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Indices of Quality and Maturity for Different Commercial Sizes of Pea Seed for Canning

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ABSTRACT

*Four sizes of pea (*Pisum sativum, *L.), chosen according to a Spanish commercial classification for canning, were analysed for tenderometer value (TV), moisture, crude protein (CP) content, total sulphur (TS) content, C/N and N/S ratios and albumin protein (AP) content. Modifications of the albumin fraction were studied by SDS-PAGE (10% acrylamide w/v). CP alone can be used as a quantitative index to distinguish growth period (SF–FN size) and maturation period (MD size). During the growth period TV is related significantly with CP and TS, and TV can be used to estimate the pea protein content and the pea protein quality. AP determination appears to be a useful confirmation of pea quality because it is simultaneously an index of the qualitative (N/S ratio) and quantitative (C/N ratio) modifications of the pea protein in the growth period of the pea seeds between 4·7 mm (SF size) and 8·8 mm (FN size). The 23 000 MWt polypeptide remained fairly constant between sizes and the 43 000 MWt was the most heavily stained.*

INTRODUCTION

In quality control and classification of vegetables, there is a need for objective methods to determine quality. Chemical and physical methods for indirectly determining the quality of peas for processing have been studied. Alcohol insoluble solids, dry matter, sugar content and tenderometer value were found to be relevant for measuring the growth and maturity of peas for canning and freezing (Ottosson, 1958). More recently sensory and chemical/physical quality criteria for establishing the maturity of peas intended for

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freezing have been studied (Martens, 1986). To reduce sampling to practicable levels during commercial operation, a maturity index is needed that will allow us to foresee the effects of handling on the final quality of the product (Shewfelt, 1984). In Spain, peas are processed according to commercial characteristics, mainly based on pea size, but nutritional quality of these sizes has not been thoroughly investigated. The proteins of pea (*Pisum sativum* L.) seeds are a source of food for man and monogastric animals. Similar to other pulse seeds, pea proteins are comparatively low in sulphur amino acids (Bhatty & Christison, 1984). However, of all pea protein fractions, the albumin protein fraction combines high levels of sulphur amino acids with a high lysine content and as a protein fraction fully satisfies the WHO (1973) requirements pattern in terms of g of amino acids/100 g protein. It has been suggested that, when considering protein quality, more emphasis should be given to the nutritionally superior value of albumin fraction (Bajaj *et al.*, 1971; Schroeder, 1982). In addition, to determine pea protein quality, it has been proposed that other indices should be measured, such as crude protein, total sulphur content and protein sulphur, and carbon/nitrogen (C/N) and nitrogen/sulphur (N/S) ratios (Schroeder, 1982).

Variations in tenderometric value indicate substantial variations in pea quality (Martens, 1986). The aim of this investigation was to study the modifications of some indices of pea protein quality (crude protein, total sulphur content, C/N and N/S ratios and albumin protein content) as a function of tenderometric value to see whether tenderometric value is an adequate predictor of pea protein quality.

MATERIALS AND METHODS

Materials

Pea seeds (cultivar Manuela) were cultivated in Cartagena (Spain). 2250 to 2500 kg/ha of the complex 8/24/8 (N/P/K) was used. Following the Spanish commercial classification for canned peas, the peas were graded by diameter screening to obtain the following sizes: super-fine (SF, 4·7–7·5 mm), very fine (VF, 7·6–8·2 mm), fine (FN, 8·3–8·8 mm) and middle (MD, 8·9–10·2 mm). Samples were taken at each size (approximately 1 kg) throughout three consecutive days.

Methods

Pea tenderness determination

Tenderometric value (TV) was determined using a Bertuzzi field tenderometer (Brugueiro-Milano). This was carried out as soon as pea seeds were picked using whole seed peas for each determination.

Moisture determination and preparation of dry defatted meal
The dehulled pea seeds were freeze-dried and moisture determined (AOAC, 1984). Freeze-dried pea seeds were ground in a Culatti mill to a fine powder (0·4 mm screen), then blended with ice-cold acetone to defat. The defatted material was air-dried overnight at room temperature and stored in a closed container at 4°C until required.

Determination of crude protein (CP) content
The Micro-Kjeldahl method (AOAC, 1984) was used to determine crude protein (N × 6·25), using 1 g of pea meal.

Determination of total sulphur (TS) content
Determination was carried out according to Norrisk and Hutton (1977) by X-ray fluorescence spectrometry. All measurements were made on 2·5 g of pea meal with a Phillips Pw/400 wavelength dispersive X-ray spectrometer (Eindhoven, Holland) interfaced with a Digital PDP 11/23 computer. A scanning target X-ray tube was used for excitation. Vacuum path was used throughout. Table 1 summarizes the instrumental conditions.

C/N ratio determination
On 1 to 3 mg of pea meal, carbon and nitrogen were determined using an elemental analyzer, Perkin-Elmer Model 240C (Nolwalk, CT, USA) following the manufacturer's recommendations.

Extraction of pea albumin
Albumin fraction was obtained by the procedure of Schroeder (1982). The fat-free meal was extracted twice with 0·1M phosphate buffer (pH 7·2) containing 0·5M NaCl on an end-over-end shaker, with a final weight/volume ratio of 1/25 (w/v) for meal to extraction buffer. The suspension was stirred for 1·5 h at 20°C. Extracts were centrifuged at 5000 rpm for 20 min at

TABLE 1
X-ray Fluorescence Spectrometry Instrumental Conditions

Analytical line	K_α
Wavelength (Å)	5·373 1
Analysing crystal	Ge
2θ	110·69
Detector	F
Collimator	C
Counting time (seg.)	200
Kv/mA	40/60

F, flow counter using Ar-CH_4 (90/10) gas.
C, coarse (550 μm).

20°C. The combined supernatants of two extracts were filtered through two layers of cheesecloth pre-wetted with extraction buffer, and the samples were dialysed against running tap water (with two changes of distilled water) for 72 h at 4°C. The dialysed extract was centrifuged at 10 000 rpm for 30 min at 4°C and the clean supernatant frozen.

Protein content of albumin (AP) pea fraction
A portion of each one of the clear supernatants, obtained by the extraction of pea albumin, was analysed by the phenol-Lowry micromethod (Bensadoun & Weinstein, 1976), scanned at 750 nm, and expressed as mg of protein/ml of pea albumin extract.

Polyacrylamide gel electrophoresis
Electrophoretic analyses of albumin sample on SDS polyacrylamide gels were performed as described by Weber *et al.* (1969). Albumin samples were first dissociated at 90–95°C with SDS in the presence of 2-mercaptoethanol (sample buffer). Fifty micrograms of protein were loaded on each gel (set from 10% (w/v) acrylamide). A tube amperage of 7 mA was applied. Electrophoresis was stopped when the tracking dye approached the bottom of the gel, which took about 5 h. The gels were examined for protein bands by the use of protein-specific stains with 0·2% Coomassie Brilliant Blue. The excess of stain was removed by sequential washing with glacial acetic acid/ethanol/water (first with 1·5:20:28·5 by volume, for 1 h, second with 1·75:15:32·5 by volume, for 1 h, repeated one more time and finally with 2:10:38 by volume for 1 h) until bands were clearly defined.

Evaluation of the electropherograms
Estimates of the MWt for individual polypeptides were obtained by averaging three determinations, using the protein standard of known MWt electrophoresed under identical conditions. The following polypeptides were used as MWt standards: phosphorylase b of rabbit muscle (94 000 MWt), albumin of bovine serum (67 000 MWt), ovoalbumin (43 000 MWt), carbonic anhydrase of bovine erythrocyte (30 000 MWt), trypsin inhibitor of soybean (20 100 MWt) and α-lactalbumin of bovine milk (14 400 MWt). Mobilities of the electrophoretic bands were measured relative to that of tracking dye, the relationship being between molecular weight and relative mobility. The optical densities evaluation of the protein band gels were scanned at 530 nm on the Profil Ecran® Fluo densitometer-integrator.

Statistical analyses
Statistical analyses were performed using the SYSTAT program (Wilkinson, 1986) on a M-240 Olivetti PC computer.

RESULTS AND DISCUSSION

Study of pea quality characteristics

Results of moisture content, TV, CP content, TS content, C/N and N/S ratios and AP content of pea seeds for different sizes are shown in Table 2.

The TV varied between 96·4 ± 2·9 and 126·0 ± 6·0 and increased with pea size and decreased with moisture (Table 2). TV/pea size/moisture relationships have been widely studied (Ottoson, 1958; Shams & Thompson, 1987; Geervani & Devi, 1988). In accordance with the demand of the Spanish

TABLE 2
Characteristics of Pea Seed for Each Size[a,b]

Size	*Moisture* (%)	*TV*	*CP*[c] (%)	*TS*[c] (%)	*C/N*[c] *ratio*	*N/S*[c] *ratio*	*AP*[d]
SF	77·3 ± 2·1	96·4 ± 2·9	25·4 ± 5·0	0·333 ± 0·003	9·97 ± 0·42	12·26 ± 0·59	1·01 ± 0·14
VF	75·9 ± 2·5	101·6 ± 3·8	30·8 ± 4·7	0·329 ± 0·004	11·16 ± 0·46	11·27 ± 0·38	1·23 ± 0·06
FN	72·9 ± 1·7	113·2 ± 4·9	38·6 ± 6·2	0·323 ± 0·003	12·14 ± 0·29	10·63 ± 0·59	1·39 ± 0·05
MD	71·6 ± 1·8	126·0 ± 6·0	27·7 ± 4·7	0·319 ± 0·002	11·03 ± 0·34	11·73 ± 0·40	2·00 ± 0·23

[a] Mean ± standard deviation.
[b] Size abbreviations: SF, superfine (4·7–7·5 mm); VF, very fine (7·6–8·2 mm); FN, fine (8·3–8·8 mm); MD, middle (8·9–10·2 mm).
[c] On dry weight.
[d] AP is expressed as mg of protein in ml of extract.

market, the larger peas (TV > 140 and size > 10·2 mm) are not used for canning but for other industrially processed foods, such as beikost, precooked meals, etc. It is considered (Kay, 1985) that peas for canning should give a reading of 115–20 TV. Following these criteria, in the present study we have considered SF and VF sizes as peas too immature for canning and FN and MD as mature peas.

CP values (Table 2) showed a trend to increase from SF size (25·4% ± 5·0) to FN size (38·6% ± 6·2) and decrease in MD size (27·7% ± 4·7). Geervani and Devi (1988) considered that values of protein content for immature peas (14–18 days after flowering) are lower than for mature peas (10 days on the plant until the green colour of the pod disappeared). This relationship could be established in the present study when we considered SF–FN sizes, but not when we consider all sizes, because CP decreases from FN to MD size. This decreasing can be considered to establish the pea maturity consolidation. So CP alone can be used as a quantitative index to distinguish growth period (SF–FN size) and maturation period (MD size). Due to low variance of carbon content in different sizes (41·05% ± 1·14), C/N ratio increased from

SF to FN, decreasing in MD size (Table 2), following the same trend that was described for CP. The correlation of these two indices (CP and C/N ratio) is positive and significant ($p \leq 0{\cdot}05$) and, consequently, as with CP, the C/N ratio alone can be used as a quantitative index to distinguish growth period and maturation period. On the other hand, it has been suggested (Schroeder, 1982) that the C/N ratio is negatively correlated with CP content when some kinds of peas are studied: primitive pea forms, field peas, and round and wrinkled garden peas.

The TS content as a percentage of dry weight of meal ranged from $0{\cdot}333\% \pm 0{\cdot}003$ to $0{\cdot}319\% \pm 0{\cdot}002$ (Table 2), decreasing as the pea size increased ($p \leq 0{\cdot}001$). Schroeder (1982) described a negative correlation of seed weight with TS, due to the small-seeded primitive forms having a higher TS content. AP content ranged between $1{\cdot}01$ mg/ml $\pm 0{\cdot}14$ and $2{\cdot}00$ mg/ml $\pm 0{\cdot}23$, increasing as pea size increased (Table 2). The same trend has been found with seed weight and albumin content by Schroeder (1982) in different pea forms: primitive, field peas, round garden peas and wrinkled garden peas. TS content and AP show a negative correlation ($p \leq 0{\cdot}001$). Sulphur losses with increased pea size must be due to legumin (the most sulphur-rich amino acid component of the globulin fraction), which decreases as albumin fraction increases (Schroeder, 1982) and to AP which increases as size increases (Table 2). So, AP appears to be a useful index of pea quality because it is simultaneously an index of the qualitative (N/S and AP correlation) and quantitative variations (C/N and AP correlation) of the pea protein in the growth period of the pea seeds between 4·7 mm (SF size) and 8·8 mm (FN size) (Fig. 1).

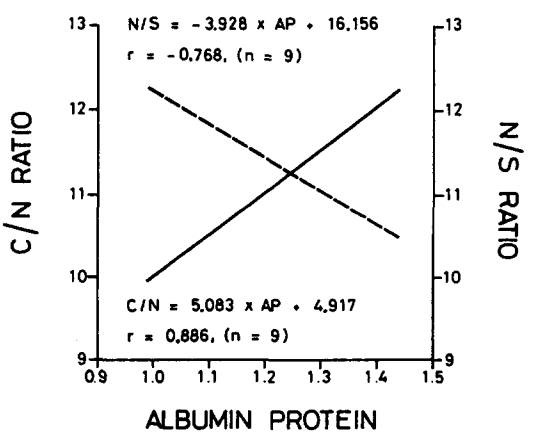

Fig. 1. Linear regressions from SF size (4·7–7·5 mm) to FN size (8·3–8·8 mm) of albumin protein (AP) and C/N ratio (C/N) (solid line) and albumin protein (AP) and N/S ratio (N/S) (dashed line). AP is expressed as mg of protein in ml of extract, and C/N and N/S ratio as dry weight.

SDS-PAGE test of albumin pea protein

On SDS-PAGE the albumin fraction of the different pea seed sizes are resolved into polypeptide bands, and the optical density of each polypeptide band is shown in Table 3. The number of electrophoretic bands was 10 for all sizes. Grant *et al.* (1976) reported ten bands too, using SDS-PAGE. However, Bhatty (1982) reported a higher number of bands (25 bands) following the same electrophoretic system used in the present study. Fox *et al.* (1964) observed 24 bands but in this case using a non-dissociating, discontinuous buffer disc electrophoresis system. The densitometric scanning of polyacrylamide gel electrophoretic patterns of the total albumin reveals seven bands after staining with Amido Black when distilled water is used for extraction (Lástity *et al.*, 1986).

TABLE 3
Optical Density Reading at 530 nm for each Polypeptide Band of Albumin Fraction for each Pea Size[a]

Polypeptide MWt band	*SF*	*VF*	*FN*	*MD*
80 000	0·240	0·480	0·708	0·843
66 000	0·373	1·162	1·255	1·277
43 000	1·204	1·855	1·941	1·782
40 000	0·703	0·809	1·466	1·320
38 000	0·516	0·811	1·437	1·311
23 000	0·634	0·720	0·523	0·484
14 000	0·161	0·186	0·418	0·254
10 000	—	—	0·375	0·195

[a] Size abbreviations: SF, superfine (4·7–7·5 mm); VF, very fine (7·6–8·2 mm); FN, fine (8·3–8·8 mm); MD, middle (8·9–10·2 mm).

The band intensities remain almost constant among sizes because the total amount of protein used for electrophoresis was always the same (50 μg). However, all sizes contained a heavily stained band mainly in the middle of the gel, and it is represented as 43 000 MWt. This band of higher optical density (Table 3) can be related with the band whose Rm value is 0·61 described by Lástity *et al.* (1986) as the major albumin component. The 43 000 MWt polypeptide increased its optical density during the growth period (from SF size to FN size), but decreased in the maturation period (from FN size to MD size) (Table 3). In pea seeds of Feltham variety, two closely related major albumin proteins have been isolated by Croy *et al.* (1984) using SDS-PAGE: the larger protein, designated PMA-L, has

53 000 MWt and consists of two 25 000 MWt subunits, and the smaller, PMA-S, has 48 000 MWt and contains two 24 500 MWt subunits. Both proteins contained significant amounts of sulphur amino acids.

In the high molecular weight area, the polypeptides of 66 000 MWt and 80 000 MWt increased, and at the middle of the gel the 38 000 MWt and 40 0000 MWt increased (Table 3). Peptides of large MWt, as described by Murray (1979), had a relatively gradual decline, not fast, during imbibition of the seeds.

Another heavily stained polypeptide, especially in SF and FN sizes, was 23 000 MWt, which was considered by Murray (1979) and Schroeder (1984) the most persistent polypeptide during germination. This polypeptide was fairly constant in the four pea sizes (Table 3). The polypeptides of low MWt (14 000 and 10 000) appeared in the larger sizes (FN and MD samples, Table 3). These low MWt proteins may be related with Psa LA described by Gatehouse (1985) on SDS-PAGE in 17% (w/v) polyacrylamide gel.

Martens (1986) considered that TV alone was a relevant but not an adequate predictor of internal pea quality. However, this investigation has demonstrated that the pea protein quality can be estimated during the growth period of the seed (from SF size to FN size) as a function of TV,

TABLE 4
Linear Regression of Tenderometric Value (TV) and Pea Protein Quality Indices

Index	*Linear regression*	*Pearson's coefficient*
Crude protein	$CP = 0{\cdot}51 \times TV - 21{\cdot}85$	0·771*
Total sulphur	$TS = 5{\cdot}11 \times 10^{-4} \times TV + 0{\cdot}38$	−0·892**
Albumin protein	$AP = 0{\cdot}02 \times TV - 0{\cdot}71$	0·862**

* Significant at $p \leq 0{\cdot}05$; ** Significant at $p \leq 0{\cdot}01$.
CP, crude protein; TS, total sulfur; AP, albumin protein.

because CP and TS are a function of TV (Table 4). A confirmation of pea protein quality can be realized by AP determination, because C/N and N/S ratios are a function of AP (Fig. 1). In terms of pea size, the values of these indices define the FN and MD sizes as mature stages and make them suitable for canning purposes, because peas which are too soft (immature peas) can be dissolved in can liquids during processing (Martin, 1977). On the other hand, SF and VF sizes, immature stages, are still building up their structures (Miller & Spencer, 1974) with changes in their polypetide composition as is shown by electrophoretic patterns.

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