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ARTICLE

Protein content correlates with starch morphology, composition and physicochemical properties in field peas

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Abstract: Protein and starch are two major components in field peas. In this study, we investigated the starch morphologies, compositions, and thermal properties between high protein peas (approximately 30%) and other market types of field peas (yellow, green, maple, and marrowfat peas, with approximately 23% protein contents). For the shape and size, high protein peas had the compound starch granules that could be easily fragmented into small irregular and polygonal granules, whereas other pea types had oval or kidney-like starch granules with high percentage of large granule sizes. High protein peas had significantly lower starch contents (27.2%–34.2%) than other pea types (45.5%–47.4%). However, the amylose content (74.6%–89.2%) in high protein peas were significantly higher that of other pea types (50.1%–54.1%). Our differential scanning calorimeter (DSC) data showed that the onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) of starch gelatinization in high protein peas were significantly higher than those of other pea types, whereas the enthalpy change (ΔH) of high protein peas was significantly lower than those of other pea types. The unique properties of high protein peas characterized in this study provided useful information to further improve pea quality.

Key words: Pisum sativum, protein, starch, SEM, DSC.

Résumé : Les protéines et l'amidon sont deux grandes composantes du pois. Dans le cadre de cette étude, les auteurs ont examiné la morphologie, la composition et les propriétés thermiques de l'amidon des variétés de pois à haute teneur en protéines (30 % environ) et des autres variétés commerciales (pois jaune, vert, perdrix, et Marrowfat, contenant approximativement 23 % de protéines). En ce qui concerne la forme et la taille, les variétés riches en protéines sont celles qui comptent les granules d'amidon composé pouvant le plus facilement se fragmenter en petits granules polygonaux irréguliers, les autres variétés ne renfermant que des granules d'amidon ovales ou réniformes et une forte proportion de granules de grosse taille. Les variétés très protéinées contenaient sensiblement moins d'amidon (27,2 % à 34,2 %) que les autres sortes de pois (45,5 % à 47,4 %). Toutefois, la concentration d'amylose (74,6 % à 89,2 %) des pois à haute teneur en protéines était significativement plus élevée que celle relevée chez les autres cultivars (50,1 % à 54,1 %). Les données obtenues avec le calorimètre différentiel à balayage indiquent que la température initiale, la température maximale et la température terminale de la gélatinisation de l'amidon sont significativement plus élevées chez les pois très riches en protéines que chez les autres types, alors que la variation d'enthalpie (ΔH) est significativement plus faible chez les pois très protéinés, comparativement aux autres variétés commerciales. Les propriétés uniques des pois à haute teneur en protéines caractérisées dans cette étude fournissent des informations utiles en vue d'une amélioration de la qualité de cette culture. [Traduit par la Rédaction]

Mots-clés : Pisum sativum, protéines, amidon, microscopie électronique à balayage, calorimètre différentiel à balayage.

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Introduction

Field pea (*Pisum sativum* L.) is cultivated in many regions of the world and its production ranks fifth in the world for food legumes after soybean, peanut, drybean, and chickpea. Canada is the largest field pea producer and exporter in the world, with an annual acreage of approximately four million acres and an annual production of almost three million tons. Field pea is mainly used as a protein source, as it has a relatively rich and unique protein profile, different from other natural protein sources (APGC 2010). Pea protein is valued for its high digestibility (90%–95%) and has less allergenic responses and no negative health controversies. It is gluten-free, low in the sulfurous amino acids (cysteine and methionine), but rich in lysine, an essential amino acid for human health (Pownall et al. 2010). Compared to cereals (almost deficient in lysine), pea contains significantly high lysine (approximately 7% of protein). The high lysine content in pea grains and fractions greatly improves their nutritional values and the combination of rice and pea protein has been widely used in health foods to achieve a superior amino acid profile (Sánchez-Lozano et al. 2009). Field peas normally contain approximately 23% protein, but novel pea germplasms which contain approximately 30% protein have been discovered (Bing 2007, 2010*a*). After a long term of AAFC field pea breeding practice, advanced pea lines with approximately 30% protein, plus good agronomic traits, have been developed (Bing 2010*b*, 2012).

According to the position of diffraction peaks, starch can be divided into three main polymorphs, namely A, B, or C type patterns (Cairns et al. 1997; Bogracheva et al. 1998). The A type pattern (e.g., waxy maize starch) is more dense than the B type pattern (e.g., potato starch). The C type pattern is between A and B polymorphs and pea starch is a typical C polymorph (Cairns et al. 1997; Bogracheva et al. 1998). Starch granules mainly consist of linear amylose and branched amylopectin. Amylopectin is one of major components in field pea starch and its average molecular weights vary from 10^{-7} to 10^{-9} Da (Aberle et al. 1994). Amylopectin consist of a backbone of (1→4)-α-D-glucose residues connected the branch chain through (1→6)-α-linkage. Pea starch is obtained as the byproduct of protein extraction, so field pea is considered to be a relatively cheap source of starch compared with corn, wheat, and potato (Ratnayake et al. 2002). Pea starch not only can be extensively used in food industry, but also can be processed into nanocomposites (Ma et al. 2008; Yu et al. 2009). Therefore, it is essential to explore the relationship between the protein and starch contents and study the physicochemical properties of high protein peas to explore their potential utilization.

Although field pea has been identified as a health food with a low glycemic index (GI) for decades, the information on contents and compositions of its protein and starch has been limited to only a few germplasm, cultivars or varieties (de Almeida Costa et al. 2006; Hoover et al. 2010). Several pea market types (including yellow, green, maple, and marrowfat peas) are available for commercial production and various milling products that contain pea fractions (flour, starch, protein, fiber, etc.) have been developed and characterized. However, only limited amounts of pea protein and starch fractions are currently used as food ingredients in the world market. In this study, we investigated protein contents and starch morphology, compositions and physiochemical properties in field pea germplasm, cultivars, and breeding lines to explore knowledge gaps that prevent the full utilizations of pea grains and fractions in functional food production.

Materials and Methods

Plant materials

54 field pea germplasm, cultivars or breeding lines of 5 pea types including 14 yellow peas, 11 maple peas, 7 green peas, 9 high protein peas, and 13 marrowfat peas collected from the AAFC field pea breeding program were analyzed in this study. The detailed information of materials is shown in Table 1.

Near infrared spectroscopy for protein content

Approximately 10 to 15 intact seeds from each sample were randomly selected from 54 individual field peas and analyzed by NIR for the determination of protein and starch contents. The monochromator NIR System (Model 6500 NIR Systems, Inc., Silver Springs, MD, USA) was used with a small ring cup (Ref. IH-0307, NIR Systems), equipped with a microsample insert (Ref. IH-0337, Ø18.5 mm). The reflectance spectra (log 1/R) from 400 to 2500 nm were recorded at 2 nm intervals. For calibration, only the spectral data from 1100 to 2500 nm were used. All the measurements were done in triplicate.

Isolation and purification of starch granules

Pure starch granules were isolated from mature field pea seeds as described by Li et al. (2012). Two seeds were steeped in 1 mL H_2O at 4 °C overnight and then ground in mortar with pestle. The slurry of each sample was transferred into a microtube and centrifuged. The pellet was then resuspended in H_2O and overlaid on 80% wt vol^{-1} cesium chloride followed by centrifuge. The purification procedure with cesium chloride was repeated twice. The granule pellet was washed twice with 0.8 mL wash buffer (62.5 mmol L^{-1} Tris-HCl, pH 6.8, 10 mmol L^{-1} EDTA, 4% SDS, and 5% β-mercaptoethanol), once with H_2O and once with acetone. The starch granules were air-dried and stored at −20 °C.

Determination of total starch and amylose contents

Total starch content of flour sample was measured using the AOAC Method 2002.2 (K-RSTAR, Megazyme, Ireland) according to the provided protocol. The percent amylose content was determined by the iodine binding

Table 1. The detailed information of materials.

ID	Name	Type	Protein (%)	Starch (%)	Amylose (%)	Breeder or origin	Pedigree
FP-62	Agassiz	Yellow pea variety	27.08	44.01	52.73	AAFC	MP1392/Grande
FP-2	CDC Golden	Yellow pea variety	24.20	49.73	47.03	CDC	MCPRV
FP-52	P0718-105	Yellow pea line	24.95	48.11	48.98	AAFC	RCPC
FP-53	P0725-105	Yellow pea line	21.44	57.53	50.27	AAFC	RPPC
FP-54	P0730-105	Yellow pea line	25.08	45.74	52.99	AAFC	Agassiz//Polstead/CDC715S-4
FP-55	P0730-110	Yellow pea line	24.02	46.17	52.58	AAFC	Agassiz//Polstead/CDC715S-4
FP-56	P0731-125	Yellow pea line	25.88	45.75	52.08	AAFC	Agassiz//Reward/Canstar
FP-57	P0735-101	Yellow pea line	22.75	50.72	44.32	AAFC	CDC Treasure//Polstead/CDC715S-4
FP-58	P0735-122	Yellow pea line	24.31	45.59	47.58	AAFC	CDC Treasure//Polstead/CDC715S-4
FP-59	P0736-111	Yellow pea line	25.05	45.33	51.06	AAFC	CDC Treasure//Reward/Canstar
FP-60	P0739-114	Yellow pea line	23.64	46.45	51.86	AAFC	Cutlass//Polstead/CDC715S-4
FP-61	P0748-101	Yellow pea line	25.92	45.16	49.51	AAFC	Hugo//Reward/Agassiz
FP-63	Peace River	Yellow pea variety	22.69	48.45	53.90	AAFC	P9561098//Eclipse/MP1566
FP-64	Reward	Yellow pea variety	22.94	46.60	51.52	AAFC	4-0359.016/MP1491
FP-4	CDC Striker	Green pea variety	26.63	46.61	54.14	CDC	MCPRV
FP-34	CDC647-1	Green pea line	28.50	44.66	54.79	CDC	Unknown
FP-24	Cooper	Green pea variety	26.38	44.86	53.91	LNL	Baccara/Cebeco 92585
FP-6	Mendel	Green pea variety	25.40	47.62	54.18	AAFC	9427004/Carneval
FP-21	P0707-102	Green pea line	24.74	48.79	56.37	AAFC	Agassiz//Polstead/CDC715S-4
FP-22	P0707-105	Green pea line	24.62	46.59	59.76	AAFC	Agassiz//Polstead/CDC715S-4
FP-15	P0709-106	Green pea line	22.66	50.02	47.41	AAFC	Cooper/CDC Patrick
FP-7	Courier	Maple pea variety	22.65	46.50	53.29	CFRNZ	Unknown
FP-9	P0609-08	Maple pea line	22.31	49.85	49.03	AAFC	CDC Acer/Reward
FP-35	P0845-13	Maple pea line	27.28	42.13	53.22	AAFC	LAN3017/CDC Acer
FP-40	P0845-16	Maple pea line	26.08	46.66	50.76	AAFC	LAN3017/CDC Acer
FP-39	P0845-18	Maple pea line	26.55	46.15	58.83	AAFC	LAN3017/CDC Acer
FP-36	P0846-03	Maple pea line	27.15	44.43	58.99	AAFC	LAN3017/Courier
FP-37	P0846-06	Maple pea line	27.32	42.46	58.60	AAFC	LAN3017/Courier
FP-41	P0846-08	Maple pea line	24.60	46.62	51.45	AAFC	LAN3017/Courier
FP-42	P0846-12	Maple pea line	24.55	48.39	46.72	AAFC	LAN3017/Courier
FP-43	P0846-14	Maple pea line	25.07	47.87	51.22	AAFC	LAN3017/Courier
FP-38	P0848-03	Maple pea line	24.38	44.53	55.91	AAFC	LAN3019/Courier
FP-51	Kahuna	Marrowfat pea variety	27.74	43.59	49.02	TPBDM	Unknown
FP-50	LAN 2032	Marrowfat pea line	26.50	42.88	50.34	LNL	Unknown
FP-44	LAN3017	Marrowfat pea line	26.26	45.71	55.10	LNL	Kabuli/Tamora
FP-16	P0716-105	Marrowfat pea line	27.32	44.68	50.14	AAFC	Kabuli/Tamora
FP-17	P0716-109	Marrowfat pea line	25.71	47.24	45.22	AAFC	Kabuli/Tamora
FP-45	P0716-13	Marrowfat pea line	25.72	46.10	51.95	AAFC	Kabuli/Tamora
FP-46	P0716-14	Marrowfat pea line	24.64	48.82	48.95	AAFC	Kabuli/Tamora
FP-47	P0716-17	Marrowfat pea line	26.00	44.63	52.91	AAFC	Kabuli/Tamora
FP-48	P0717-05	Marrowfat pea line	26.75	47.86	48.33	AAFC	Kahuna/Tamora
FP-49	P0717-06	Marrowfat pea line	26.12	45.89	48.83	AAFC	Kahuna/Tamora

(*continued*).

Table 1. (*concluded*).

ID	Name	Type	Protein (%)	Starch (%)	Amylose (%)	Breeder or origin	Pedigree
FP-18	P0717-108	Marrowfat pea line	25.72	47.30	55.14	AAFC	Kahuna/Tamora
FP-19	P0717-109	Marrowfat pea line	25.75	46.08	47.49	AAFC	Kahuna/Tamora
FP-20	P0717-112	Marrowfat pea line	26.54	45.55	52.68	AAFC	Kahuna/Tamora
FP-31	MI3391	High protein germplasm	34.87	27.22	79.83	GC	Unknown
FP-32	P0538-24	High protein line	32.99	29.59	86.02	AAFC	MI3391/CDC647-1
FP-29	P0538-60	High protein line	33.29	29.24	79.22	AAFC	MI3391/CDC647-1
FP-30	P0538-7	High protein line	32.62	27.49	89.18	AAFC	MI3391/CDC647-1
FP-12	P0540-41	High protein line	31.71	32.18	74.56	AAFC	MI3391/Reward
FP-33	P0540-59	High protein line	32.98	32.52	85.83	AAFC	MI3391/Reward
FP-27	P0540-70	High protein line	32.66	30.75	84.26	AAFC	MI3391/Reward
FP-28	P0540-9	High protein line	31.79	29.63	87.60	AAFC	MI3391/Reward
FP-13	P0540-91	High protein line	31.64	34.20	76.74	AAFC	MI3391/Reward

Note: AAFC, Agriculture and Agri-Food Canada; CDC, Crop Development Centre, University of Saskatchewan; LNL, Limagrain, the Netherlands; CFRNZ, Crop & Food Research, New Zealand; TPBDM, Toft Plant Breeding, Denmark; GC, Germplasm Collection, MCPRV, CDC Mozart/3/Carneval/PI251051//Radley/CDC Vienna; RCPC, Reward/Canstar//Polstead/CDC1308T-10; RPPC, Reward/P9904601//Polstead/CDC715S-4.

assay as described by He et al. (2012) and Zhu et al. (2008) with some modifications. Ten mg of starch granule sample was first suspended in 95% ethanol and dissolved in 1 mL of 1 N NaOH. The solution was then diluted 10 times with H_2O and neutralized with 0.1 N HCl. Finally, the solution was diluted to a final 0.25 mg mL^{-1} as starch stock solution. Blue color was developed by incubating 0.1 mL of starch stock solution with 1.8 mL water and 0.1 mL of KI–I_2 solution (2% potassium iodide and 0.2% iodine, wt vol^{-1}) at room temperature for 30 min. The absorbance was recorded at 625 nm with spectrophotometer. Amylose content in starch was calculated by the following formula based on the established amylose standard curve.

$$\text{Amylose (\%)} = (1.574885 \times \text{Absorbance } 625 - 0.29545) \times 100$$

Morphological observation of starch granules

The starch granules were placed on aluminum stubs with a double-adhesive tape and the granular morphology was imaged by using a scanning electron microscopy (SEM) (Hitachi S570, Hitachi High Technologies, Tokyo, Japan) at an accelerating voltage of 10 kV, equipped with Quartz PCI software for digital image acquisition (Quartz Imaging, Vancouver, Canada). Starch granular sizes and distributions were evaluated with a flow particle image analyzer (FPIA-3000, Sysmex Corporation, Japan). Starch samples (50 mg mL^{-1}) were vigorously vortexed in 1.5 mL microfuge tubes and 400 μL of the solution was added to the circulating sample intake module of the equipment. A minimum of 2500 starch granules were analyzed in each replication (He et al. 2012).

Characterization of starch thermal properties

Thermal properties of native, gelatinized starch were analyzed using a differential scanning calorimeter (DSC 2920, TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system (RCS). The starch sample (10 mg) was precisely weighed into the aluminum Tzero pan (TA Instruments, USA) and mixed with 20 μL H_2O at a starch:water ratio of 1:2. The pan was sealed and equilibrated at room temperature for 1 h. The heating rate was at 10 °C min^{-1} over the temperature range of 30 °C–100 °C. The instrument was calibrated using indium and an empty pan as reference standards. Enthalpy change (ΔH), gelatinization onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) were measured using the Universal Analysis 2000 v 4.7A software (TA Instruments, USA).

Amylopectin analysis for degree of polymerization

Amylopectin was debranched using isoamylase (Jane et al. 1999; Zheng et al. 2015). Branch chain-length distribution of amylopectin was then analyzed using a capillary electrophoresis (CE) system (PA800plus, Beckman Coulter Inc., Ontario, Canada) as follows: 5 mg of starch

Fig. 1. Percentages of protein, total starch, and amylose contents in starch granules among 5 types of field peas. Figure appears in colour on the Web.

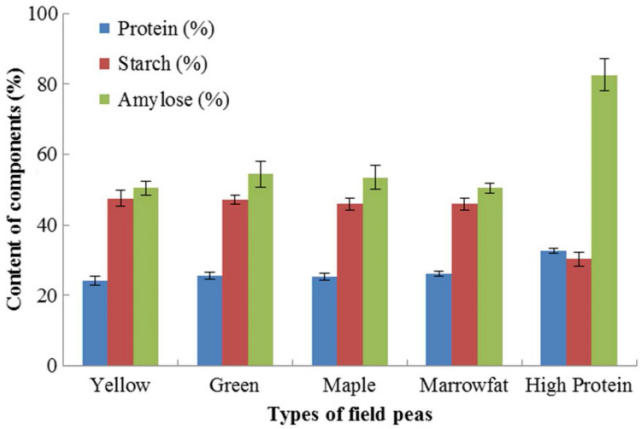
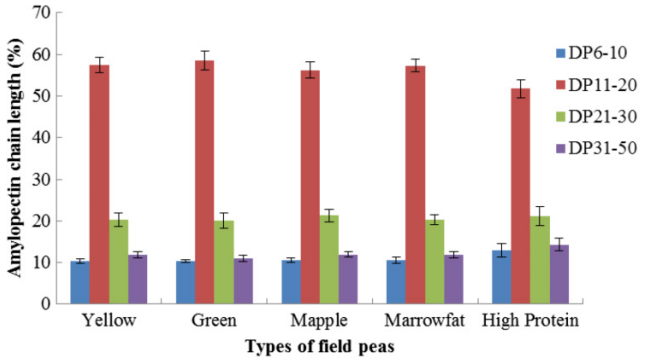


Fig. 2. Relative distributions of amylopectin chain lengths in starch granules of 5 types of field pea samples. Degree of polymorphism (DP) was categorized into 4 groups (DP 6–10, DP 11–20, DP 21–30, DP 31–50). Figure appears in colour on the Web.



was suspended in 5 mL H_2O in a 50 mL glass test tube and heated at 130 °C for 30 min with intermittent vortexing. One mL of the mixed solution was transferred into a 2 mL tube, and then 55 μL of 1 M sodium acetate (pH 4.0) and 4 units of isoamylase (Megazyme, Ireland) were added. The reaction mixture was incubated at 40 °C for 4 h before the reaction was stopped by heating at 95 °C for 20 min. The digested mixture was then freeze-dried and re-dissolved in 1 mL H_2O by heating at 95 °C for 5 min. Ten μL of re-dissolved solution was vacuum-dried and labeled with 8-amino-(1,3,6)-pyrenetrisulfonic acid (APTS) using the Carbohydrate Labeling Kit (Beckman Coulter Inc., Ontario, Canada). The labeled carbohydrate chains were separated by the CE and detected through a laser induced fluorescent (LIF) quipped detector and analyzed for the degree of polymerization (DP) values with the 32 Karat software provided by Beckman Coulter Inc.

Statistical analysis

All experiments were carried out in triplicate and the data were expressed as means ± standard deviations (SD). Difference between various groups was assessed by one-way analysis of variance (ANOVA). If $p < 0.05$, the results were considered to be significant. The analysis of data was performed by statistics analysis system SAS®9.3.

Results

Compositions of field pea

We used NIR to analyze the protein and starch contents in pea seeds. As shown in Fig. 1, the mean protein contents were 24.28%, 25.56%, 25.26%, and 26.21% for pea types of yellow, green, maple, and marrowfat, respectively. The mean protein content of high protein peas was 32.73%, in which MI3391 (FP-31) and P0538-60 (FP-29) contained 34.87% and 33.29%, respectively, significantly higher than those of other pea types. By contrast, the total starch contents were 47.52%, 47.02%, 45.96%, and 45.87% for pea types of yellow, green, maple, and marrowfat, respectively. However, the total starch content of high protein peas was 30.31%, in which MI3391 and P0538-60 only had 27.22% and 29.24%, respectively, significantly lower than those of other pea types. These results indicated that there was a negative correlation between protein and starch contents in field peas (i.e., the higher the protein, the lower the starch, or vice versa).

We found that there were significant differences in amylose contents between high protein peas and other pea types. As represented in Fig. 1, the mean amylose contents in starch granules of yellow, green, maple, and marrowfat peas were about 50.46%, 54.37%, 53.46%, and 50.47%, respectively. However, the mean amylose content in starch granules of high protein peas was approximately 82.58%, in which the lines P0538-7 (FP-30) and P0540-9 (FP-28) had 89.18% and 87.6%, respectively. Our results indicated that the amylose contents of high protein peas were significantly higher than those of other pea types, but there was no significant difference in amylose contents among yellow, green, maple, and marrowfat peas.

The degree of polymerization (DP) of amylopectin in field peas was analyzed and the results were shown in Fig. 2. The majority of amylopectin chain lengths in starch granules of field peas ranged from 11 to 30 glucose units. The percentages of DP 11–30 were 77.76%, 78.73%, 77.5%, 77.55%, and 72.7% for yellow, green, maple, marrowfat, and high protein types, respectively. In addition, the percentages of DP 11–20 in high protein peas were significantly lower than that in other pea types.

Morphological observation of starch granule

We observed the morphology of starch granules in field peas under SEM and found that the granular sizes and shapes were significantly different between high protein peas and other pea types. In general, field peas had starch granules with oval or kidney-like shapes, but

Fig. 3. SEM images (×1000) of starch granules isolated from low protein pea lines CDC Golden (FP-2) and CDC Striker (FP-4), and high protein pea lines P0540-41 (FP-12) and P0540-91 (FP-13).

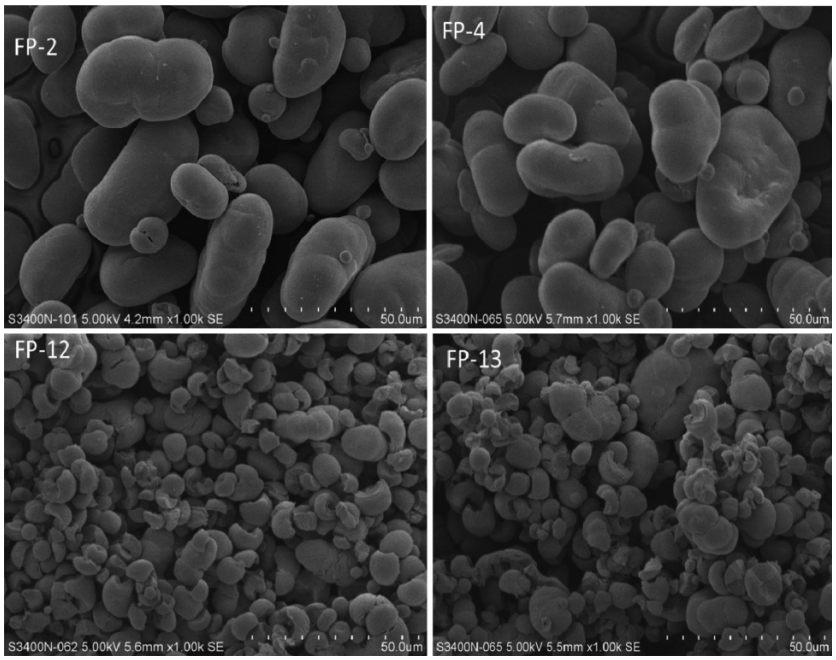
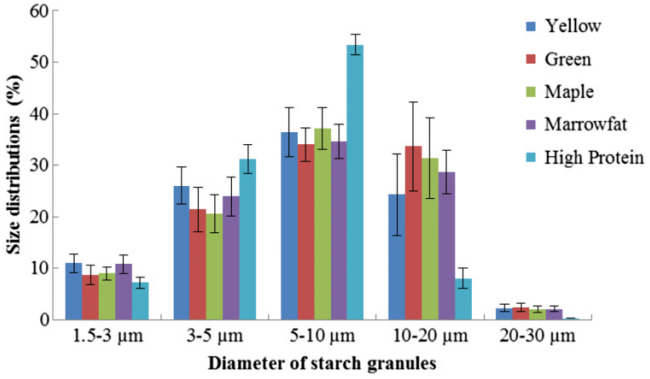


Fig. 4. Size distributions of starch granules among 5 types of field peas. Figure appears in colour on the Web.



the starch granules in high protein peas showed the compound structure with irregular and polygonal shapes (Fig. 3). There were some fissures on the granule surfaces of most pea starches from yellow, green, maple, and marrowfat peas, whereas the cracks were much deep and obvious on the granule surfaces of high protein peas. The large compound granules of high protein peas were easily subdivided and fragmented into several small irregular and polygonal granules along the cracks.

The granular diameters of pea starch were determined and their distribution profiles were shown in Fig. 4. The majority of starch granules in high protein peas ranged from 3 to 10 μm in diameter, whereas the sizes of starch granules in other pea types were mainly distributed at 5–20 μm. From 5 to 10 μm, there were approximately 36.44%, 34%, 37.09%, 34.57%, and 53.37% of total starch granules for pea types of yellow, green, maple, marrowfat, and high protein peas, respectively, in which the granule percentage in high protein peas was significantly higher than that in other pea types. However, the granule percentage of 10–20 μm diameters in high protein peas was significantly lower than that in other pea types. Overall, the granular size of high protein peas was significantly smaller than those of other pea types, but there were no significant differences in granular shapes and sizes among yellow, green, maple, and marrowfat peas.

Thermal property of pea starch

The thermal property of pea starch was measured by DSC. As shown in the Table 2, the peak temperature (T_p) and enthalpy change (ΔH) of starch gelatinization in yellow, green, maple, and marrowfat pea peas were distributed in 66.76–67.41 °C, and 4.91–5.3 J g^{-1}, which indicated that there was no significant difference in starch thermal properties among these pea types. However, the T_p and ΔH values of high protein peas were 79.8 °C and 1.99 J g^{-1}, respectively. Our data analysis showed that the T_o, T_p, and T_c of high protein peas were significantly higher than those of other pea types, whereas the ΔH of high protein peas was significantly lower than those of other pea types. In addition, the temperature range of starch gelatinization in high protein peas was significantly wider than those of other pea types.

Discussion

Pea starch is difficult to isolate and purify, because field pea contains a large amount of insoluble flocculent

Table 2. Starch thermal properties of different types of field peas.

	T_o (°C)	T_p (°C)	T_c (°C)	T_c–T_o (°C)	Enthalpy (J/g)
Yellow	62.17 ± 0.78b	67.20 ± 1.37b	75.48 ± 1.82b	13.32 ± 1.17b	5.30 ± 1.89a
Green	61.64 ± 1.54b	66.76 ± 1.63b	74.70 ± 1.57b	13.06 ± 0.54b	4.91 ± 0.41a
Maple	61.83 ± 0.66b	66.99 ± 1.00b	75.02 ± 1.20b	13.20 ± 0.79b	5.17 ± 0.28a
Marrowfat	62.04 ± 1.42b	67.41 ± 2.10b	75.20 ± 2.12b	13.16 ± 0.74b	5.08 ± 0.38a
High protein	69.64 ± 4.43a	79.80 ± 3.17a	89.9 ± 3.17a	20.26 ± 4.26a	1.99 ± 0.15b

Note: Different letters indicate the statistical significance at $p < 0.05$. T_o, T_p, and T_c mean onset, peak, and conclusion temperature (°C) of starch gelatinization.

protein and fiber, which may combine together with starch granules to interfere with starch precipitation and sedimentation (Reichert and Youngs 1978; Schoch and Maywald 1968). The most common method for pea starch extraction is air classification, which can be used to separate starch from the protein matrix (Ratnayake et al. 2001; Tyler et al. 1981). However, the purity of starch is very low by using this method: only approximately 65% for pea starch (Comer and Fry 1978). Wet milling is another method for pea starch isolation. The starch purity extracted by wet milling is dramatically higher than that by air classification. Colonna et al. (1981) reported that the starch can be up to 93.8%–96.7% by wet milling from smooth peas. In addition, Meuser et al. (1995) developed a process to isolate starch from wrinkled pea and the purity of starch can reach up to 89%. Considering that our materials included both smooth and wrinkled peas and a consistent method is essential for different pea types, we used the density gradient centrifugation method to isolate pea starch in this study. Our results demonstrated that this approach is suitable for the isolation of pure starch granules from all pea types.

The granular morphology of pea starch is various, such as oval, round, spherical, irregular or polygonal shapes. In this study, we found that the granular sizes and shapes are different between high protein peas and other pea types. The high protein peas synthesize the unique compound starch granules, significantly different from other pea types. We have observed the fissures on the granule surfaces, which is consistent with previously reported results (Gujska et al. 1994). Similar to the results of Bertoft et al. (1993), there are two different populations in the size distribution of pea starch in our study. As the compound granules can be fragmented into small irregular and polygonal granules, the size of starch granules in high protein peas are generally smaller than that in other pea types, which is similar to previous reports (Bertoft et al. 1993; Stute 1990).

Protein and starch are two major biomolecules in pea seeds. The starch contents of smooth and wrinkled peas range from 32.7% to 42% (Ratnayake et al. 2001; Wang et al. 2011) and 18% to 22% (Ratnayake et al. 2002), respectively. These reports suggested that the starch contents of smooth peas are significantly higher than that of wrinkled peas, which is not consistent with our results, since high protein peas used in this study include not only wrinkled peas but also smooth peas. High amylose content is a typical characteristic of pea starch. The ratio of amylose to amylopectin is one of the main distinctions between smooth peas and wrinkled peas. The amylose content in starch granules of smooth peas range from 33.1% to 48.8% (Barron et al. 2000; Biliaderis et al. 1981; Colonna and Mercier 1984; Colonna and Mercier 1985; Czuchajowska et al. 1998), whereas the corresponding values of wrinkled peas are from 64% to 88% (Biliaderis et al. 1981; Colonna and Mercier 1984; Colonna and Mercier 1985; Praznik et al. 1994). Our study further indicated that the amylose content in high protein peas is significantly higher than those in other pea types. Previous reports (Hizukuri et al. 1989) indicated that the branch points are not randomly distributed in the amylopectin: they are clustered and form crystalline lamellar domains among adjacent linear segments. Our results suggested that the branch chain sizes of amylopectins are concentrated 11 to 30 glucose residues, which have some differences with that reported by Hizukuri (1985).

When starch is heated in the presence of excess water, its crystalline structure undergoes a change from order to disorder and the starch granules swell to a high degree. This gelatinization phenomenon is an important starch property, widely explored for starch functionalities in the food industry (Bogracheva et al. 1998). DSC has been widely used to determine the starch gelatinization. It has been reported that the T_p and ΔH values of smooth pea starch are from 60 °C–67.5 °C, and 14.1–22.6 J g^{-1} (Davydova et al. 1995; Ratnayake et al. 2001), respectively, whereas the corresponding values of wrinkled pea starch are 133 °C and 2.9 J g^{-1} (Colonna et al. 1981). The T_p of smooth pea starch are significantly lower than that of wrinkled pea starch. Our results reveal that the T_o, T_p, and T_c of high protein peas are significantly higher than those of other pea types and the ΔH of high protein peas is significantly lower than those of other pea types, which is similar with those of wrinkled peas. The gelatinization and swelling properties have some relations to the amylopectin structure, starch composition, and granule architecture (Tester 1997). The different thermal properties identified in this study may be due to the differences in

amylopectin architecture between high protein peas and other pea types.

Conclusion

The morphologies and thermal properties of starch granules in high protein peas are significantly different from those in yellow, green, maple, and marrowfat peas. The starch content is negatively correlated to protein content in field peas, which is informative for variety selection and quality improvement. Compared to other pea types, the high protein peas have lower starch content but high amylose content. Further studies will be valuable to elucidate the genetic mechanisms on different physicochemical properties of pea starch, as well as to clarify the relationship between the starch and protein contents at the molecular level among different pea types.

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