

## Total antioxidant capacity in different pea (*Pisum sativum*) varieties after blanching and freezing

Jessica Nilsson<sup>a</sup>, Rolf Stegmark<sup>b</sup>, Björn Åkesson<sup>a,\*</sup>

<sup>a</sup> Biomedical Nutrition, Lund Institute of Technology, Lund University, Lund, Sweden

<sup>b</sup> Findus R&D AB, Bjuv, Sweden

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### Abstract

Thirty-five varieties of the green pea (*Pisum sativum* L.) were analysed for their total antioxidant capacity (TAC). After blanching and freezing, the water-soluble fraction had, on average, a TAC of 0.61(0.22)  $\mu\text{mol/g}$  (mean(SD)) and the water-insoluble fraction a value of 0.23(0.08)  $\mu\text{mol/g}$ . There was a significant correlation between the TAC in the water-soluble and water-insoluble fractions ( $r = 0.41$ ;  $p < 0.001$ ). The peas had been grown during two consecutive years and were harvested at two different periods during the second year, 1999. Regarding the antioxidant capacity in both the water-soluble and the water-insoluble extract, there was a significant difference between the varieties but not between the harvest periods. A significant correlation ( $r = 0.72$ ) between TAC in the water-soluble fraction and ascorbic acid content was found. Ascorbic acid accounted for a large part of the water-soluble antioxidant capacity. Further studies are necessary to reveal other compounds explaining the variation in pea antioxidant capacity and the mechanisms involved. Measurement of TAC may be a convenient route for the selection of pea varieties with optimal functional and health effects.

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**Keywords:** Green peas; *Pisum sativum*; Total antioxidant capacity (TAC); FRAP; Variety; Cultivation conditions

### 1. Introduction

Green peas (*Pisum sativum* L.) have a nutritionally favourable composition with respect to macronutrients: they have a low fat content, are high in fibre, and protein (National Food Administration, 2002), and they contain starch with a low glycemic index (Foster-Powell & Miller, 1995). Among micronutrients, peas have high contents of ascorbic acid,  $\beta$ -carotene, thiamine and riboflavin and, compared to other vegetables, peas are rich in iron (National Food Administration, 2002). Moreover, several reports have shown that other antioxidants are present in peas, some of which are, so far, poorly characterised. Alonso, Orúe, and Marzo (1998) analysed polyphenols in peas and found that their concentrations varied according to the treatment of the

peas. Among individual compounds, the content of quercetin in peas was found to be 0.15 mg/100 g (Ewald, Fjellkner-Modig, Johansson, Sjöholm, & Åkesson, 1999). Some of the polyphenol compounds seemed to be bound to a pea superoxide dismutase that could act as a carrier (Nice, Robinson, & Holden, 1995). Regarding amino acids, Zamora, Alaiz, and Hidalgo (1999) found that pyrrolylnorleucine was a normal component of foods, acting as a natural antioxidant.

Dietary antioxidants protect against reactive oxygen species in the human body by several mechanisms. An increased intake of antioxidants may therefore have a number of health effects, such as reducing the incidence of cancer and cardiovascular diseases (Diplock et al., 1998; Halliwell & Gutteridge, 1999). Due to the detection of many new bioactive compounds in food with possible antioxidant activity, and the increased interest in the relationship between antioxidants and disease risks and mechanisms, there is a need to establish simple methods to get an overall measure of the amount of

\* Corresponding author. Tel.: +46 46 222 95 81; fax: +46 46 222 38 53.

E-mail address: [bjorn.akesson@kc.lu.se](mailto:bjorn.akesson@kc.lu.se) (B. Åkesson).

antioxidants and their activity in different foods. Several methods for measuring the total antioxidant capacity (TAC) have been developed during the past few years (Benzie & Strain, 1996; Cao, Sofic, & Prior, 1996; Lissi, Salim-Hanna, Pascual, & del Castillo, 1995; Miller, Diplock, & Rice-Evans, 1995). In this report, the ferric reducing/antioxidant power (FRAP) assay was selected for a detailed study of the TAC value of green peas in relation to variety and harvest periods. In the FRAP method, any substance that can donate electrons and has a half-reaction reduction potential lower than that of the  $\text{Fe}^{3+}/\text{Fe}^{2+}$  redox couple will be reactive (Benzie & Strain, 1996; Buettner, 1993). Previous evaluations of methods based on this principle have shown them to give reproducible results, be linear over a wide concentration range, be relatively easy to operate, and suitable for measuring TAC in different matrices (Benzie & Strain, 1996; Chen, 2002). In two parallel studies the FRAP method was used for analysis of TAC in strawberries and *Brassica* vegetables (unpublished data).

## 2. Materials and methods

### 2.1. Chemicals

Trolox<sup>®</sup> (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 97% and TPTZ (2,4,6-tripyridyl-*s*-triazine) >98% were purchased from Sigma–Aldrich (St. Louis, USA), ferric chloride from ICN Biomedicals Inc. (USA), acetic acid (glacial, p.a.) and acetone (p.a.) from Merck (Darmstadt, Germany), sodium acetate from BDH Chemicals Ltd. (UK), oxalic acid from Tamro MedLab (Sweden) and the Tillman reagent (2,6-dichlorophenol indophenol) from Merck Eurolab (Belgium).

### 2.2. Samples

Thirty-five varieties of peas (*Pisum sativum* L.) were grown at Findus R&D AB, Bjuv, Sweden. In 1998, they were grown from late April to mid June and in 1999 from late April to mid June but also from early June to early August. All pea samples were harvested at a tenderometer value close to 100, threshed, blanched (97 °C, 100 s), brine-graded in 10.9% (w/v) NaCl for density fractionation and finally frozen within 1–2 h after the threshing. They were stored at –20 °C for TAC analysis and at –30 °C for ascorbic acid analysis.

### 2.3. Sample preparation

Frozen green peas (20 g) were thawed in an equal weight of acetate buffer (0.1 mol/l, pH 5.0) for 1 h and then the mixture was homogenised with a rotating-blade homogeniser for 2 min. The homogenates were centrifuged at 26,000g for 30 min at 4 °C in a Beckman J2

centrifuge (Beckman, Palo Alto, CA, USA). The supernatants were frozen and stored in aliquots at –80 °C until analysis. One gramme of the remaining pulp was extracted with 8 ml of acetone for 30 min at room temperature with occasional mixing and then centrifuged at 1200g for 10 min at room temperature in a Beckman GPR centrifuge. The acetone extracts were stored at –80 °C until analysis.

### 2.4. Analytical methods

#### 2.4.1. Assay of total antioxidant capacity

The assay used was the FRAP procedure (Benzie & Strain, 1996). The FRAP reagent was made as follows: 25 ml of 0.1 mol/l acetate buffer (pH 3.6) were mixed with 2.5 ml of 10 mmol/l TPTZ in 40 mmol/l HCl and 2.5 ml of 20 mmol/l  $\text{FeCl}_3$  in water. Sample (30  $\mu\text{l}$  and 90  $\mu\text{l}$  of water were added to 900  $\mu\text{l}$  of reagent. As a blank, 900  $\mu\text{l}$  of reagent together with 120  $\mu\text{l}$  of water were used. The absorbance readings were made at 593 nm every 20 s for 10 min with an Ultrospec 3000 UV/Vis spectrophotometer (Pharmacia Biotech, Sweden) thermostatted at 25 °C. All samples were analysed in duplicate, on two different days. Five different concentrations of Trolox<sup>®</sup> (100, 250, 500, 750, 1000  $\mu\text{mol/l}$ ) were used to construct a standard curve and the results for the samples were expressed as  $\mu\text{mol/g}$  (edible part). Because of the limited stability of the FRAP reagent, it was prepared several times during the day. If necessary, dilutions of the samples were made with 0.1 mol/l acetate buffer (pH 3.6). To establish the inter- and intra-assay variations, water-soluble extracts from ten of the varieties were analysed on two occasions, using three dilutions (3, 2 and 1.5 times) and duplicate analyses.

#### 2.4.2. Ascorbic acid analysis

Frozen peas (20 g) were homogenized for 90 s in 20 ml of oxalic acid (6 %, w/v). The homogenate was diluted to 100 ml with distilled water and centrifuged for 10 min at 2000 rpm. The supernatant were filtered and 10 ml of the filtrate were titrated with the Tillman reagent. The resistance of the solution decreased as soon as there was an excess of Tillman's reagent, and the electric potential dropped. Ascorbic acid was determined in peas grown during the early period in 1999. Dehydroascorbic acid was not determined in this study.

For determination of the dry matter content, approximately five gramme of peas were dried at 70 °C for 2 days and then for 2 h at 105 °C. The peas were allowed to reach room temperature in a desiccator and were then weighed.

### 2.5. Statistical analyses

All data were subjected to analysis of variance (ANOVA) and significant differences were then assessed by multiple comparisons with Tukey's test. Linear cor-

relation coefficients were calculated. Tests giving  $p < 0.05$  were considered statistically significant.

### 3. Results

#### 3.1. TAC in green peas

The water-soluble TAC in buffer extracts from peas ranged from 0.20 to 1.29  $\mu\text{mol/g}$  among all samples with a mean value (SD) of 0.61 (0.22)  $\mu\text{mol/g}$  (Table 1). The values for the water-insoluble TAC in the acetone extract ranged from 0.05 to 0.46  $\mu\text{mol/g}$ , with a mean of 0.23 (0.08)  $\mu\text{mol/g}$ , which was significantly lower than that in the water-soluble part ( $p < 0.001$ ).

For the water-soluble extracts from ten varieties analysed in duplicate on two occasions, there was an average difference between the duplicates of 6.5% and, between the two occasions, of 8.6%. When three different concentrations of the extracts were analysed there was an average difference of 7.8% between the final TAC values multiplied with the dilution factors (not a significant difference).

#### 3.2. TAC in pea varieties

For the water-soluble TAC, the mean values for pea varieties ranged from 0.31 to 0.97  $\mu\text{mol/g}$  and there were significant differences between the varieties ( $p = 0.002$ ) but not between the three harvest periods ( $p = 0.16$ ) (Table 1). The same observation was found for the water-insoluble TAC values, with significant difference in TAC values among the varieties ( $p = 0.001$ ) but not among the three harvest periods ( $p = 0.08$ ). The mean values for water-insoluble TAC in pea varieties ranged from 0.11 to 0.38  $\mu\text{mol/g}$ .

#### 3.3. Ascorbic acid in pea varieties

The ascorbic acid content in peas ranged from 0.40 to 1.48  $\mu\text{mol/g}$ , with a mean value (SD) of 0.93 (0.26)  $\mu\text{mol/g}$

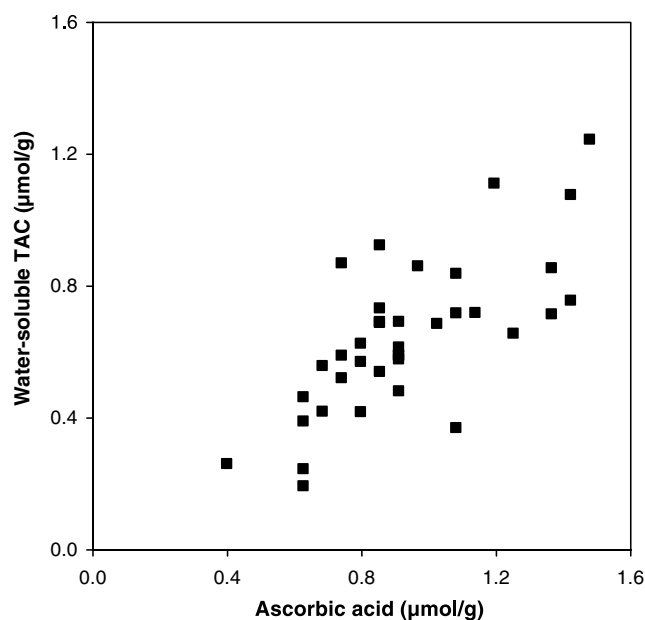


Fig. 1. Correlation between water-soluble TAC values ( $\mu\text{mol/g}$ ) and ascorbic acid ( $\mu\text{mol/g}$ ) in the peas harvested during the early period in 1999. The linear correlation coefficient  $r$  was 0.72 ( $p < 0.001$ ).

g in the peas grown during the early harvest period in 1999. It was positively correlated to the water-soluble TAC values in peas from the same period,  $r = 0.72$  ( $p < 0.001$ ) (Fig. 1). The ascorbic acid content was at least as high as the water-soluble TAC, indicating that ascorbic acid constituted a major part of this TAC fraction.

#### 3.4. Dry matter

Results on the dry matter content in peas showed no significant differences between the different varieties ( $p = 0.14$ ) but a significant difference between the three harvest periods ( $p = 0.014$ ) (Table 1). The content of dry matter was 0.7% higher in peas harvested early in 1998 than in those harvested late in 1999.

Table 1

Total antioxidant capacity ( $\mu\text{mol/g}$ ) in water-soluble and water-insoluble fractions, and percentage of dry matter in green peas (*Pisum sativum* L.) for the total material and the different harvest periods during 1998 and 1999

Samples	Water-soluble TAC			Water-insoluble TAC			Dry matter		
	Mean (SD)	Range	<i>n</i>	Mean (SD)	Range	<i>n</i>	Mean (SD)	Range	<i>n</i>
Total	0.61 (0.22)	0.20–1.29	108	0.23 (0.08)	0.05–0.46	105	18.3 (1.1)	15.9–22.2	110
1998 early	0.57 (0.25)	0.21–1.29	35	0.23 (0.08)	0.05–0.46	35	18.6 (1.3)	16.1–22.2	36
1999 early	0.65 (0.23)	0.20–1.25	37	0.21 (0.08)	0.08–0.35	36	18.4 (1.0)	16.8–20.9	37
1999 late	0.60 (0.14)	0.41–0.91	36	0.25 (0.09)	0.09–0.43	34	17.9 (1.0)	15.9–20.4	37
	<i>p</i> value								
Variety	0.002			0.001			0.14		
Harvest period	0.16			0.08			0.014		

The  $p$  values from statistical analysis of the differences in means between different varieties and harvest periods were obtained by ANOVA.

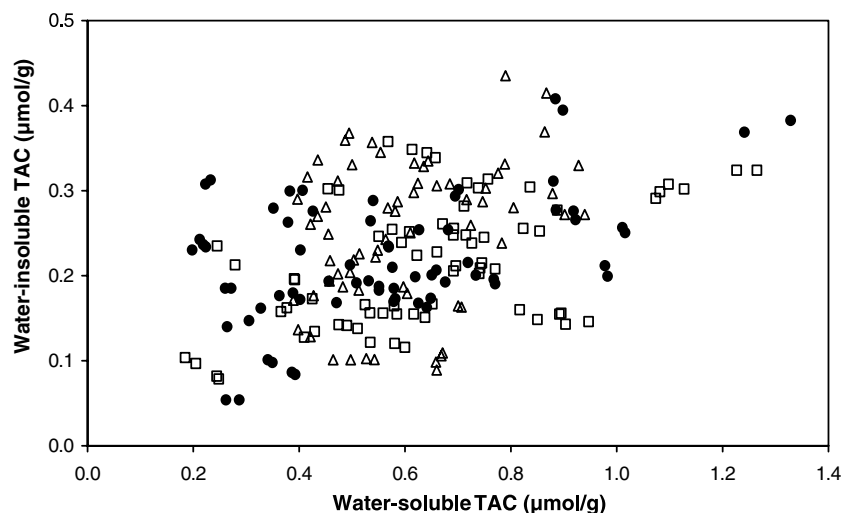


Fig. 2. Correlation between TAC values ( $\mu\text{mol/g}$ ) from the water-soluble part and from the water-insoluble part. The linear correlation coefficient  $r$  was 0.41 ( $p < 0.001$ ) for the total material. Symbols: (●) early period in 1998; (□) early period in 1999; (△) late period in 1999.

Table 2

Linear correlation coefficients between the antioxidant capacities in the subgroups of green peas

	Water-soluble TAC			Water-insoluble TAC		
	Early 1998	Early 1999	Late 1999	Early 1998	Early 1999	Late 1999
<i>Water-soluble TAC</i>						
Early 1998	–	0.34*	0.21	0.48**	0.28	0.21
Early 1999		–	0.40*	0.03	0.52**	0.18
Late 1999			–	–0.17	0.41*	0.32
<i>Water-insoluble TAC</i>						
Early 1998				–	0.13	0.21
Early 1999					–	0.62***
Late 1999						–

\* The correlation was significant at the 0.05 level.

\*\* The correlation was significant at the 0.01 level.

\*\*\* The correlation was significant at the 0.001 level.

### 3.5. Associations of TAC between harvest periods and pea fractions

In the total material, a correlation coefficient of 0.41 ( $p < 0.001$ ) was observed between the values for water-soluble and water-insoluble TAC (Fig. 2). The water-soluble TAC for the early periods in each year was significantly correlated with the corresponding water-insoluble TAC (Table 2). There were also significant correlations between the TAC of the same varieties between the early period 1999 and the late period 1999, both for the water-soluble fraction  $r = 0.40$  ( $p < 0.05$ ) (Fig. 3a) and for the water-insoluble fraction  $r = 0.62$  ( $p < 0.001$ ) (Fig. 3b). The correlation coefficients between TAC in 1998 samples and 1999 samples were not significant in most cases. There were no significant correlations between the dry matter content and the TAC value, either for the water-soluble,  $r = -0.08$  ( $p = 0.42$ ) or the water-insoluble TAC,  $r = -0.17$  ( $p = 0.078$ ).

## 4. Discussion

### 4.1. Type of TAC assay

Oxidative reactions may affect the quality and functionality of peas in a number of ways. This may be important for several aspects of food and nutritional quality, such as the formation of aroma volatiles (Jakobsen, Hansen, Christensen, Brockhoff, & Olsen, 1998). Thus, it is important to explore and evaluate different markers of antioxidant content and oxidative reactions. The FRAP method used in the present study measures antioxidants as reductants in a redox-linked colorimetric reaction (Benzie & Strain, 1996). Other methods used for analysing TAC are more indirect and assay the inhibition of generated free radicals. Some methods use a lag-phase type of measurement that was difficult to standardise in previous experiments. In a recent version of the ABTS method the scavenging of a free radical is measured directly (Re et al., 1999). For the

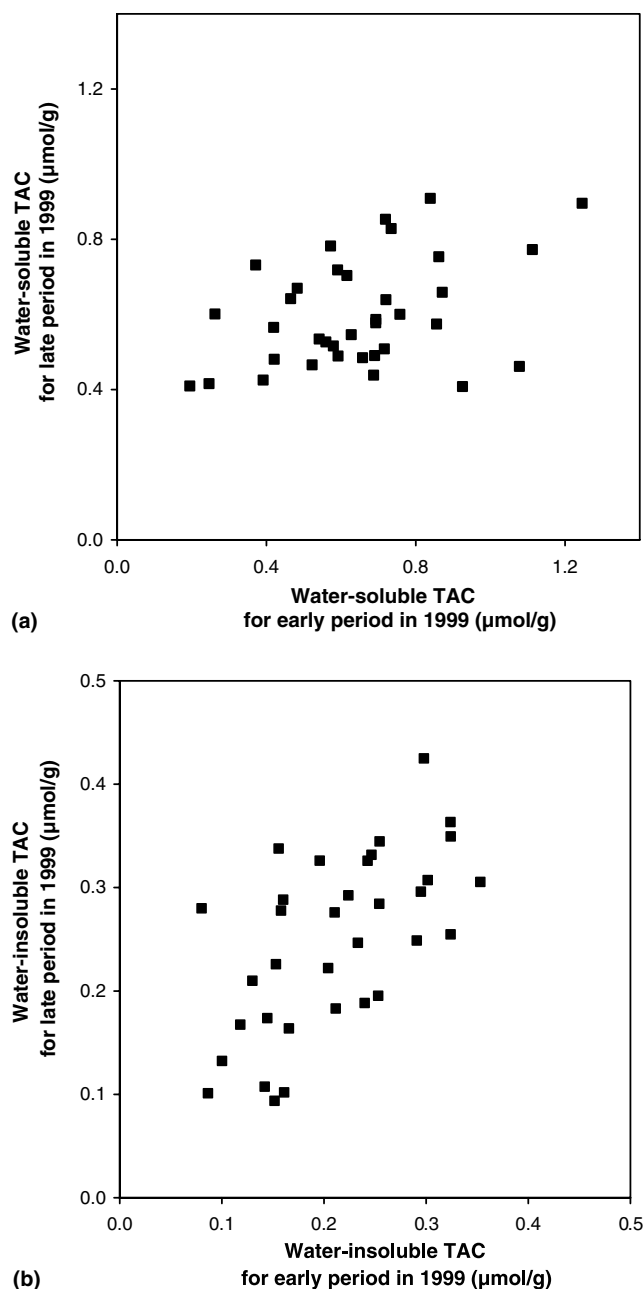


Fig. 3. Correlation between TAC values ( $\mu\text{mol/g}$ ) from early period 1999 and late period 1999. (a) Water-soluble TAC. The linear correlation coefficient  $r$  was 0.40 ( $p < 0.05$ ). (b) Water-insoluble TAC. The linear correlation coefficient  $r$  was 0.62 ( $p < 0.001$ ).

FRAP method used in the present report, the low between-day and between-duplicates imprecision and the linear dependence of the results with sample dilution indicated a good analytical performance for the assay of pea extracts.

#### 4.2. Factors influencing TAC in peas

Regarding the two fractions resulting from the sample preparation, the water-soluble fraction would be

expected to contain ascorbic acid and the acetone-soluble fraction would be expected to contain lipid-soluble antioxidants such as  $\beta$ -carotene and  $\alpha$ -tocopherol. This made possible an assessment of the antioxidant content in two biologically important fractions, although the reactivity of individual compounds must also be taken into account. Previously, ascorbic acid and  $\alpha$ -tocopherol were shown to have the same activity in the FRAP assay as the reference compound Trolox (Benzie & Strain, 1996) but different estimates of the reactivity of  $\beta$ -carotene have been published (Hunter & Fletcher, 2002; Pulido, Bravo, & Saura-Calixto, 2000). It has also been shown that sulphur-containing amino acids and thiols are inactive in the FRAP assay. In general, TAC in the water-soluble fraction was three-fold higher than that in the water-insoluble fraction. Anyhow, there was a positive correlation between TAC in these two fractions, which may have several explanations. Since both water-soluble and water-insoluble antioxidants are probably involved in the protection of the plant against the oxidative stress, a parallel variation in the contents of water-soluble and water-insoluble antioxidants may be a physiological response. The correlation found was probably not related to variations in dry matter content since there was no correlation between the amount of dry matter and TAC in either fraction. To some extent the correlation might be influenced by inevitable cross-contamination between the two extracts.

#### 4.3. Antioxidant compounds in green peas

The present report shows that both the water-soluble and water-insoluble TAC varied significantly among different varieties of peas. For water-soluble TAC this was mainly due to the content of ascorbic acid in the peas. Also, previously, it has been shown that the amount of ascorbic acid varies among pea varieties (Savage & Deo, 1989). Our study confirms this finding and similar magnitudes of the concentrations of water-soluble TAC and ascorbic acid show that ascorbic acid was a main contributor to TAC. The strong correlation between water-soluble TAC and ascorbic acid content is compatible with this finding. The reason why the measured ascorbic acid content in some samples reached a higher value than water-soluble TAC may be that the pea samples for ascorbic acid analysis were stored for a shorter time and at a lower temperature than those used for TAC analysis. Loss of ascorbic acid in frozen vegetables has been shown to depend on both temperature and time (Giannakourou & Taoukis, 2003). Moreover, it is possible that some of the variation in water-soluble TAC will depend on other constituents not so thoroughly examined. The content of quercetin in blanched peas was found to be 0.15 mg/100 g (5 nmol/g) by Ewold et al. (1999), which would make a small contribution. In addition, some antioxidant polyphenols might be found,

in both the water-soluble and in the water-insoluble fractions.

Regarding the fat-soluble antioxidants, blanched frozen green peas have an approximately 500 times lower content of  $\alpha$ -tocopherol (2 nmol/g) than of ascorbic acid (1  $\mu$ mol/g). The low concentration of  $\alpha$ -tocopherol indicates that  $\alpha$ -tocopherol could account for only a small fraction of the water-insoluble TAC and that the main contribution to this fraction must arise from compounds other than the fat-soluble vitamins. As mentioned above, quercetin and other flavonoids would be expected to contribute (Ewald et al., 1999). Hunter & Fletcher (2002) reported that chlorophyll could contribute to the TAC in peas, as measured by the FRAP assay. Recently, Troszyńska, Estrella, López-Amóres, & Hernández (2002) found that an acetone extract of pea seed coats contained a number of phenolic compounds, among which apigenin glycosides dominated. Additional analyses of antioxidants are important to reveal the proportion of TAC accounted for by other compounds. It is also interesting to explore the use of different extraction techniques and TAC assays, which might give complementary information.

#### 4.4. Comparison of TAC in different foods

FRAP method has previously been used for the analysis of TAC in blueberries, blackberries, black currant (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002), different *Rubus* species (Deighton, Brennan, Finn, & Davies, 2000), rosehips (Gao, Björk, Trajkovski, & Uggla, 2000) and guava (Jiménez-Escrig, Rincón, Pulido, & Saura-Calixto, 2001), but few data on green peas are available. TAC in all these samples showed a large variation (between 0.2 and 157  $\mu$ mol/g). In addition, different beverages have been tested, e.g. black and green tea (Benzie & Szeto, 1999) and fruit juices (Gardner, White, McPhail, & Duthie, 2000; Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000), showing TAC values between 1.2 and 11.4  $\mu$ mol/ml). In parallel studies, we have compared TAC methods for studies on milk (Chen, 2002). Very recently, a study of the TAC in a wider range of dietary plants, using the FRAP assay, was published (Halvorsen et al., 2002). They found the antioxidant capacity of peas to be 0.6  $\mu$ mol/g, using our method of expressing the data, which is very similar to the results shown in the present report. Hunter and Fletcher (2002) found the total water-soluble antioxidant activity in frozen peas to be 1.75  $\mu$ mol ascorbic equivalents/g and the total lipid-soluble activity to be 0.13  $\mu$ mol ascorbic equivalents/g although different extraction techniques were used from those in the present study. Both the present study, and that of Halvorsen et al. (2002), thus show that green peas have a low total antioxidant capacity compared to that in many other vegetables. Among pulses, fava bean had the highest

TAC value (1.9  $\mu$ mol/g) and, compared to vegetables, the TAC value in peas was similar to those in cabbage (0.9  $\mu$ mol/g), endive (1.0  $\mu$ mol/g) and aubergines (1.7  $\mu$ mol/g) while the highest TAC value in vegetables, 3.0  $\mu$ mol/g, was found in chilli pepper (Halvorsen et al., 2002). Although the content of ascorbic acid and TAC in peas is moderate, an intake of, for instance, 100 g of peas could contribute approximately 1/3 of the recommended ascorbic acid intake and probably also a sizeable part of other antioxidants. Selection of pea varieties with high antioxidant content is thus important from this point of view.

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