Int. Agrophysics, 1994, 8, 311-317

CHANGES IN MICROSTRUCTURE AND PHYSICAL PROPERTIES OF GREEN PEAS DURING RIPENING

J. Sadowska, J. Fornal, B. Kaczyńska, B. Jacórzyński

Division of Food Science, Centre for Agrotechnology and Veterinary Sciences, Polish Academy of Sciences, Tuwima 10, 10-718 Olsztyn, P.O.Box 5, Poland

A b s t r a c t. Seeds of two Polish varieties of green pea, Polar and Werona, were investigated. Seeds were collected from 17th June to 6th July 1992, i.e., from 14th to 38th day after flowering. Dry mass, size, mass of 1000 seeds, share of cotyledon mass in seeds and hardness of seeds (measured as force and energy to puncture a seed) were determined. Microstructure of seeds was analysed in SEM type JSM 5200. Seeds of Werona variety ripened earlier than those of Polar. During ripening the mass of 1000 seeds and seed size of Werona variety were lower than the respective values for Polar variety, but its mass cotyledon share was higher. The cotyledons and seed coats of Polar seeds were also harder than those of Werona at the same ripening phase. Initially, up to day 22 after flowering for Polar and day 24 for Werona the maximum of puncture stress was equal to the pucture stress for seed coat. After that period the maximum value depended on increasing cotyledon hardness. The difference in hardness of the examined green pea varieties is reflected in their structure. Particularly clear differences, concerning both thickness and microstructure elements, were observed in the seed coat.

K e y w o r d s: green peas seeds, microstructure, physical properties

INTRODUCTION

High nutritional value of green peas is limited by a number of natural antinutritional factors such as amylase inhibitors, phytins, phenolic compounds as well as goitrogenic and flatulogenic agents. The latter are being formed during the last phase of pea ripening, so it is not recommended to harvest too ripe seeds [5]. Attempts are also to cultivate varieties with prolongated ripening time what will permit to obtain seeds rich in protein and some mineral elements, such as iron, cooper or zinc and with reduced content of antinutritional and flatulogenic components. Establishing of a simple index of ripeness phase is very important to determine technological usability of seeds. Objective estimation of the ripeness stage based on determining of the content of dry matter or the level of characteristic chemical compounds (mono- and polysaccharides) [5] cannot be applied in industry due to the long time of analysis. It seems, that step change of the values of some mechanic properties of seeds can compose such index [2,4]. Choice of proper property and determination of a relevant standard range of its values for practical purposes requires studies on these values variability for different varieties grown in diverse conditions for several years. The aim of the study was to determine the changes in selected physical properties and microstructure of green pea seeds during ripening.

MATERIAL AND METHODS

Seeds of two Polish early green pea varieties Polar (standard) and Werona were investigated. Samples of seeds were collected from 17th June which was the 14th (for Polar) and 19th (for Werona) day after flowering. The following values were determined:

- dry matter content in seeds;
- mass of 1 000 seeds;
- linear dimensions of seed, i.e., length measured along the axis parallel to hilum and thickness measured along the axis perpendicular to hilum;
- seed hardness measured as puncture stress and puncture energy at the following working parameters of INSTRON 1011: cylindrical penetrator with 1.25 mm^2 cross-section area, load transducer capacity of 50 N, crosshead speed 10 mm/min;
- seed coat and cotyledon share in total seed mass. Ten gram sample was soaked in water (1:5) for 18 h and next seeds were hulled manually. Seed coats and cotyledons were dried separately in a laboratory drier to constant mass.

The microstructure of seeds was examined in a scanning electron microscope JSM 5200 at 15 kV voltage.

RESULTS AND DISCUSSION

Changes in physical and mechanical properties of green peas were analysed with regard to the number of days after flowering irrespective of the sampling time. Dimensions of single seed and mass of 1 000 Polar variety seeds (Table 1) were increasing till 26th day after flowering. Between 26th and 29th day after flowering these values dropped and remained on a constant level till the last day of sampling (33rd day after flowering). Maximum dimensions and mass of 1 000 seeds of Werona variety (Table 1) were found on 31th day after flowering. Also in this case between 31st and 34th day after flowering the determined values dropped and remained at constant level till the end of experiment. During ripening Polar seeds were always slightly larger and heavier than Werona ones, but this difference disappeared by the day of harvest. Still, the mass of 1 000 seeds of Werona variety remained lower, which at similar sizes most likely resulted from their significantly higher content of dry matter. Change in dry matter content in Polar seeds (Table 1) can

Table 1. Changes of some physical properties of Polar and Werona green pea seeds during ripening

Day after flowering	Size		Mass of 1 000 seeds (g)	Dry matter content (%)	Share of seed coat (%)
	length (mm)	thickness (mm)			
			Polar		
14	7.2	6.5	211.4	20.4	51.1
16	7.6	7.1	262.8	21.3	31.1
19	9.0	7.8	387.1	24.2	27.4
22	9.0	8.3	442.3	27.2	18.1
26	10.1	9.6	485.6	28.8	16.8
29	9.1	9.0	435.4	34.2	13.8
33	8.6	7.6	428.7	35.8	13.4
			Werona		
19	7.5	6.0	192.7	23.2	33.6
21	8.2	7.0	276.1	25.7	21.5
24	8.6	7.4	355.6	26.8	17.0
27	9.1	7.8	426.5	35.5	16.1
31	9.8	8.3	443.6	37.5	11.9
34	8.8	7.6	375.4	40.2	11.5
38	8.8	7.6	384.9	48.0	11.3

be described as follow:

$$DM = 8.136 + 0.849 \cdot D^* \quad (1)$$

where DM is dry matter content (%), and D^* is the number of days after flowering. High values of correlation and determination coefficients (respectively, 0.988 and 97.7 %) confirm the truthfulness of the accepted formula in the investigated range of water content in seeds, i.e., 23.0-48.0 %. Respective equation for changes in dry matter content in Werona seeds (Table 1) for water content range from about 20.0 to 36.0 % is:

$$DM = -1.441 + 1.27 \cdot D^* \quad (2)$$

In this case correlation and determination coefficients were 0.982 and 96.4 %, respectively. Higher curve slope for Werona seeds confirms that during ripening the increase of dry matter content in seeds of this variety was actually higher. The share of the seed coat continously diminishes during seeds ripening of both varieties (Table 1). Yet, up to 26th day after flowering the seed coat share is higher in Werona variety and after that point in Polar variety. A distinct increase in the cotyledon share was observed on 24th (Polar) and 29th (Werona) days after flowering. After that time, the percentage of the seed coat and cotyledon remained at a constant level, what seems to confirm technological ripeness of seeds harvested later. The share of the seed coat in the Polar seeds was generally higher during all ripening period.

To eliminate the difficulties in measuring of seed mechanical properties [2,3] (dimensions of seeds change during ripening and compression), a classical pucture test was applied to determine seed hardness. As the investigated seeds differed in thickness during various phases of ripening, the values of puncture stress measured for the whole seeds are not quite comparable, especially in puncture energy. Determined values were converted to 1 mm of seed thicknes what partly allowed to neutralize this size difference. The results for whole seeds and converted to 1 mm of seed thickness are presented in Table 2. A typical force-deformation curves for puncture of seeds in various phases of ripening were presented in Fig. 1. Up to 22nd day (Polar) and 24th day (Werona) after

Table 2. Changes of mechanical properties of Polar and Werona green pea seeds during ripening

Day after flowering	Puncture strain (%)	Puncture stress			Puncture energy		
		seed (N/mm^2)	coat (N/mm^2)	cotyl. (N/mm^2)	seed (mJ/mm)	coat (mJ)	cotyl. (mJ/mm)
				Polar			
14	17.4	2.6	2.4	1.0	0.9	0.9	0.8
16	19.2	3.6	3.6	1.1	2.0	2.1	1.6
19	10.5	3.7	3.7	2.2	2.9	3.5	2.3
22	14.0	5.4	5.4	3.4	4.4	3.2	3.5
26	11.0	6.3	5.4	4.9	5.6	6.3	4.3
29	13.8	7.3	7.0	5.4	6.1	4.9	5.6
33	15.3	7.4	7.0	5.4	5.7	4.7	5.1
				Werona			
19	17.8	3.1	3.1	1.9	0.9	1.0	1.1
21	16.7	3.8	3.8	2.5	2.0	2.0	2.5
24	15.3	5.4	4.3	4.2	2.9	5.7	3.8
27	14.9	6.4	4.7	4.9	4.4	5.9	4.3
31	13.7	8.0	5.5	6.2	5.6	6.8	5.5
34	17.1	8.2	6.8	6.1	6.1	5.9	6.1
38	14.5	8.9	7.2	7.0	5.7	3.9	6.1

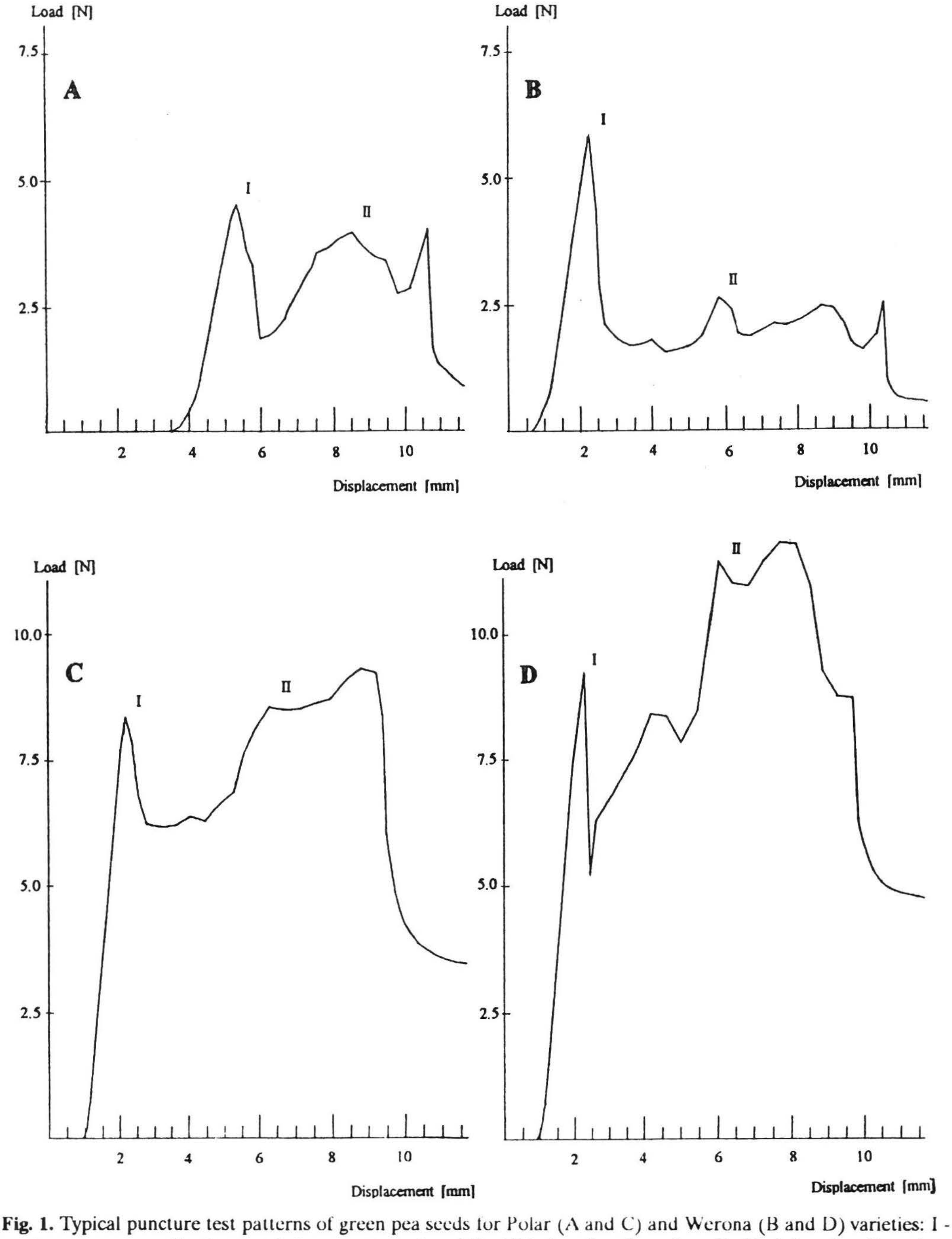

Fig. 1. Typical puncture test patterns of green pea seeds for Polar (A and C) and Werona (B and D) varieties: I - seed coat puncture; II - first cotyledon puncture; A and B - 19th day after flowering; C - 33rd day after flowering; D - 38th day after flowering.

flowering the value of maximum puncture stress of seeds was equal to puncture stress of seed coat (Figs 1A and 1B). After that time, the maximum value of puncture stress depended only on the increased hardness of cotyledons (Figs 1C and 1D).

At the beginning of ripening higher values of maximum puncture stress and puncture stress for cotyledon were found for seeds of Polar variety (Table 2). However, between 27th and 29th day after flowering these stresses for seeds of Werona variety reached higher values (Table 2) thus the hardness of cotyledons of ripe Polar seeds was lower. That result suggests different rate of cotyledon ripening of these varieties. The values of puncture stress for the seed coat during all ripening period were higher for Polar variety what points to its higher thickness and mechanical resistance. It seems important that in spite of a continously increase of puncture stress for whole Polar seeds, from 29th day after flowering the values of puncture stresses for seed coats and cotyledons remain at a constant level unlike in Werona seeds (Table 2).

Similarly for stress, initially more energy was used to puncture the cotyledons of Polar seeds, but in the final phase of ripening the puncturing of Werona seeds required more energy. Puncture energy consumption did not increase continuously; maximum energy needed for puncturing of the whole seeds and cotyledons of Polar and Werona varieties were observed on 29th and 34th day after flowering, respectively. Maximum energy used for puncturing of the seed coat already on 26th and 31st day after flowering were observed (Table 2). The deformation and strain values measured at seed coat failure decreases as ripening proceeds (Table 2), which means that seed elasticity diminishes. This fact and dramatic drop in seed coat puncture energy confirm a significant change in rheological character of the seed coat. From being thick (compared with seed thickness) and elastic (resistant to greater stresses) the seed coat becomes thinner and

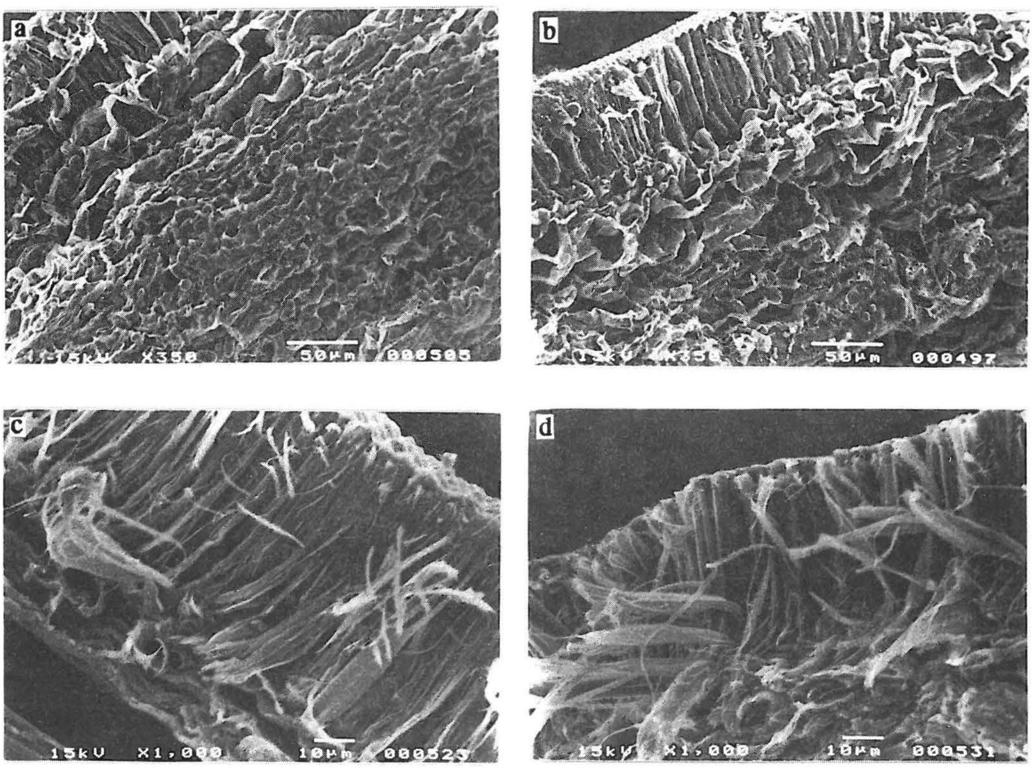

Photo 1. Seed coat microstructure of green peas at various days after flowering for: var. Polar, 16th day, (a); var. Verona, 21st day, (b); var. Polar, 33rd day, (c); var. Werona, 38th day, (d).

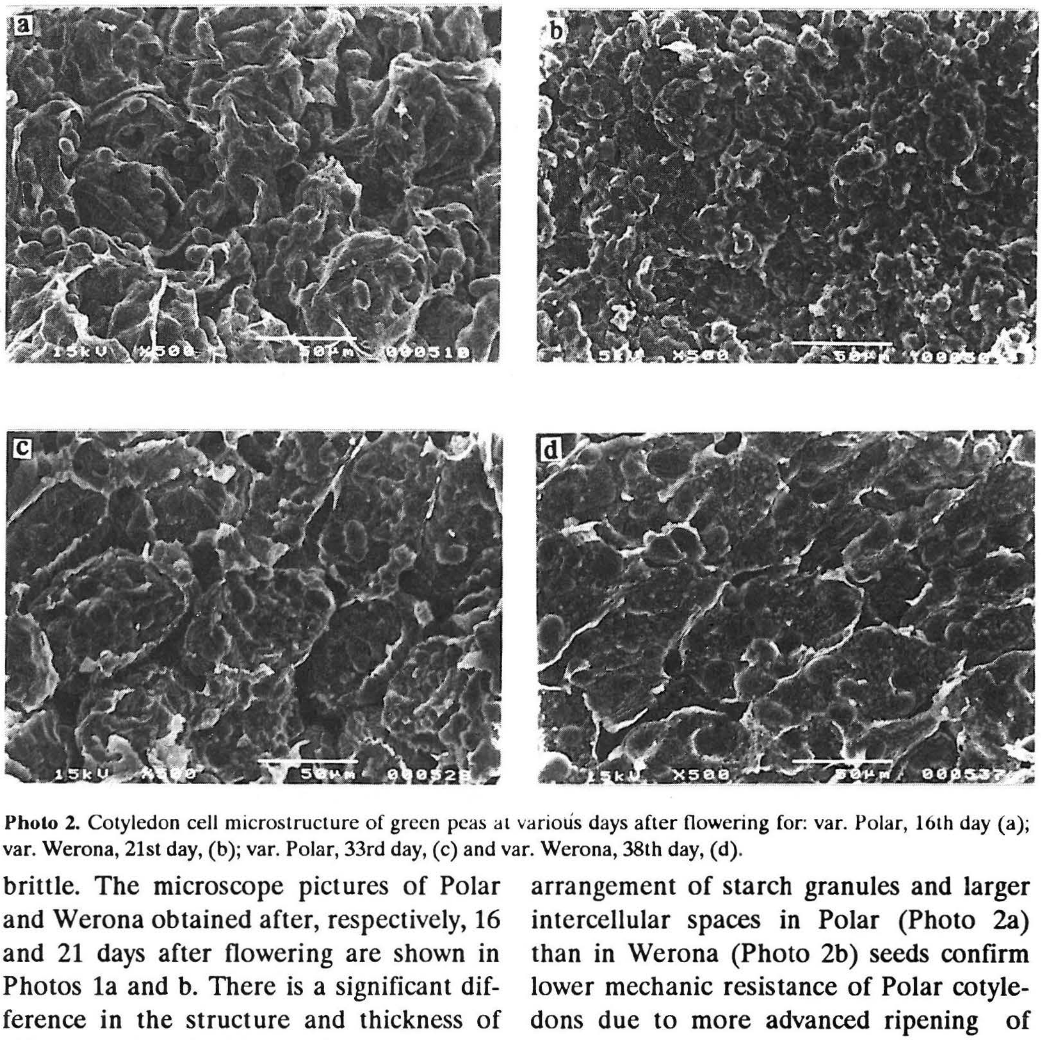

Photo 2. Cotyledon cell microstructure of green peas at various days after flowering for: var. Polar, 16th day (a); var. Werona, 21st day, (b); var. Polar, 33rd day, (c) and var. Werona, 38th day, (d).

brittle. The microscope pictures of Polar and Werona obtained after, respectively, 16 and 21 days after flowering are shown in Photos 1a and b. There is a significant difference in the structure and thickness of this part of seed which decides percentage of the seed coat in the whole seed (Table 1) and which results from the various phase of ripeness. A sponge-like character of the seed coat, made mainly of parenchyma, decides the low values of stress at which the seed coat gets punctured (Table 2). In the spongy cells of parenchyma starch granules were observed, which so far have not been reported elsewhere. Even greater diversification was noticed in the structure of cotyledon cells in seeds of both varieties (Photo 1b), which was generally similar to bean structure at the same phase of ripeness [1]. Poorer filling of cells with protein, looser arrangement of starch granules and larger intercellular spaces in Polar (Photo 2a) than in Werona (Photo 2b) seeds confirm lower mechanic resistance of Polar cotyledons due to more advanced ripening of these seeds at sampling. Fracture of a ripe seed coat requires slightly greater stress but the value of used energy is lower than in the case of seeds in earlier phase of ripening. This is mainly caused by completely developed compact structure of palisade layer. Similar seed coat structure of both pea varieties from last sampling (Photos 1c and d) explains why puncture stress were almost the same for both varieties. Clear difference of the values of cotyledon puncture stress (Table 2) results from the persisting differences in cotyledon structure of both varieties. The cells of Polar variety are characterized by clearly formed starch granules braided by structureless protein substance and numerous large intercellular spaces (Photo 2c). In the cells of Werona variety in a later phase of ripening the spaces considerably decreased what resulted in forming of a very compact structure, more mechanical resistant. Additional element causing greater mechanical resistance of ripe Werona cotyledons is the presence of granular protein bodies combined with structureless protein substance and tightly braided around starch granules (Photo 2d). Such picture of cell structure is typical for other legume seeds over 30 days after flowering [1].

CONCLUSIONS

1. Apart from ripeness phase, a clear variety effect on the size and mass of 1000 seeds was observed. Seeds of Polar variety are greater, have higher mass of 1 000 seeds and cotyledon share.

2. Changes in seed hardness during ripening proceed at various rate: at the same stage of ripening Polar seeds are initially harder but later they are exceeded by Werona seeds. The most significant for mechanic properties on whole seeds in the initial stage of ripening is the seed coat, and later - cotyledon structure.

3. Great hardness of ripe Werona seeds results from their different structure. Especially important is the small share of intercellular spaces and presence of granular protein bodies.

REFERENCES

1. **Hughes J.S., Swanson B.G.:** Microstructural changes in maturing seeds of common bean (Phaseolus vulgaris L.). Food Microstructure, 4(2), 183-189, 1985.
2. **Hoser A., Dobrzycki J.:** Rheological and structural characteristics of green peas. Żywienie Człowieka i Metabolizm, 10(4), 237-282, 1983.
3. **Grochowicz J., Nadulski R.:** The mechanical properties of some leguminous plant seeds. Proc. 3rd Int. Conf. Agric. Materials, Prague, 289-292, 1985.
4. **Jech J., Sloboda A., Sosnowski S.:** The effect of physicomechanical properties on the designing of machinery intendent for legume harvest. Zemedelska-Technika, 37, 9, 491-499, 1991.
5. **Jacórzyński B., Osucha A.:** Characteristics of sugars in green legume seeds. Polish J. Food Nutr. Sci., 2/43, 1, 93-103, 1992.