.190666
O-fermented	0.623818	-0.192604
Off-odour	-0.990318	-0.130390
F-green-pea	0.923146	-0.167276
F-sweet	0.923659	-0.332630
F-astringent	-0.985134	0.032334
F-bitter	-0.969023	0.181032
F-starch	0.944896	-0.245135
Aftertaste	-0.931582	-0.111732
T-juiciness	0.936282	-0.189777
T-fibrousness	-0.963927	-0.224723
T-flourness	0.923378	-0.305340

*- C=colour, O=odour, F=taste, and T=texture

TABLE 4. Changes of the content of polyphenols in extracts of pea sprouts and nitrogenous compounds in pea sprouts during germination of pea*,**.

Germination period (days)	Total phenolics (mg/g extract)	Total nitrogen (g/kg DM)	Non-protein nitrogen	
			(g/kg DM)	(% of total nitrogen)
Ungerminated	5.73 ± 0.12^{a}	34.70 ± 0.10^{a}	3.50 ± 0.00^{a}	10.09
2	5.75 ± 0.17^{a}	39.40 ± 0.20^{c}	5.20 ± 0.10^{c}	13.20
3	7.13 ± 0.01^{b}	39.40 ± 0.20^{c}	6.90 ± 0.00^{d}	17.52
4	8.71 ± 0.17^{c}	39.80 ± 0.80^{c}	7.60 ± 0.10^{e}	19.10
5	9.23 ± 0.04^{d}	40.90 ± 0.70^{d}	9.50 ± 0.10^{f}	23.23
6	10.13 ± 0.36^{e}	41.10 ± 0.30^{d}	10.40 ± 0.10^{g}	25.30
7	11.00 ± 0.12^{f}	42.30 ± 0.10^{e}	13.70 ± 0.20^{h}	32.39

* – Mean chemical analysis ratings of lentil sprouts and their standard deviations, four replicates. ** – Values followed by the same letters in the same column are not significantly different ($p<0.05$).

The obtained chemical data were related to the sensory evaluation and subjected to a statistical analysis. The statistically significant linear correlation ($p<0.01$) was found for overall quality *versus* total phenols; overall quality *versus* total nitrogen; and overall quality *versus* NPN (Figure 3a, b, c). The correlation coefficients between those parameters were $r=-0.960$, $r=-0.938$, and $r=-0.937$, respectively. The above-presented results confirmed previous findings which indicated a negative effect of phenolic compounds on the sensory quality of mung bean sprouts [Troszyńska *et al.*, 2006].

It is well known that sensory activity of phenols as well as nitrogenous compounds depends not only on their relative concentration, but is also strictly connected with their chemical structure. For example, astringency of tannins increases with their degree of polymerization [Peleg *et al.*, 1999; Lesschaeve & Noble, 2005]. In addition, small difference in conformation can produce significant differences in sensory

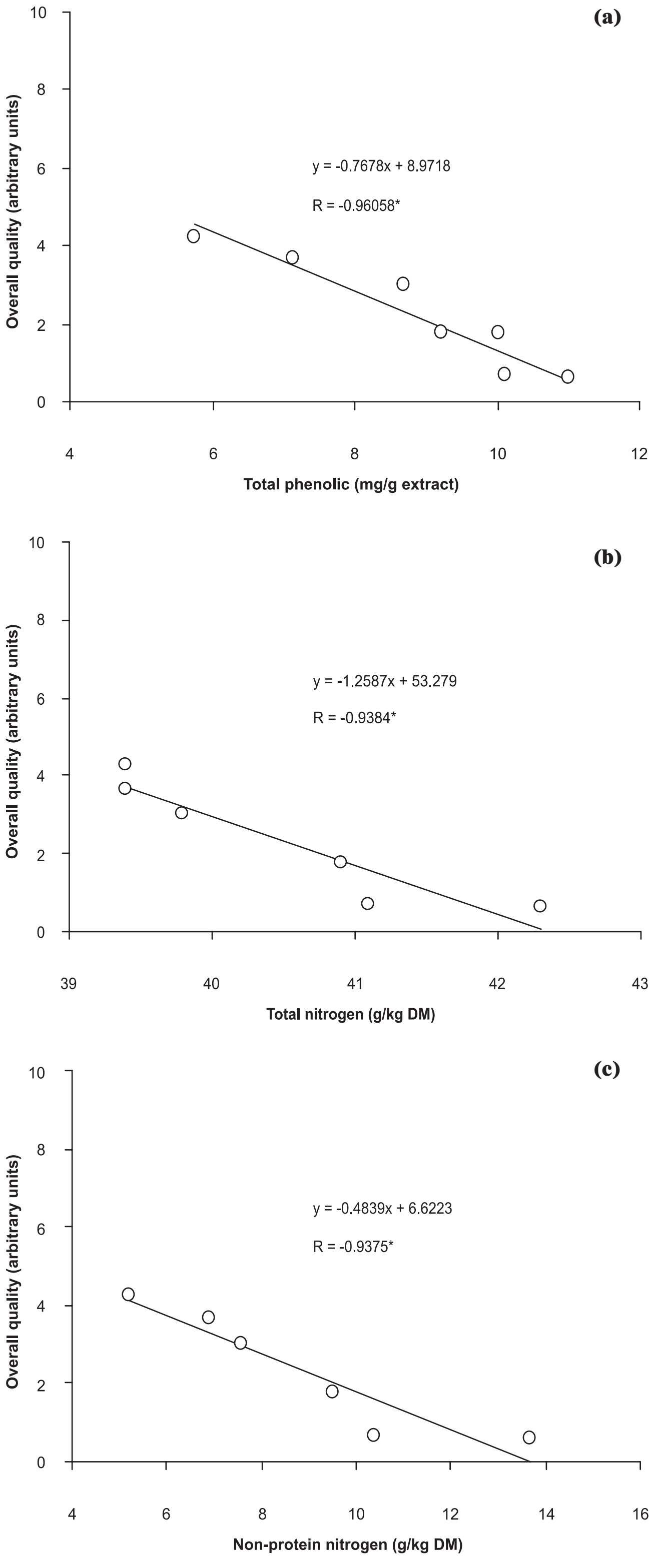

FIGURE 3. Correlation between sensory and chemical results for total phenolics (a), total nitrogen (b), and non-protein nitrogen (c); * significant at α 0.01.

properties. A comparison of equal weights of catechin and epicatechin, which are chiral isomers, indicated that epicatechin was characterised by higher intensity of astringency [Kielhorn & Thorngate, 1999]. Moreover, like many of the astringent compounds, these two monomers were also bitter. According to literature, bitter taste can also be elicited by phenolic acids [Peleg & Noble, 1995] and peptides containing hydrophobic amino acids [Korhonen *et al*., 1998], which can be released during the germination process. Changes in the composition of both the individual phenolics and some of the non-protein nitrogen compounds were observed during the germination of different species of legume seeds [Bartolome *et al*., 1997; López-Amóres, 2006; Rozan *et al*., 2001; Kuo *et al*., 2004; Urbano *et al*., 2005]. Unfortunately, so far very little is known about their sensory activity. In order to use the sprouts as functional food more detailed studies on the sensory properties of these wholesome compounds are requested. It is caused by the fact that the food-choice process is mainly based on sensory quality.

CONCLUSIONS

The results obtained from this study reveal the importance of the germination time for the content of phenolics and nitrogenous compounds as well as the sensory quality of pea sprouts. The germination for 2 days (48 h) was the most suitable for providing the highest overall quality of sprouts while the phenolics and nitrogenous compounds were found at the lowest concentration. The elongation of the germination time caused an increase in the content of these compounds and deterioration of the sensory quality of products. It indicates that the phenolics and nitrogenous compounds can be a limiting factor in the utilisation of fresh sprouts as functional food.

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