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Content of low molecular weight carbohydrates in vining peas (*Pisum sativum*) related to harvest time, size and brine grade

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Abstract

Knowledge of how the content of low molecular weight carbohydrates (LMWC), including the raffinose family oligosaccharides (RFO; raffinose, stachyose and verbascose), changes in unripe peas (*Pisum sativum)* around harvest time, is today limited. An early and a late maturing cultivar of peas were harvested with 24 h of each other on seven different occasions, of which three were chosen for this study. The pea samples were divided into three groups by size and brine grading, and then analysed with regard to the content of low molecular weight carbohydrates. The content of raffinose and stachyose increased in all groups during a period of 5 days. Peas that sank (sinkers) in the brine grading test had a higher content of raffinose and stachyose and a lower content of sucrose than floating peas, indicating that sinkers have reached a greater degree of maturation. The content of verbascose remained the same or decreased during the study period. In conclusion, the date of harvesting green peas for freezing affects the contents of RFO.

Keywords: Low molecular weight carbohydrates; Raffinose family oligosaccharides; α-Galactosides; Vining peas

1. Introduction

Legume foods are a rich and inexpensive source of protein, carbohydrate, vitamins and minerals, and have played an important role in human nutrition since ancient times. In addition, legume seeds have good storage properties and some of these crops can even be grown in arid conditions. However, one problem that is believed to limit the consumption of legume seeds is the associated discomfort of flatulence. This colonic gas production is mainly caused by the high content of α-galactosides, also known as the raffinose family oligosaccharides (RFO). RFO are synthesized by a group of galactosyltransferases, which add galactose moieties to sucrose, yielding raffinose (α-D-galactopyranosyl-(1-6)-α-D-glucopyranosyl-(1-2)-β-D-fructofuranoside), and its higher homologues including stachyose and verbascose (Peterbauer et al., 2001; Sprenger & Keller, 2000). These oligosaccharides are known to accumulate in legume seeds during maturation and are then used as an energy source during germination.

The accumulation of α-galactosides has also been related to the ability of legume seeds to survive when exposed to desiccation (Bailly et al., 2001; Blackman, Obendorf, & Leopold, 1992). A proposed mechanism for this is that RFO have the capacity to maintain the integrity of cell membranes by a direct interaction with the phospholipid headgroups, thus replacing water upon dehydration (Crowe, Hoekstra, Nguyen-Khanh, & Crowe, 1996). Moreover, it has been suggested that RFO contributes to the cold hardiness of legume seeds, protecting them from damage caused by cold (Bachmann, Matile, & Keller, 1994; Gilmour, Sebolt, Salazar,

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Everard, & Thomashow, 2000). Since starch synthesis is sensitive to low temperatures, RFO will provide a very important carbon supply at such times (Chatterton, Harrison, Thornley, & Bennett, 1990).

The lack of the enzyme α-galactosidase in the gastrointestinal (GI)-tract of monogastric animals makes RFO indigestible and susceptible to colonic fermentation, producing short-chain fatty acids (SCFA) and gases. These properties may result in undesired flatulence, but also a potential prebiotic effect (Trindade, Abratt, & Reid, 2003), by specifically stimulating the growth of remedial species of the colonic microflora. Additionally, there is increasing evidence that the different SCFA produced have several beneficial health effects. Butyric acid, for instance, is the main energy source for the colonic mucosa (Clausen & Mortensen, 1994) and is believed to provide protection against colon diseases such as cancer (Archer et al., 1998; Augeron & Laboisse, 1984; Hague et al., 1993) and distal ulcerative colitis (Cummings, 1997; Scheppach et al., 1992). The lowering of pH resulting from the production of SCFA may, through an inhibition of bacterial transformation of primary to the co-carcinogenic secondary bile acids, also have a protective effect (Christl et al., 1997). Furthermore, it has been proposed that other SCFA, such as propionic acid, improve glucose tolerance (Thorburn, Muir, & Proietto, 1993), inhibit hepatic cholesterogenesis (Chen, Anderson, & Jennings, 1984) and enhance immune function (Schley & Field, 2002). Interestingly, different types of carbohydrates give rise to different patterns of SCFA profiles (Berggren, Björck, & Nyman, 1993; Henningsson, Björck, & Nyman, 2002). Some types of starches, raffinose and food items, such as peas, containing high amounts of RFO have been shown to give high proportions of butyric acid upon fermentation in the hindgut of rats (Berggren et al., 1993; Goodlad & Mathers, 1990; Henningsson, Nyman, & Bjorck, 2001). Thus, there is reason to believe that a regular consumption of moderate amounts of RFO and other indigestible carbohydrates producing specific SCFA will contribute to a desirable effect on gut health.

Vining peas (*Pisum sativum*) have a low content of RFO, since the seeds are harvested fully developed, but early in the maturation process (Ekvall, Stegmark, & Nyman, 2005). During seed development up until harvest of vining peas, sucrose is accumulated in the seeds. Maturation of the seeds mainly takes place after harvest. Maturation is characterized by declining contents of sugars and an accumulation of starch and RFO (Daveby, Abrahamsson, & Åman, 1993; Ottosson, 1958).

The purpose of the present investigation was to extend our knowledge concerning the distribution of RFO and other low molecular weight carbohydrates (LMWC) in vining peas during the early maturation process occurring in times of harvest and, in this way, study the possibility of increasing the content of RFO, without compromising the sweetness of the peas. Two cultivars differing in maturity were chosen and divided into three fractions according to their size and density, a procedure generally used when processing peas for different purposes.

2. Materials and methods

2.1. Materials

Samples of vining peas (*P. sativum*) were provided by Findus R&D AB, Bjuv, Sweden. The samples comprised two cultivars; an early maturing cultivar (X475) harvested during week 27 (end of June/beginning of July) and one late maturing cultivar (Finett) harvested during week 30 (late July). The peas were grown (25 m^2) in Bjuv, southern Sweden, during 2000 and harvested (0.5 kg/m^2) on seven occasions with an interval of approximately 24 h (Table 1). The threshed peas were first divided into small and large peas by sieve sizing and then blanched (97 °C, for on average 100 s, exact time is dependent on size and tenderometer value) in a 40 L steam heated water tub. The large peas were then brine graded in NaCl (10.9% w/v) and divided into two fractions according to their density (floaters and sinkers). This division corresponds with commercial classification. The large floaters are usually regarded as the highest quality, and reach the market as frozen peas. The taste of large sinkers is generally less sweet, and these are therefore graded as seconds, and normally used in mixtures of frozen semi-manufactured articles or for canning. The small peas are often marketed frozen as small peas or ''petits pois''. After blanching the different pea samples were frozen (−20 °C) in packages of 75 g, freeze-dried and stored (−20 °C) until their content of LMWC was analysed. Analysis was decided to be performed on pea samples harvested day 2, 5 and 7.

2.2. Analysis of LMWC

The freeze-dried samples were ground into powder using a Tecator sample mill (Foss Tecator, Höganäs, Sweden). Samples of 0.2 g were placed in test-tubes and 1 ml arabinose (5 mg/ml) was added as internal standard. In order to ensure enzyme inactivation, 12 ml of 95% (v/v) ethanol was added to obtain an ethanol concentration of 50% (v/v). Extraction was allowed to proceed for 30 min at room temperature, with stirring every 10 min on a vortex. Cell wall material was then removed by centrifugation (3000*g*, 10 min).

The proteins in the supernatant were precipitated with ethanol (80%, v/v) for 30 min at −20 °C. To separate the protein from LMWC the samples were centrifuged at 3000*g* and 0 °C for 20 min. One millilitre of the supernatant was then transferred to a round flask,

Table 1
The content of LMWC (g kg^{-1} DM) according to pea size and brine grading in an early and a late cultivar of vining peas (*Pisum sativum*, X475 and Finett) harvested at three occasions during seven days[A,B]

	Early maturing cultivar (X475)						Late maturing cultivar (Finett)					
	Small		Large floaters		Large sinkers		Small		Large floaters		Large sinkers	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
myo-Inositol	7.7^{a}	7	4.6^{b}	18	4.6^{b}	13	5.6^{a}	28	4.0^{ab}	14	3.8^{b}	5
Sorbitol	1.7^{c}	39	3.8^{b}	36	5.0^{a}	32	2.2^{b}	36	4.3^{a}	7	4.1^{a}	12
Glucose	4.0^{a}	24	1.9^{b}	28	1.3^{b}	36	2.2^{a}	36	1.2^{b}	16	1.2^{b}	20
Fructose	5.0	4	2.5	19	1.9	24	4.0	24	1.1	58	1.1	8
Sucrose	273^{a}	4	235^{b}	6	210^{c}	2	254^{a}	15	255^{ab}	10	213^{b}	16
Raffinose	1.2^{b}	20	1.7^{b}	21	3.8^{a}	51	1.7^{b}	56	1.9^{b}	39	4.3^{a}	37
Stachyose	0.6^{b}	71	0.7^{b}	40	2.3^{a}	71	1.4^{b}	87	1.7^{b}	61	4.6^{a}	58
Verbascose	1.5^{a}	12	0.7^{b}	15	0.8^{b}	24	2.4^{a}	70	0.9^{b}	10	0.9^{b}	28
RFO	3.4^{b}	16	3.1^{b}	24	6.9^{a}	50	5.5^{b}	16	4.4^{b}	38	9.8^{a}	41
LMWC	286^{a}	4	243^{b}	6	221^{c}	3	267^{a}	14	262^{ab}	9	225^{b}	14

[A] Means of the three different harvesting occasions.
[B] Means in the same row within each cultivar (early maturing and late maturing) followed by different superscript letters are significantly different according to a general linear model ($p < 0.05$).

taken into dryness by evaporation at 40 °C, and redissolved in 5 ml water. Low-density particles were removed by filtration through a 0.45 μm Millex-HV filter (Millipore Corporation, Bedford, USA). A portion (10 μl) of the sample was then analysed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). NaOH (15–200 mmol) was used as the eluent at a flow rate of 1.0 ml/min.

Heat units were used as a measure to estimate the degree of maturation of the peas (Fig. 1). Heat units were calculated as accumulated degrees of temperature (Celsius) between the day's mean temperature ((max + min)/2) and the plant's minimum growth temperature (2.7 °C for these peas). The tenderometer test was used to estimate the degree of maturation. This method gives an indirect measure of the content of starch, which correlates well with the degree of maturation since starch also accumulates during maturation of peas (Ottosson, 1958). The tenderometer test was performed on the peas in bulk and on the fraction of small peas, but not individually on the two fractions of large peas (Fig. 1).

2.3. Statistical evaluation

All analyses were performed at least in triplicate, with an average error of less than 3%. For statistical evaluation of the results the general linear model (GLM) was used followed by a pairwise comparison with Tukey's method. Mean values were considered significantly different at $p < 0.05$. The coefficient of variation (CV; standard deviation divided by the mean value) was used to describe the differences (including errors) in the content of LMWC between different days. All statistical analyses were processed with MINITAB 13 statistical software (Minitab, 2000).

3. Results and discussion

3.1. General

The average content of LMWC in the total plant material of peas included in this study was 250 g kg^{-1} dry matter (DM) (data not shown). The overall dominating

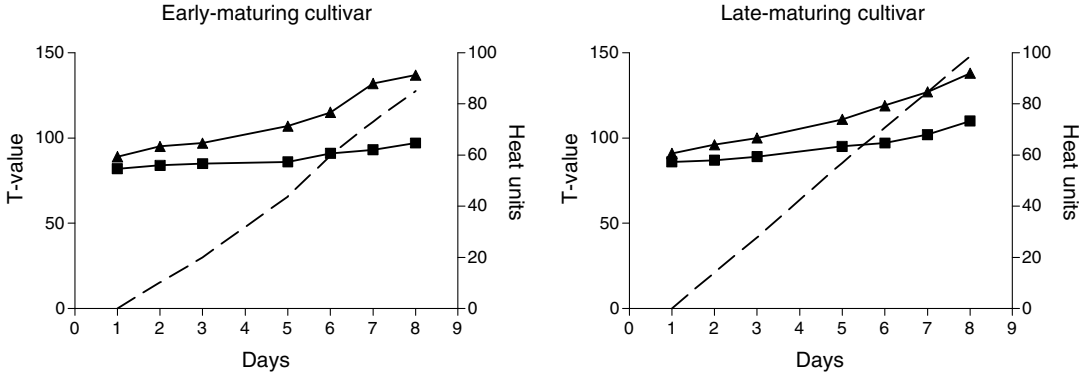

Fig. 1. Tenderometer (T) values (full lines) and heat units (broken lines) for the two cultivars during the period of study. T-values are given for peas in bulk (▲) and for the fraction of small peas (■).

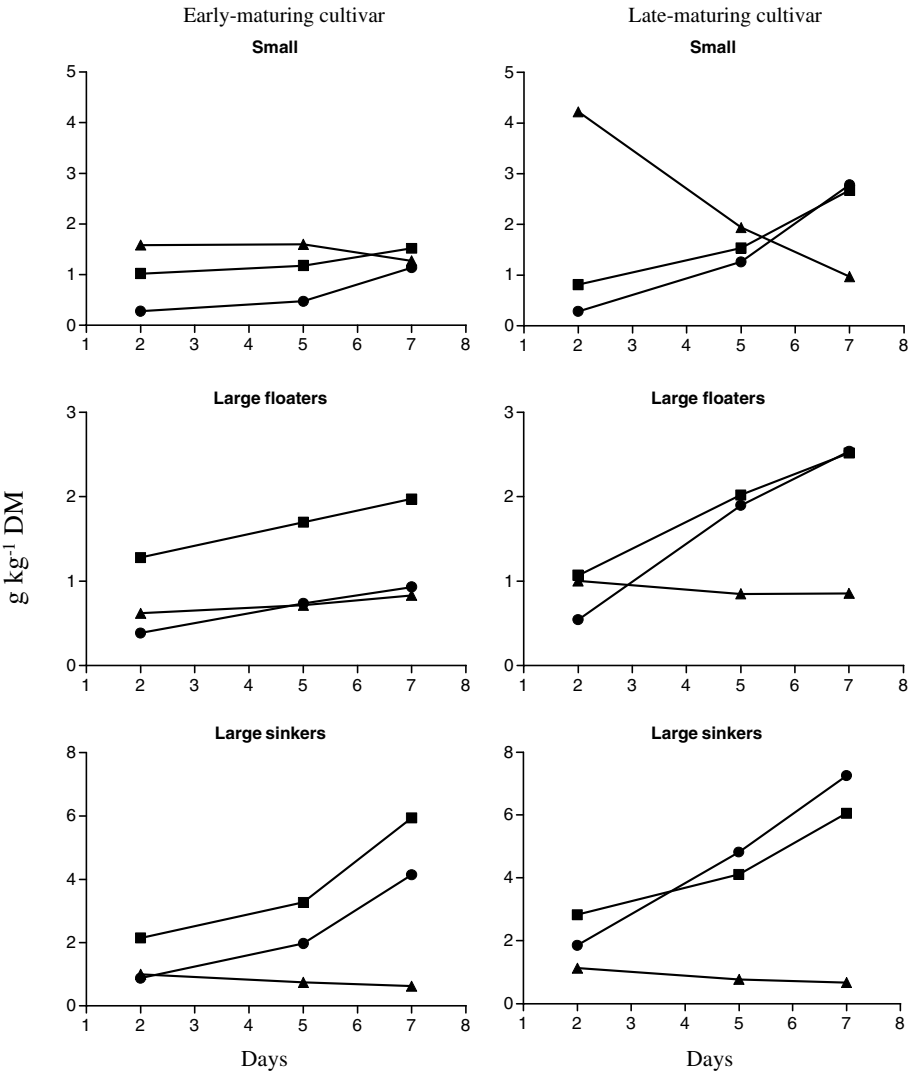

Fig. 2. Changes in the content of RFO in vining peas as a function of the degree of maturation: (■) raffinose, (●) stachyose, (▲) verbascose.

sugar was sucrose, which accounted for 96% of the LMWC and 24% of the dry matter. The amounts of glucose and fructose were, on average, 2.0 and 2.8 g kg^{-1}, respectively, while among the sugar alcohols, sorbitol was present at 3.5 g kg^{-1} and a substance identified as *myo*-inositol at 5.0 g kg^{-1}. The content of RFO was 5.5 g kg^{-1} with the average distribution: raffinose 44%, stachyose 35% and verbascose 22% (see Fig. 2).

3.2. Effect of size

The content of the different LMWC was similarly affected by the size of the peas in both the late and the early maturing cultivar (Table 1). The average content of LMWC was higher ($p < 0.05$) in small peas (276 g kg^{-1} DM) than in large peas (238 g kg^{-1} DM) due to a higher content of especially sucrose, but also of glucose and fructose in the smaller peas (Table 1). The difference between small and large peas is probably not only due a difference in size, but also to difference in degree of maturation between small and large peas, since the peas grow larger during maturation. This corresponds well with the fact that the content of sucrose is known to decrease during maturation (Ottosson, 1958). The small peas of the present study were also found to have a lower tenderometer value than large peas (Fig. 1), confirming the lower degree of maturation in small peas.

Furthermore, the size of the peas had diverse effects on the oligosaccharides. Higher contents of raffinose were found in large peas and, to some extent, also of stachyose. Interestingly, verbascose was less abundant in large peas than in small ones ($p < 0.05$).

Interestingly, for both cultivars the content of *myo*-inositol was higher and that of sorbitol lower in small peas than in large peas ($p < 0.05$). If this effect has to do with maturity or has other explanations, can only be speculated upon. Regarding the fact that *myo*-inosi-

tol is involved in the synthesis of RFO (Bachmann et al., 1994; Peterbauer, Mucha, Mach, & Richter, 2002), one could interpret these results as mobilization of the plant for an upcoming accumulation of raffinose and stachyose. The content of *myo*-inositol has recently been reported to affect the accumulation of RFO (Karner et al., 2004). *myo*-Inositol is one of the constituents of the compound galactinol (*O*-α-D-galactopyranosyl-(1 → 1)-L-*myo*-inositol), which acts as a galactose donor to form raffinose from sucrose and stachyose from raffinose (Hoch, Peterbauer, & Richter, 1999).

3.3. Effect of density in large peas

Brine grading is performed as a step in the production line. The peas that sink are denser due to a higher content of starch. It is believed that this higher content of starch can be ascribed to a greater degree of maturation, since starch, in conformity with RFO, accumulates during maturation (Ottosson, 1958). Large peas that sank in the brine grading test had an average content of sucrose more than 10% lower than large peas that floated ($p < 0.05$) (Table 1). The amounts of raffinose, stachyose and sorbitol were generally greater in sinking peas than in floaters, which agrees well with the conclusion that large sinkers are more mature than large floaters.

3.4. Effect of maturation

The content of α-galactosides in peas is known to increase during maturation. This accumulation has been observed to begin approximately 37 days post-anthesis (Frias et al., 1996). The present study was concentrated on the content of LMWC in the peas during this specific period. From the results we can conclude that the amounts of raffinose and stachyose indeed accumulated during this period, starting from a very low concentration on the first day investigated (only about 28 days post-anthesis) (Fig. 2). The increase was, however, comparatively low and not a reason to postpone harvest to a later date, in order to achieve a higher content of these sugars with the aim of improving colonic health. Surprisingly, verbascose did not follow the pattern observed for raffinose and stachyose. In contrast this carbohydrate seemed rather to decrease in the material investigated (Fig. 2). Verbascose has been reported to accumulate later in the maturation process than raffinose and stachyose (Frias et al., 1996), but there should not be any α-galactosidase activity or breakdown of this carbohydrate at all in this early stage. The synthesis of verbascose does not follow the same pattern as raffinose and stachyose, and considerable variation in the content of verbascose in seeds has been observed (Frias et al., 1999). The enzyme, earlier referred to as verbascose synthase, has recently been identified as a multifunctional stachyose synthase (STS) (Peterbauer et al., 2002), synthesizing both stachyose and verbascose. The synthesis of verbascose takes place in half reactions that first yield stachyose and then the higher homologue verbascose. Since the half reactions are reversible, stachyose can yield both raffinose and verbascose. The variation in the content of verbascose in legume seeds might be due to a variation in the ability of STS to synthesize this carbohydrate. This variation may be due to growing conditions (Ekvall et al., 2005) and/or heredity (Peterbauer et al., 2003). Furthermore, recent studies have demonstrated that verbascose, in peas, could be synthesized through a galactinol-independent pathway (Peterbauer et al., 2002), which may explain why the synthesis of verbascose does not follow the same pattern as for raffinose and stachyose. Our findings corroborate the theory that the accumulation of verbascose takes place later in the seeds of peas. The observed decrease in the amounts of verbascose has yet to be explained.

The content of sucrose in the early maturing cultivar seemed to be less affected during the maturation process (Fig. 3). However, in the late maturing cultivar the amount of sucrose decreased during this period from a mean of 254.4 ± 25.9 g kg^{-1} DM to a mean of 225.7 ± 31.6 g kg^{-1} five days later (Fig. 3). The decrease occurred earlier in large peas than in small peas. The content of sucrose is expected to fall during the on-going ripening process. This tendency is, however, difficult to

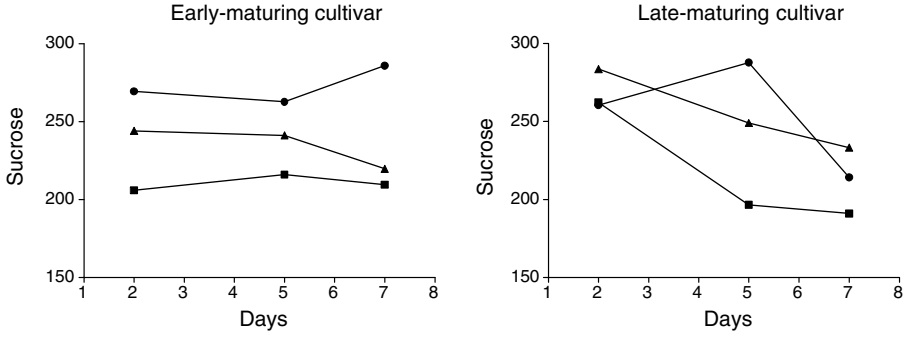

Fig. 3. Changes in the content of sucrose (g kg^{-1} DM) during one week following harvest: (●) small, (▲) large floaters, (■) large sinkers.

observe during such a short period of time (5 days). Photosynthesis and sucrose synthesis are likely to proceed simultaneously, during this short period of time, with the accumulation of starch and RFO. Sucrose, as well as glucose and fructose, will probably therefore be highly metabolized in this early stage of ripening. This would explain our contradictory results on the content of sucrose. The decrease in this sugar may not be significant until later in the ripening process (Ottosson, 1958).

In conclusion, the accumulation of RFO in peas begins with a synthesis of raffinose and stachyose, while the content of verbascose is believed to increase at a later stage in the maturation. Peas with a high degree of maturation are more dense (sinkers) and contain more raffinose and stachyose, but less sucrose. Although the considerable changes in the content of RFO, these were too low to be of any nutritional significance and therefore there is no reason to postpone the harvest to a later date, for vining peas harvested at this early stage.

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