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ScienceDirect
Food Chemistry 103 (2007) 875–884
Food Chemistry
www.elsevier.com/locate/foodchem

Correlations of ingredients with sensory attributes in green beans and peas under different storage conditions

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Received 10 October 2005; received in revised form 18 April 2006; accepted 21 September 2006

Abstract

The total ascorbic acid contents and the antioxidant capacity in green beans and in peas were measured during deep-frozen storage and compared to storage at 4 °C and 20 °C. In green beans only, the flavonols quercetin and kaempferol were measured. The results were correlated with sensory attributes which were evaluated at the same storage stages. The total ascorbic acid content is a good parameter for the storage time of both vegetables and showed, for peas, a positive correlation with the attribute ''sweet taste'' and a negative correlation with the attribute ''musty'' odour. The total ascorbic acid content of green beans was positively correlated with a ''squeaky'' texture and negatively with a ''musty'' taste. The flavonol content and the antioxidant capacity were more stable during the storage process and showed less correlation with sensory attributes.

Keywords: Green beans; Peas; Storage; Total ascorbic acid; Flavonols; Antioxidant capacity; Sensory attributes; Correlation

1. Introduction

Numerous parameters (e.g. vitamins, sugars, titratable acids, the fatty acid profile, chlorophylls and the amino acid content) in vegetables are known to be affected by various storage conditions. The quality of vegetables is related both to analytical parameters with nutritive or chemo-protective properties and to sensory attributes, which represent acceptance by the consumer.

It is assumed that some chemical parameters may change in the same manner as important sensory attributes, because some chemical parameters, such as antioxidants and reductones, may play a protective role for fruits and vegetables. By protecting the plant material from physiological decay, these ingredients may also be important for the preservation of main sensory attributes.

The most important of these chemical parameters in green beans and peas are vitamin C, the flavonols quercetin and kaempferol, β-carotene and some organic acids (e.g. *p*-coumaric acid or ferulic acid).

A considerable body of work has already been published on the determination of vitamin C during storage in green beans and peas (Aparicio-Cuesta, Rivas-Gonzalo, Santos-Buelga, & Garcia-Moreno, 1989; Bloeck, Iseli-Winter, Perren, Escher, & Solms, 1986; Charoenrein & Reid, 1989; Sanchez-Mata, Camara, & Diez-Marques, 2002; Szoke & Aldor, 1964) and it is already well-known that the vitamin C content of peas and green beans can easily be preserved by deep-frozen storage. But many publications on this issue are old and report results, which were accomplished with antiquated photometric methods instead of the modern HPLC-methods. Modern methods of vitamin C determination distinguish between different ascorbic acid isomers (D- and L-forms as well as dehydro-forms or isoascorbic-acid-forms). The correct measurement of the total vitamin C activity includes (in addition to the ascorbic acid

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doi:10.1016/j.foodchem.2006.09.039

content) the content of dehydroascorbic acid, which is regarded to have the same vitamin C activity.

Other very important substances for the human diet, which have chemoprotective properties are the polyphenols. This class of compounds can be divided into a large number of subclasses. One of them, the flavonols, features a very high antioxidative potential (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995) and they are largely supplied by the consumption of green beans (Price, Colquhoun, Barnes, & Rhodes, 1998). The flavonols in green beans, which are based on two aglycones, quercetin and kaempferol, are present only in the form of a multiplicity of glycosides and as phenolic acid conjugates. As the degree of glycosylation may be important for the stability and ability of these compounds to cross the intestinal wall, several efforts have been made so far, not only to determine the kind and the amount of the aglycone (Hertog, Hollman, & Kromhout, 1993), but also to quantify the different conjugates (Hempel & Boehm, 1996; Price et al., 1998).

The antioxidative capacity can be measured by several analytical assays which mostly consist of a photometric reaction that affects a high number of substances but mainly compounds with hydroxyl groups such as polyphenols and other antioxidative active chemicals (Huang, Ou, & Prior, 2005).

The most common modern methods are electron-transfer assays (e.g. ferric reducing/antioxidative power test – FRAP) and hydrogen atom transfer reactions (e.g. oxygen radical absorbance capacity – ORAC). The FRAP-assay has the advantage, that it is easy and very rapid, which makes it highly suitable for complex samples such as fresh vegetables (Benzie, Chung, & Strain, 1999) with many possible redox-reactions. Furthermore, it is sensitive and there are fewer side-reactions, compared for example to the method of Folin–Ciocalteu, which involves reactions with proteins or some basic chemicals (Schild & Enders, 1936).

It is of fundamental importance to provide a large amount of data during the whole storage period in order to evaluate correlations between sensory attributes and chemical parameters. In this work, the main sensory parameters, the content of vitamin C, flavonols and the intensity of antioxidative capacity were measured at each stage of post-harvest storage (starting from identical raw material) to see if there was a correlation (positive or negative) for the whole storage period.

2. Materials and methods

2.1. Harvesting, processing and sample-storage

Green beans of the variety Paulista were provided by Elbtal Tiefkühlkost Vertriebs GmbH and harvested in early August 2003 in Lommatzsch, Germany. Peas of the variety Tristar were harvested in late June 2003, also in Lommatzsch, Germany and directly subjected to the industrial washing process. The so-prepared peas and green beans of one batch were then either transported on the day of harvest in cool-boxes to the Faculty of Life Sciences, Hamburg University of Applied Sciences for the storage at 4 °C and 20 °C, respectively, or blanched under defined conditions (at 95 °C for 50 s (peas) and 70 s (green beans)) and then submitted to the deep-frozen storage at −18 °C or −25 °C (by shock-freezing) in Lommatzsch under the same conditions as in common industrial production. After the defined storage period (0, 4, 8 and 12 months) the deep-frozen material was then also transported from Lommatzsch to Hamburg, always within one day under refrigerated cooling.

If not immediately analysed, the material was kept refrigerated at −45 °C (at which temperature no changes should occur for at least 12 months, according to Bognár and Wolf (2002).

After 4, 7 and 14 days at 4 °C, or 4 and 7 days at 20 °C, respectively, the peas and the green beans were analysed, either raw or cooked, after a defined cooking stage, to simulate domestic processing. The deep-frozen stored peas and green beans were also analysed raw or cooked. One part was processed immediately after deep-freezing (stage: 0 m), and the other parts of the pool after 4, 8 and 12 months, respectively.

The defined cooking process was accomplished as follows: peas were cooked in portions of 500 g with 100 g water and 2 g salt; this took 5 min for the peas stored at 4 °C or 20 °C, and for the deep-frozen ones, only 2 min, because of the additional blanching step. The green beans were cooked in portions of 500 g with 300 g water and 2 g salt; for the fresh green beans, this took 8 min, and for the deep-frozen ones 10 min.

For chemical analysis (vitamin C, flavonols and antioxidative capacity), a representative amount of 100 g of the unprocessed and the cooked material, respectively, was freeze-dried to assure the comparability of long-time stored (and thus dehydrated) states with more aqueous states (e.g. cooked stages). The freeze-dried material was thoroughly pulverised in a mortar and analysed for the amounts of ascorbic and dehydroascorbic acid, the flavonols quercetin and kaempferol and the antioxidative capacity. For the comparison of the flavonolglycoside profile and for the evaluation of the dependency on the variety, two retail products were obtained from a local supermarket and analysed additionally.

2.2. Sensory evaluation

Sensory evaluation was only done after cooking; each stage of the stored vegetables was separated into one portion to be chemically analysed and another portion which was sensory-evaluated by a trained panel with 12 judges. With the quantitative descriptive analysis, the character and intensity of defined attributes were measured in three replications. The panel selected 27 descriptive attributes for cooked peas and beans, concerning appearance, odour, taste and texture. The intensities of the attributes were measured on a 10-point-scale; for orientation the scale was divided in the middle. During their training, the panel-

lists learned to give homogeneous ratings. They also worked out detailed definitions for each attribute.

The results were subjected to a statistical analysis of variance (ANOVA) with the least-significant difference (LSD) test.

2.3. Chemical analysis

2.3.1. Reagents and chemicals

All reagents were from Sigma Aldrich (St. Louis, MO, USA), except for acetonitrile which was from Acros (NJ, USA). Water was always bidistilled and demineralised. For the analysis of the flavonolglycosides, quercetin-3-*O*-rutinoside (rutin) was from Roth (Karlsruhe, Germany), kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, quercitrin (quercetin-3-*O*-rhamnoside) and quercetin-3-*O*-glucoside from Extrasynthèse (Genay, France). 2,4,6-Tri-2-pyridinyl-1,3,5-triazine for the measurement of the antioxidative capacity was purchased from Fluka.

2.3.2. Ascorbic acid and dehydroascorbic acid contents

The extraction of a sample of 200 mg ground freeze-dried plant material was carried out with 10 ml *m*-phosphoric acid–perchloric acid mixture (2,5 g *m*-phosphoric acid and 3% perchloric acid in 100 ml water) to stabilise the ascorbic acid and simultaneously precipitate the proteins. The suspension was ultrasonicated in 10 ml tubes for 15 min and then centrifuged at 3600*g*.

To record the sum of dehydroascorbic acid and ascorbic acid, a pre-reduction step was performed by a 250-fold excess (compared to the amount of dehydroascorbic acid in the sample) of dithiothreitol in 1 M di-potassium hydrogen phosphate buffer (adjusted to pH 7.0 with phosphoric acid). There were no significant differences in the results by the reduction step as long as a 50-fold excess was exceeded. After 30 min and centrifugation the supernatant was diluted 3:10 with *m*-phosphoric acid–perchloric acid mixture.

The total ascorbic acid content was measured by determining the content of ascorbic acid and the content of dehydroascorbic acid together after a pre-reduction step. Ascorbic acid content and total ascorbic acid content were both determined by HPLC. The supernatants were microfiltered and aliquots of 20 μl injected into an HPLC system consisting of a thermostatted RP-18 column (Lichrospher® 100, 5 μm, 4.6 × 250 mm), an electrochemical detector (EC 2000 TSP, glassy-carbon working potential: 0.85 V Ag/AgCl range 50 nA, Filter 0.1 s, offset 0%, cell-distance: 120 μm, $T = 23$ °C), an UV detector (243 nm) and an isocratic pump with a flow rate of 1 ml/min. The eluant was a 45 mM sodium dihydrogen phosphate buffer adjusted to pH 2.0 with *o*-phosphoric acid.

All samples were measured in triplicate.

2.3.3. Flavonols

The native flavonolglycosides were extracted according to a method of DuPont, Mondin, Williamson, & Price (2000). This involved an extraction step of the sample of 150 mg freeze-dried vegetable-powder with 10 ml 70% methanol (v/v). The suspension was agitated for 15 min and ultrasonicated for 15 min and centrifuged at 3600*g*. The supernatant was transferred to a 10 ml tube and the methanol was evaporated under nitrogen. The remaining 3 ml solution were filled up to 10 ml and 5 ml were applied on a polyamide 6 (Chromabond®, Macherey & Nagel, Düren, Germany, 500 mg/3 ml) solid phase extraction cartridge (which was equilibrated with 3 ml methanol and 6 ml water). The cartridge was then washed with 6 ml water. The neutral flavonolglycosides were eluted with methanol and the acidic flavonolglycosides were eluted with methanol/ammonia (25%) (99.5/0.5 v/v). The fractions were evaporated to dryness and readjusted to 1.5 ml with dimethylformamide/water (33/66 v/v).

Identification was achieved by retention time, co-chromatography, spectral data and mass spectrometry data. Chromatographic separations were carried out on a HPLC-DAD-MS system (Hewlett-Packard 1100 Series, Böblingen, Germany), which consisted of a thermostatted RP-18 column ($T = 35$ °C, column: Prodigy® (Phenomenex, Aschaffenburg, Deutschland) 100 A, 250 × 3 mm, 5 μm) with a flow rate of 0.58 ml/min with the following solvent system: eluant A: 2% tetrahydrofuran, 0.1% trifluoro acetic acid in water (v/v/v); eluant B: acetonitrile; 5% B in A holding for 2 min, within 8 min to 16% B, within 3 min to 17% B, within 7 min to 18% B, within 10 min to 21% B and within 5 min to 30% B, then, for equilibration within 1 min, back to 5% B and holding for 4 min. The monitoring wavelengths were 345 and 260 nm, respectively. Mass spectrometry was carried out with an electrospray source in the positive ionisation mode. Typical tuning parameters were as follows: Nebuliser pressure 46 psi, drying gas flow 12.5 ml/min, drying gas temperature 290 °C, capillary voltage 3800, fragmentor voltage 45, threshold 120, gain 3 within a mass range of 200–1000 for the scan mode. As there are many different flavononoid–aglycones for vegetables reported in the literature, the $M + H^+$-masses of the main flavonoids in vegetables apigenin, kaempferol, luteolin, quercetin and myricetin, combined with the $M + H^+$-masses of the main glycosides (xylosylrutinosides, glucosylrutinosides, rutinosides, diglucosides, monoglucosides and monorhamnosides) were adjusted in the single ion mode.

Quantification was carried out with internal standards (kaempferol 3-*O*-glucopyranoside for the kaempferolglycosides, and quercitrin for the quercetinglycosides, both of which were not detected in green beans). The kaempferolglycosides and the quercetinglycosides were summed and calculated as their aglycones.

All samples were measured in triplicate.

2.3.4. Antioxidative capacity

The antioxidative capacity was measured by the FRAP Test. According to Bub et al. (2000), the quantification was performed with plate-readers. For the extraction, which was a variation of the method of Nilsson, Stegmark, and

Åkesson (2004), 0.2 g of the freeze-dried vegetables was weighed into a Pyrex glass and 10 ml of water were added. The suspension was shaken for 20 min in an automatic shaker at 400 min^{-1} and centrifuged for 15 min at 3600*g*. The supernatant was kept at −45 °C before analysis.

The FRAP-reagent was prepared as follows: 0.3123 g of 2,4,6-tri-2-pyridinyl-1,3,5-triazine were dissolved in 4 ml of 1 M hydrochloric acid and made up to 100 ml with water (TPTZ-solution); 0.5406 g ferric chloride (-six-hydrate) was dissolved in 100 ml water (ferric chloride solution); 10 ml TPTZ-solution and 10 ml ferric chloride solution were added to 100 ml acetate buffer (3.1 g sodium acetate-trihydrate in 16 ml glacial acetic acid and 984 ml water).

The reaction was performed in 96-well microtitre plates at ambient temperature. About 5–50 µl of the vegetable extract were added to 150 µl FRAP-solution and the final volume was made up to 200 µl with water. After 4 minutes, the solutions were measured spectro-photometrically at 590 nm against an ascorbic acid dilution series (0–50 µl ascorbic acid (0.2 mM) for the spectro-photometric reaction).

Each sample was measured in duplicate.

For statistical comparison, the chemical results were also subjected to a test for homogeneity of variances. The test of Scheffé was applied and a level of significance of 0.05 was chosen.

2.4. Correlations with main sensory parameter

The data of the sensory evaluation and the total ascorbic acid-content were correlated by the method of partial least squares regression (PLSR). For this analysis, the software "The Unscrambler®" (Camo, Norway) was used. The PLSR is a combination of the principal component analysis and multiple correlation. The PLSR supplies the so-called "loadings" for the variables (sensory attributes and chemical parameters) and the so-called "scores" for the products (Busch-Stockfisch, 2002). The PLSR-results also yield a percentage number, which is a quality degree as to how far this model of correlation is described by the chosen two dimensions (*x* and *y* axes). The data were standardised and non-significant variables were discarded.

3. Results and discussion

3.1. Measurements of the peas

3.1.1. Ascorbic acid and dehydroascorbic acid content

In Fig. 1, the results of the determination of the ascorbic acid (lower bar) and the dehydroascorbic acid content (upper bar) in cooked peas are shown. The amount of total ascorbic acid rapidly decreased within 14 days at 4 °C; 39% of the original amount (88 mg/100 g dry matter) was lost. After 7 days at 20 °C, the amount of total ascorbic acid even decreased by 72% of the original vitamin C content. Bognár (1987) found losses of up to 12% and 43% after 3 days of storage at 4 °C and 20 °C, respectively, but it is not reported which method (determination of the total ascorbic acid content or only the ascorbic acid content) was used or whether the content was referred to the dry matter of the peas. The deep-frozen peas did not change significantly during the storage period over 12 months. Bognár (1987) reported that peas stored at −18 °C showed losses between 12% and 32% of their original total ascorbic acid content, but no standard deviations are presented in his study. Derse & Teply (1958) analysed the total ascorbic acid content, but the vegetables stored at −18 °C for 2, 5 and 11 months, were additionally subjected to an exposure at −6.6 °C (20 °F) for 1 month. They reported losses of up to 64% (without standard deviations), but no details about any kind of blanching step were reported. Furthermore, in both studies, only raw peas were measured. The advantage of measuring the cooked ones as well, is that it is possible then to establish the more realistic values of intake by the consumer. Puupponen-Pimiä et al. (2003) found 5–30% of total ascorbic acid content loss during deep-frozen storage, dependent on the cultivar. Comparable to our results, Favell (1998) found that, after 12 months at −20 °C, the decrease during deep-frozen storage was less than 10% of the original stage (after blanching). The results in the scientific literature show that methodological variances have a great impact on the obtained results.

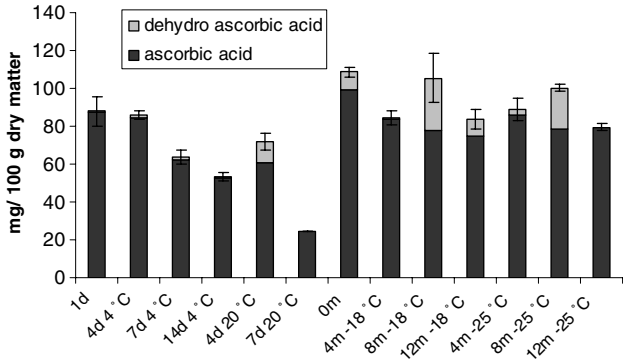

Fig. 1. Dehydroascorbic acid and ascorbic acid contents of cooked peas.

Not displayed in Fig. 1 are the contents of ascorbic acid and dehydroascorbic acid of the raw peas, because the trend for the storage losses of total ascorbic acid content for the raw peas during the storage period was the same as for the cooked ones; the losses by the cooking process were between 4.5 and 23.7 mg/100 g dry matter for the peas stored at 4 °C and at 20 °C, and between 7.2 and 37.6 mg/100 g dry matter for the deep-frozen peas (that means 3–32% loss). Hermann (1986) reported cooking losses of 26% and 32% compared to the raw product for not-stored peas and for deep-frozen peas, respectively. Bognár (1987) reported losses of about 6 mg/100 g (22% of the original content) in the blanching step. In our study, there are no observable losses attributed to the blanching process (compare 1 d and 0 m in Fig. 1). That might be explainable by the more modern industrial blanching methods and with

possible minor transport losses during the transport of the peas (for the stages 1–7d 20 °C) on the first day. Furthermore, the homogeneity of the initial total ascorbic acid content of deep-frozen vegetables (here: 0 m) can also vary to a large extent (Giannakourou & Taoukis, 2003). Comparing different storage studies, there are large variations in the kinetic behaviour of the total ascorbic acid content, highly dependent on the different cultivars analysed (Giannakourou & Taoukis, 2003).

3.1.2. Flavonols

Only small amounts (<1.05 mg/100 g dry matter) of substances with a typical UV-absorption in the DAD were detected in both fractions (neutral SPE-eluate and acidic SPE-eluate) of the pea extract, but an exact identification of flavonolglycosides was not possible and a storage trend was not measurable for this parameter. These findings are in common with findings of other authors (e.g. Hertog, Hollman, & Kahn, 1992).

3.1.3. Antioxidative capacity

The content of the antioxidative capacity is expressed in ascorbic acid equivalents (AAE) as it was determined by comparing the absorptions with those of an ascorbic acid standard curve (Fig. 2). The peas stored at 4 °C and at 20 °C showed significant losses of antioxidative capacity after 7 days and 14 days, respectively, but some standard deviations are relatively high. The losses of the deep-frozen peas seem to be smaller, comparing the different storage states with the original one (0 m). But the blanching step itself had major effects on the antioxidative capacity, which could be seen comparing the state 0 m with the state 1 d (first day for the storage at 4 °C and 20 °C, respectively). The deep-frozen peas seem to be stable for the whole storage period in terms of the antioxidative capacity.

3.1.4. Correlations with sensory parameters

The significantly changing sensory attributes of cooked peas after correlation by PLSR were "sweet", "grassy", "aromatic" and "musty" taste. The several storage stages (the products) can be characterised in a two-dimensional plot by the multi-variate calculations as seen in Fig. 3a. In Fig. 3b, the correlation of the total ascorbic acid content with the main sensory attributes is shown. In general, the chemical parameters, the attributes or the products which are located in the same area, are correlated and parameters, attributes or products which stand opposite to each other (e.g. total ascorbic acid content and "musty" taste are very different and do not correlate).

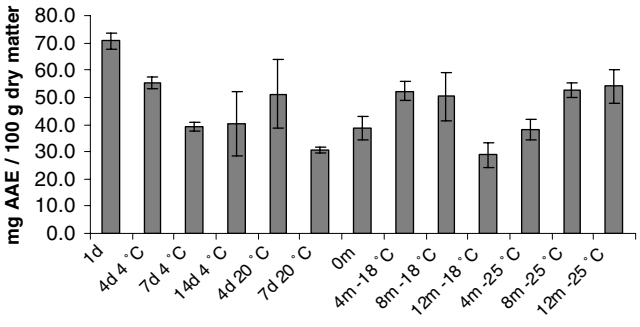

Fig. 2. Antioxidant capacity (measured by the FRAP-assay) of the aqueous pea-extracts.

As can be seen in Fig. 3a, all the peas stored at 20 °C and the peas after 14 days of storage at 4 °C, are different from the other stages. The deep-frozen peas are more similar to the fresh stored peas and have higher total ascorbic acid contents. This can be seen by projecting the position of the stages in Fig. 3a to the position of the parameter total ascorbic acid content in Fig. 3b (with respect to the different scales). In Fig. 3b, the variance of the total ascorbic acid content is given by the *y*-explanations, 69% and 5%, for the first and second dimension; this means that 74% of the variance of the total ascorbic acid content for peas is explained by the other variables.

In Fig. 3b, it can be additionally seen that the total ascorbic acid content above all correlates positively with the "sweet" taste and to a lesser extent with the attributes "grassy" and "aromatic" taste. The attribute "musty" taste is negatively correlated with the total ascorbic acid content, as it is located in the opposite quadrant.

The total coefficient of correlation, which was also calculated by the software programme "Unscrambler®", was $R = 0.83$.

The correlation of the amounts of the antioxidative capacity with the main sensory attributes is shown in Fig. 3c and d. The two stages with the smallest similarity compared with the original state (1 d) are the peas stored at 20 °C. The deep-frozen products did not show this unambiguous trend (comparing with 0 m). It can be seen that there is only an explained variance of $(24 + 16\%) = 40\%$ for the *y*-dimension.

The factor of correlation calculated by the *PLSR*-model is $R = 0.49$. This is low, which means that this model is not suitable for the description of significant coherences between sensory data and the antioxidative capacity. All the correlations displayed in Fig. 3d, despite the quantity of the specified attributes of the PLSR, are not significant. All significantly changing sensory attributes are displayed by Fig. 3d, but an interpretation of the results would be dubious.

3.2. Green beans

3.2.1. Total ascorbic acid content

In green beans, the total ascorbic acid content significantly decreased over the storage periods at 4 °C and 20 °C (Fig. 4). Already after 4 days at 20 °C, the amount was reduced to only around 20% of that on the first day. Bognár (1987) reported losses of 34% and 50% after 3 days of storage at 4 °C and 20 °C, respectively, but did not report which method (determination of the total ascorbic acid content or only the ascorbic acid content) was used or whether the content was referred to the dry matter of

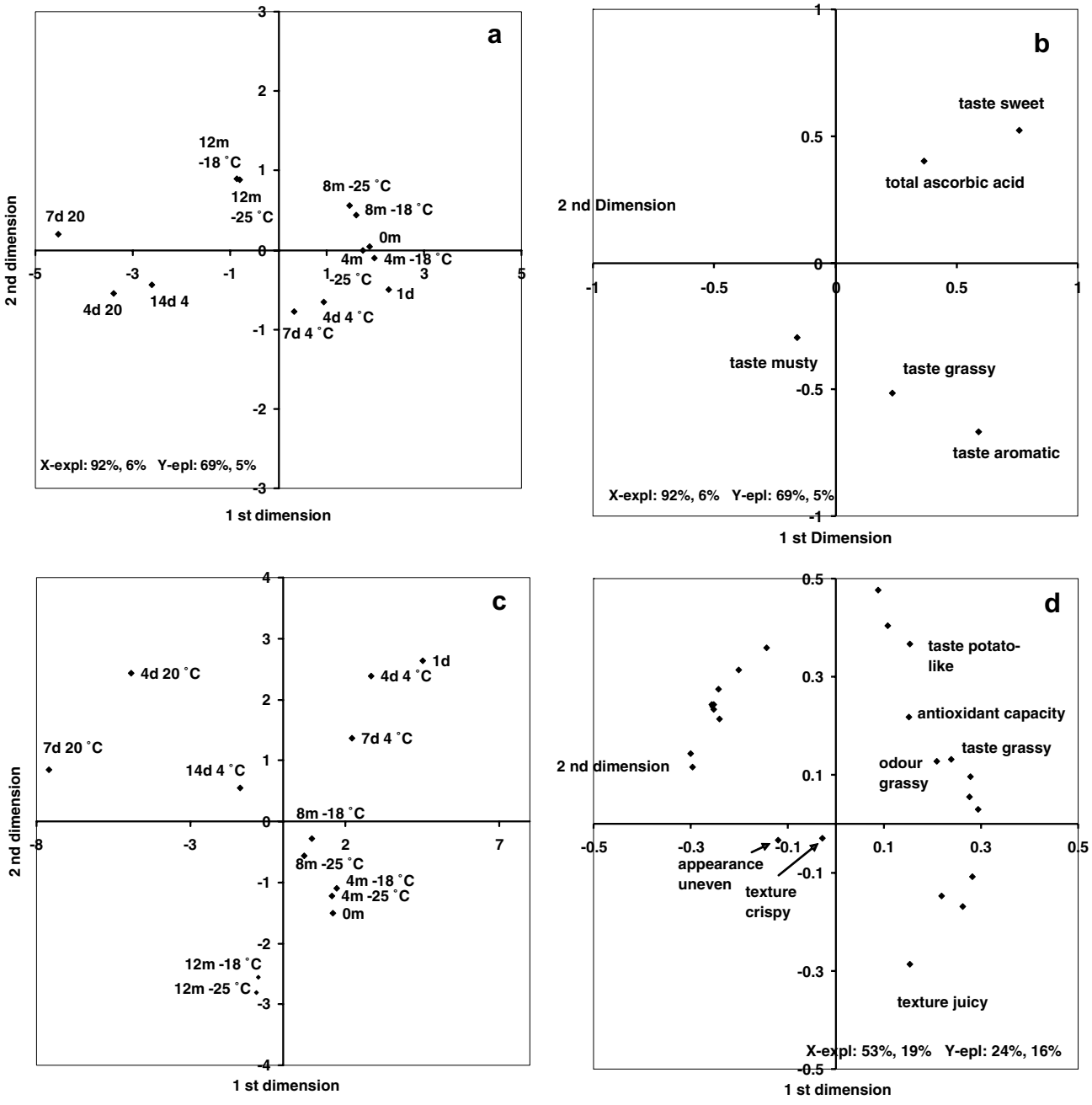

Fig. 3. *PLSR*-graphics of the correlation of peas, a: products after correlation of sensory attributes with the total ascorbic acid content, b: attributes after correlation of sensory attributes with the total ascorbic acid content, c: products after correlation of sensory attributes with the antioxidant capacity, and d: attributes after correlation of sensory attributes with the antioxidant capacity.

the green beans. Favell (1998) found that approximately 40% of the original amount of the total ascorbic acid content was lost after 3 days of storage at 4 °C. These minor deviations might be due to the differences in the experimental procedures. In addition, the different variety or other factors influencing the storage stability (relative humidity, light, oxygen status) or different pre-harvest conditions (e.g. weather, soil, fertilisation, and industrial washing) may play an important role in affecting the total ascorbic acid content of green beans.

Comparing the deep-frozen stages, it was reported that even after 12 months of storage at −18 °C, 75% of the total ascorbic acid content was left. Bognár (1987) reported losses of 22–55% after 9 months of storage at −18 °C. Favell (1998) found losses of about 20% (compared with the original state after blanching).

After a storage period of 12 months at −25 °C, around 95% of the initial total ascorbic acid content was left. Before cooking, again around 20 mg/100 g dry matter more were measured in green beans. The trend is the same

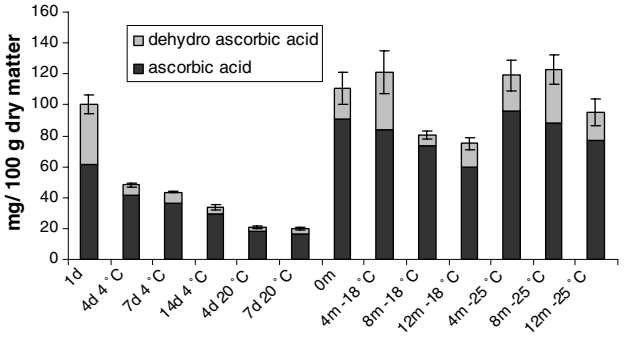

Fig. 4. Dehydroascorbic acid and ascorbic acid contents of cooked green beans.

during the whole storage time (with even smaller standard deviations), but only the results of the processed green beans are presented because only the cooked ones were correlated with the sensory attributes.

3.2.2. Flavonols

To prevent chemical degradation and to evaluate possible changes in the flavonolglycoside profile during storage, all important naturally occurring flavonolglycosides were separately identified. The flavonolglycosides identified in green beans were: quercetin 3-*O*-glucuronide and kaempferol 3-*O*-glucuronide in the acidic eluate of the polyamide-SPE. Fig. 2a shows the substances quercetin 3-*O*-xylosylrutinoside (peak 3), kaempferol 3-*O*-xylosylrutinoside (peak 6), quercetin 3-*O*-rutinoside (=rutin) (peak 7) and kaempferol 3-*O*-rutinoside (peak 9) in the neutral eluate, which have also been identified in green beans previously by Hempel and Boehm (1996) and Price et al. (1998). Besides, the existence of two other quercetin isomers (peaks 4 and 5) and two other kaempferol isomers (8 and 10) can be seen in the chromatogram of the first (neutral) SPE-eluate of a green bean-extract (Fig. 5), which

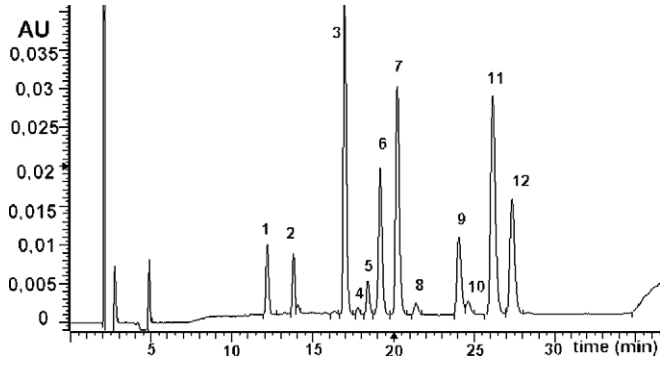

Fig. 5. Chromatogram of the first polyamide-eluate of a green bean-extract. Peaks 1 and 2: phenol carbonic acids, peak 3: quercetin-3-*O* xylosylrutinoside, peak 4: quercetin glycoside, peak 5: quercetin glycoside, peak 6: kaempferol-3-*O*-xylosylrutinoside, peak 7: quercetin-3-*O* rutinoside (rutin), peak 8: kaempferol glycoside, peak 9: kaempferol-3-*O* rutinoside, peak 10: kaempferol glycoside, peak 11: internal standard kaempferol-3-*O* glucoside, peak 12: internal standard quercetin-3-*O* rhamnoside (quercitrin).

was indicated by the typical diode array spectra and the aglyca $M + H^+$ masses 287 and 303 for kaempferol and quercetin, respectively. One of them was tentatively identified as quercetin 3-*O*-glucosyl-xylosyl-acetate. The recoveries of the flavonol glycosides were 77–102%. The response factors were 0.94 for quercetin 3-*O*-rutinoside and 1.05 for kaempferol 3-*O*-rutinoside, which were used for the calculation of every single isomer (with molar-dependent correction factors).

The percentage of each isomer of the total amount of quercetin and the distribution of the quercetinglycosides in canned green beans and in a glassware pack of green beans is shown in Fig. 6. There is no significant difference in the compositions of the different flavonolglycosides at

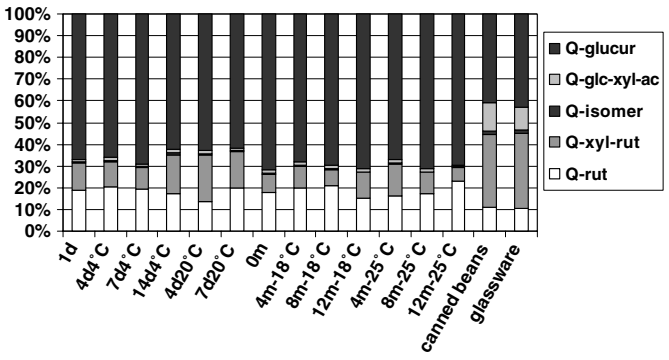

Fig. 6. Distribution of the flavonol glycoside isomers of quercetin in differently stored green beans and two retail products (canned beans and glassware packed).

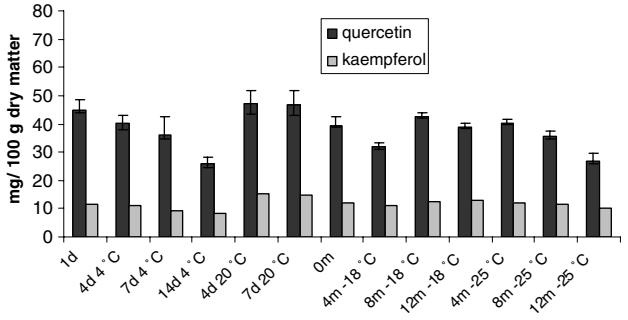

Fig. 7. Quercetin and kaempferol contents (as the sum of their glycosides) in green beans.

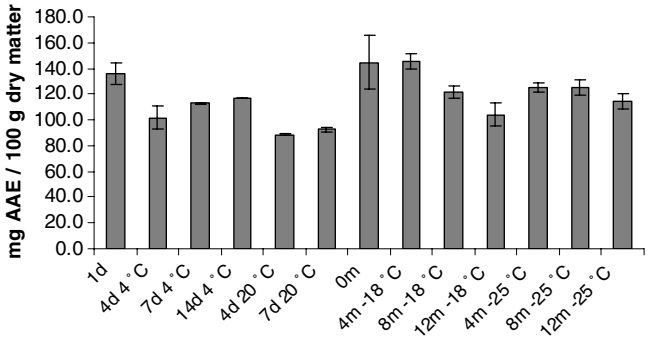

Fig. 8. Antioxidant capacity (measured by the FRAP-assay) of the aqueous green bean-extracts.

different storage stages but there is a (most probable variety-dependent) difference in the distribution of the flavonolglycosides on comparing the glassware pack and the canned beans with the stored green beans.

All peaks which could be attributed to a quercetin or kaempferol aglycone were calculated as their aglycones and summed for the calculation of the total quercetin amount and the total kaempferol amount, respectively. The trend for the content of quercetin and kaempferol, respectively, in all measured temperatures during the whole storage period is shown in Fig. 7. Only the cooked green beans are presented as only the cooked vegetables were correlated with the sensory attributes. It can be seen that a storage period of 14 days significantly reduced the amount of these flavonoids in green beans. The deep-frozen beans showed the same tendencies; the amount of quercetin was nearly the same after 12 months of storage at −25 °C compared to 14 days at 4 °C. The flavonols do not seem to be a suitable storage parameter.

After statistical calculations, there are only tendencies of a decreasing amount of quercetin and kaempferol observable within the analysed period.

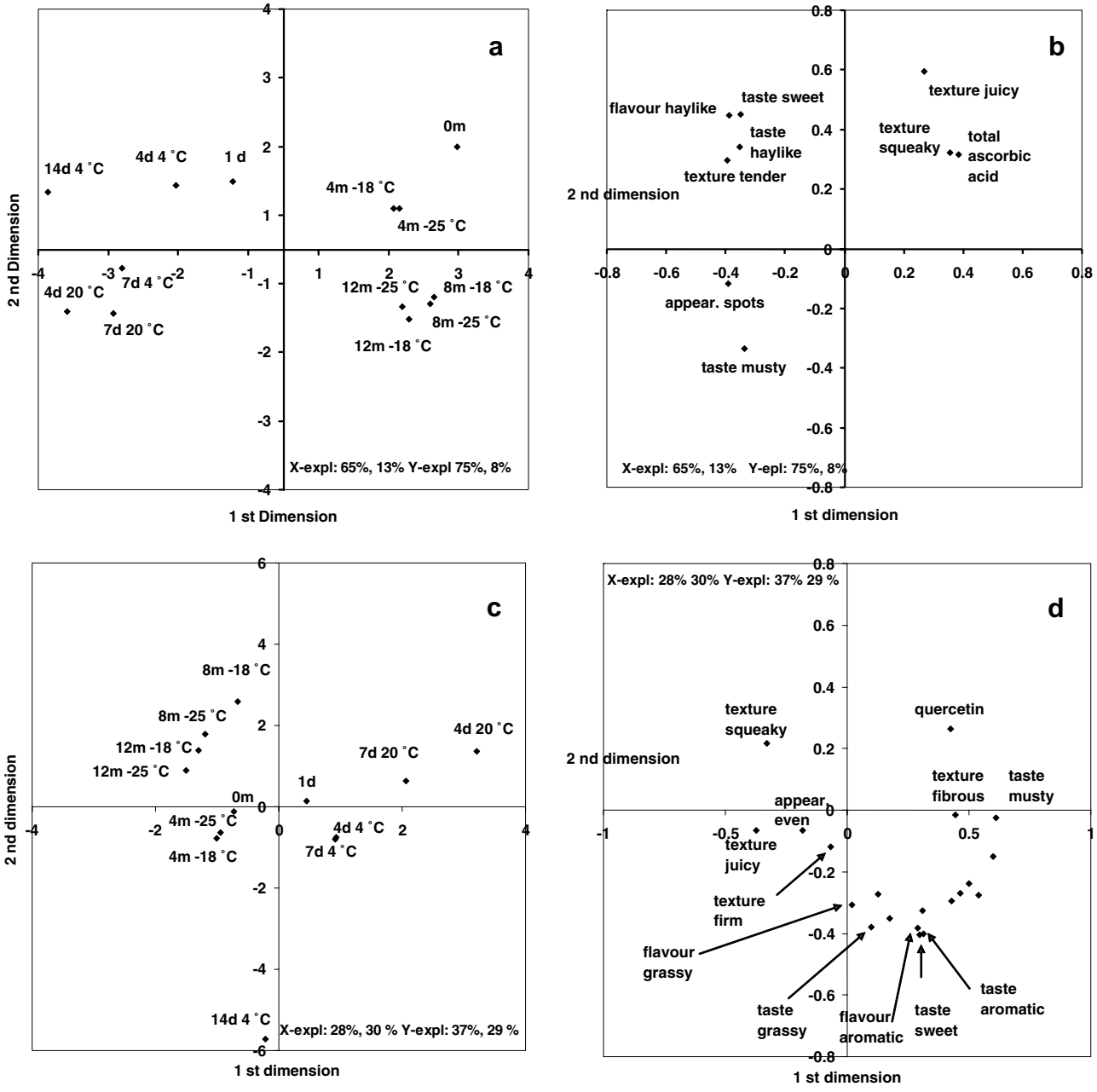

Fig. 9. *PLSR*-graphics of the correlation of green beans, a: products after correlation of sensory attributes with the total ascorbic acid content, b: attributes after correlation of sensory attributes with the total ascorbic acid content, c: products after correlation of sensory attributes with the quercetin content, d: attributes after correlation of sensory attributes with the quercetin content, e: products after correlation of sensory attributes with the antioxidant capacity, and f: attributes after correlation of sensory attributes with the antioxidant capacity.

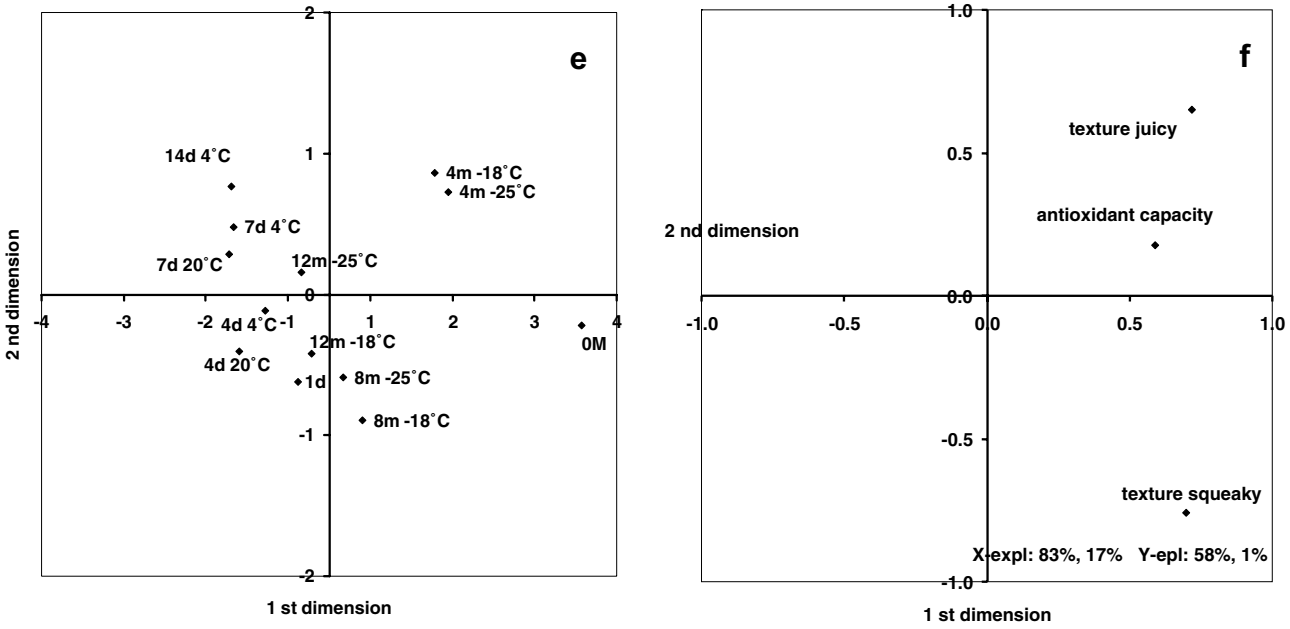

Fig. 9 (*continued*)

3.2.3. Antioxidative capacity

The antioxidative capacity was measured, according to the other parameters in the cooked green beans (see Fig. 8). The cooked green beans tend to loose antioxidative capacity during a storage period of 14 days at 4 °C and 7 days at 20 °C, respectively. The losses during deep-frozen storage seem to be less but there are no significant differences according to the statistics. There is a significant difference only between two deep-frozen states (0 m and 4 m at −18 °C) to 4 days at 20 °C and 7 days at 20 °C, respectively.

3.2.4. Correlations with sensory parameter

From the correlation graphics of the PLSR (Fig. 9), it can be seen that, again, there is a strong statistical explanation for the chemical parameter total ascorbic acid content by the sensory attributes in the correlation graphics. The *y*-explanation yields 83%, which is a relatively high correlation. The total ascorbic acid content in beans shows a high negative correlation with the "spotty" appearance and the "musty" taste (Fig. 9b) and a high positive correlation with the "squeaky" and "juicy" texture. Furthermore, beans with a high amount of total ascorbic acid have only low sensory values for a "tender" texture, a "sweet" and "haylike" taste and a "haylike" flavour.

The correlation of the total quercetin content and the sensory attributes are displayed in Fig. 9c and d. In regard to the content of quercetin, only 66% of the variance is explained. All significantly changing sensory attributes are displayed in Fig. 9b, but there are, in fact, no significantly correlated attributes, which was the result of the PLSR-calculation. The amount of kaempferol, which was also correlated with the main sensory attributes in green beans and is not visualised here, was evidently also not correlated with any of the sensory attributes.

The antioxidative capacity, measured with the FRAP-assay, showed significant correlations with the sensory attributes (see explanation factor for the antioxidative capacity of 60% in Fig. 9e and f; *R* was 0.76). In our study, a "juicy" texture is associated with a high amount of antioxidative capacity assayed by the FRAP-test and the "squeaky" texture also tends to correlate with it.

4. Conclusion

The total ascorbic acid content seemed to be the best chemical indicator for the storage period of all evaluated products.

Significant losses of the total ascorbic acid content, both in cooked peas and green beans, were determined at 4 °C and 20 °C, respectively. Deep-frozen peas and green beans showed high amounts of total ascorbic acid content and of the main, mostly positively, regarded attributes, even after a 12-month storage period.

It was possible to elaborate some striking correlations with sensory attributes of peas and green beans: the total ascorbic acid content was, amongst others, found to be positively correlated with the attribute "sweet" taste and negatively correlated with the attribute "musty" odour in peas. The total ascorbic acid content of green beans was positively correlated with a "squeaky" texture and negatively with a "musty" taste. The antioxidative capacity and the flavonol content were also determined, but none or only lower degrees of correlations with the sensory attributes were found.

Acknowledgements

This research project was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the Ministry of Economics and Labour. Project No. AiF –FV 13373 N.

References

Aparicio-Cuesta, P., Rivas-Gonzalo, J. C., Santos-Buelga, C., & Garcia-Moreno, C. (1989). Quality of deep-frozen green beans (*Phaseolus vulgaris*) subjected to different storage conditions. *Journal of the Science of Food and Agriculture, 48*, 249–259.

Benzie, I. F. F., Chung, W. Y., & Strain, J. J. (1999). "Antioxidative" (reducing) efficiency of ascorbate in plasma is not affected by concentration. *Journal Nutritional Biochemistry, 10*, 146–150.

Bloeck, M., Iseli-Winter, S., Perren, M., Escher, F., & Solms, J. (1986). Quality changes during storage of heat-sterilized vegetables. *Developments in Food Science, 12*, 393–411.

Bognár, A. A. (1987). Vitaminabbau bei der Be- und Verarbeitung von Gemüse. *Lebensmitteltechnik, 10*, 541–554.

Bognár, A. A., & Wolf, W. (2002). Zur Lagerstabilität und Qualität tiefgefrorener Lebensmittel. *Ernährung im Fokus, 2*, 143–149.

Bub, A., Watzl, B., Abrahamse, L., Delincée, H., Adam, S., Wever, J., et al. (2000). Moderate intervention with carotenoid-rich vegetable products reduces lipid peroxidation in men. *Journal of Nutrition, 130*, 2200–2206.

Busch-Stockfisch, M. (2002). Einsatz von PLS Regression zur Auswertung von Daten aus der Sensorik. In J. Meier (Ed.), *Praxishandbuch Sensorik in der Produktentwicklung und Qualitätssicherung*. Hamburg: Behr's Verlag.

Charoenrein, S., & Reid, D. S. (1989). Effect of freezing conditions and storage temperature on the stability of deep-frozen green beans. In *ACS symposium series* (Vol. 405, pp. 226–238). (Qual. Factors Fruits Veg.: Chem. Technol.).

Derse, P. H., & Teply, L. J. (1958). Effect of storage conditions on nutrients in deep-frozen green beans, peas, orange juice, and strawberries. *Journal of Agricultural and Food Chemistry, 6*, 309–312.

DuPont, M. S., Mondin, Z., Williamson, G., & Price, K. R. (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *Journal of Agricultural and Food Chemistry, 48*, 3957–3964.

Favell, D. J. (1998). A comparison of the vitamin C content of fresh and deep-frozen vegetables. *Food Chemistry, 62*, 59–64.

Giannakourou, M. C., & Taoukis, P. S. (2003). Kinetic modelling of vitamin C loss in deep-frozen green vegetables under variable storage conditions. *Food Chemistry, 83*, 33–41.

Hempel, J., & Boehm, H. (1996). Quality and quantity of prevailing flavonoid glycosides of yellow and green French beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 44*, 2114–2116.

Hermann, K. (1986). Einfluß von Lagerung, Zubereitung und Konservierung auf den Vitamingehalt von Gemüse. *Ernährungs-Umschau, 15*, 81–83.

Hertog, M. G. L., Hollman, P. C. H., & Kahn, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry, 40*, 2379–2383.

Hertog, M. G. L., Hollman, P. C. H., & Kromhout, D. (1993). Determination of potentially anticarcinogenic flavonoids in foods and preliminary results on daily intake in the Netherlands. In *Special publication – Royal society of chemistry*. (Vol. 123, pp. 198–202) (Food and cancer prevention: chemical and biological aspects).

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidative capacity assays. *Journal of Agricultural and Food Chemistry, 53*, 1841–1856.

Nilsson, J., Stegmark, R., & Åkesson, B. (2004). Total antioxidative capacity in different pea (*Pisum sativum*) varieties after blanching and freezing. *Food Chemistry, 86*, 501–507.

Price, K. R., Colquhoun, I. J., Barnes, K. A., & Rhodes, M. J. C. (1998). Composition of Flavonol glycosides in green beans and their fate during processing. *Journal of Agricultural and Food Chemistry, 46*, 4898–4903.

Puupponen-Pimiä, R., Häkkinen, S. T., Aarni, M., Suortti, T., Lampi, A.-M., Eurola, M., et al. (2003). Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *Journal of the Science of Food and Agriculture, 83*, 1398–1402.

Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidative activities of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*, 375–383.

Sanchez-Mata, M. C., Camara, M., & Diez-Marques, C. (2002). Extending shelf-life and nutritive value of green beans (*Phaseolus vulgaris* L.), by controlled atmosphere storage: micronutrients. *Food Chemistry, 80*, 317–322.

Schild, E., & Enders, C. (1936). Zur Spezifität des Phenolreagens nach Folin und Ciocalteu für die Tryptophanbestimmung. *Biochemische Zeitschrift, 286*, 220–224.

Szoke, K., & Aldor, T. (1964). Vitamin C loss in vegetables during storage. *Nahrung, 8*, 669–673.