Chaerophyllum bulbosum: a New Vegetable Interesting for its Root Carbohydrate Reserves

O.J. Ayala Garay, M. Briard, J.Y. Péron
Unité de Recherche en Génie Biologique appliqué à l'Horticulture, Inst. National d'Horticulture, 2 rue Le Nôtre, 49045 Angers Cedex 01, France

V. Planchot
Unité de Rech.sur les Polysaccharides leurs Organisations et Interactions
Inst. National de la Rech. Agron.
Rue de Géraudière BP 71627
44316 Nantes Cedex 3, France

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Abstract

Tuberous-rooted chervil (*Chaerophyllum bulbosum* L.) belongs to the *Apiaceae* family. Its root, very rich in carbohydrate reserves, is edible as a vegetable. To bring this species closer to commercialisation with the aim of enhancing the diversification of vegetable crops, a research program was initiated. One of the objectives was to characterise the root reserves. The present paper gives the results of starch and sucrose evaluation.

The root starch content at harvest time was very high (37 % dry matter, containing 76 % starch). Because this rate is exceptional for a species cultivated under a temperate climate, *C. bulbosum* could be a good option for diversification of starch sources under this climate. Results of a physical and chemical characterisation of the starch are discussed in this way.

A two-month period of cold storage (4 °C), necessary to reach the consumable stage, resulted in a dramatic change of the carbohydrate composition. After this period sucrose content reached 25 % of root dry matter (DM). Sucrose and starch (35 % of root DM) were the two major components. The combination of these two high rates in a single organ, uncommon for a vegetable, reveals the great interest of tuberous-rooted chervil for the human diet. It is, indeed, a very tasty edible root combining a very rapidly digested sugar with a more slowly digestable one, resulting in long-term availability of energy.

In conclusion, as it can be used for both industrial starch production and food production, *C. bulbosum* offers a good opportunity to diversify horticultural crops.

INTRODUCTION

Tuberous-rooted chervil is an apiaceous biennial species of which the root is edible. This species, originating from Eastern Europe, was quite popular in France during the first part of the 20th century (Bois, 1927). However, the production of *C. bulbosum* remained very limited and in 1975 it had almost disappeared. Thanks to a research program carried out since 1978 by the National Institute of Horticulture (INH) of France (Péron, 1989), this plant is presently undergoing a rapid economic development.

The root of *C. bulbosum* is often similar in shape to the 'Davanture' carrot type but very lightly coloured (Fig. 1). The root is characterised by the accumulation of starch and sucrose. At harvest time, in July, the starch content is very high, approximately 75 % of root dry matter (Garay et al., 1999).

Starch is a staple in the diet of much of the world's population, and is also widely used in the food and beverage industries, as well as having some manufacturing applications in the paper and textile industries (Slattery et al., 2000). At present the current demand for starch is met by a limited range of crops, the most important of which are potatoes, maize and wheat (Matin and Smith, 1995). After a review of the disadvantages and limitations of these starch sources Wang et al. (2000) proposed to look for new sources of starch for the daily carbohydrate intake needs of the population and for food processing. Our team wondered if tuberous-rooted chervil could be a suitable

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candidate for the diversification of starch sources.

At harvest time the root is not edible: taste is not developed. Traditionally, a storage at 10°C during four to five months is necessary to reach the stage at which it is edible. We will call this stage gastronomic maturity. Storage enables changes to occur in carbohydrate reserves responsible for taste. A threshold of sucrose root content was established by Billot et al. (1989) for this gastronomic maturity: 25 % of root dry matter (DM). Experiments with other tuber crops, like potato, showed that the temperature variation during storage produces changes in the carbohydrate composition of the tuber (Hill et al., 1996). When tubers are stored at low temperatures (4°C) they accumulate sugars, mainly sucrose, glucose and fructose. This phenomenon is known as cold sweetening. Contrary to potato, where the high sugar content is undesirable (Suttle, 1996), a rapid sweetening could be important for the tuberous-rooted chervil in order to reach gastronomic maturity more quickly.

The objectives of the proposed study were to determine the root carbohydrate content and to characterise the starch of this species at harvest time and also to study the effect of temperature on the development of the carbohydrate content of the roots during storage.

MATERIALS AND METHODS

Plant Material

Roots of the cultivar 313, issued from the INH breeding program, were used. They were grown at Angers, France. The sowing date was 14 February 2000 and harvesting occurred when the leaves became totally necrosed on 13 July 2000.

Moisture Content Measurements

Moisture content was determined by drying 5 g samples of hand-peeled root at 70 °C for 72 h. Four replications were made.

Microscopy Preparations

Histological preparation of the root tissue was carried out at harvest time. The explants were fixed for 5 h in a solution containing 1 % formaldehyde, 5 % acetic acid and 50 % ethanol. After dehydration through a graded alcohol series (from 30 to 100 %), samples were embedded in paraffin wax and cut between 10 and 20 µm thick with a Microm microtome. Sections were observed with a Leica optical microscope using polarisation filters.

Sucrose and Glucose Contents

Four weighed samples (1 g approx. of freshly-grated tissue) were ground in 10 ml of 80 % ethanol at 4°C. They were extracted twice with 40 ml of 80 % ethanol at boiling temperature under reflux for 30 min. After centrifugation (12 min at 14 000 *g*) the two extracts were adjusted to 100 ml with 80 % ethanol. Aliquots of 1.5 ml were evaporated and recaptured with 1 ml of distilled water. Sucrose and glucose determinations were carried out enzymatically as described in the protocol of the Boehringer kit (Germany).

Starch Content

The alcohol-insoluble substances (AIS) after sugar extraction were dried for 72 h at 40°C. Aliquots of approximately 100 mg of AIS were used for starch determination using a commercial assay kit from Megazyme (Ireland) according to the procedure described by McCleary et al. (1994).

Starch Isolation

170 g of hand-peeled roots were blended for 2.5 min in an Oster blender, at high speed, with three volume of distilled water. The homogenate was filtered through three sieves (500, 200 and 10 µm). The filtrate was centrifuged at 4000 g during 15 min and the

supernatant was decanted. The starch was washed several times to remove the protein layer, first with distilled water (pH 7) and then with alkaline water (pH 9). A last washing was done with 100 % ethanol to facilitate the drying. The starch was dried at 45°C for 24 h in a ventilated oven and stored at room temperature in a plastic flask. During the isolation process the integrity of the starch granules was verified. For this, microscopic observations were done with a Leica (Germany) optical microscope.

Amylose Content

Amylose content was measured according to the amperometric method described by Gérard et al. (2001).

X-ray Diffraction

X-ray diffraction analyses were performed on native starch by a method previously described in Gérard et al. (2002).

Storage Conditions

The roots were stored a week after harvesting (22 July) at 4, 10 and 16°C with constant relative humidity (98%). Root samples were collected every 15 days during 2.5 months.

RESULTS AND DISCUSSION

Carbohydrate Characteristics at Harvest Time

1. Root Dry Matter and Carbohydrate Content: Table 1 shows the principal results of root characterisation at harvest time in July.

In comparison to other tuberous crops the root of tuberous-rooted chervil has a very high dry-matter content (37.6 % of fresh matter). For example, the dry-matter content of potato is about 20 % (Gravoueille, 1996) and for carrot, an other Apiacea, it is about 11 % (Massiot et al., 1988).

The present biochemical results show that the dry matter at harvest time was essentially composed of starch. Indeed the starch content in July represented 77 % of root DM. In comparison to other starch rates reported respectively by Gravoueille (1996) and Valetudie (1992), it is higher than potato and sweet potato (70 %).

Starch yield after the procedure of purification was 69 % of root DM (Table 2). This quantity is equivalent to 90 % of the total starch quantity and we consider it a high starch yield. Because of this high yield, *C. bulbosum* is a species of great potential for industry: experiments for a possible industrial extraction of the starch could be done with cellulases, for example.

2. Shape and Size of Starch Granule: The microscopic appearance of starch granules of *C. bulbosum* is presented in Fig. 2. The granule surface appeared smooth and without evidence of fracture. This suggests that the extraction procedure was mild enough and did not produce any breakdown of the granules.

Two shapes of starch granules were observed (Fig. 2). In general, the bigger granules were circular and the smaller ones were polyhedral [in shape]. The diameter of starch granules ranged from 3 to 22 µm. In general, the starch granule shape and size depended on the species it was extracted from. Frequently only one shape was observed. For example, potato starch granules were large and oval and their diameters ranged from 15 to 100 µm (Buléon et al., 1998). However, *Myrosme cannifolia*, a tuber-producing plant of the *Marantaceae* family, also has its shape varying according to granule size: the granule diameters vary from 9 to 18 µm and the bigger granules are polyhedral, whereas smaller granules are oval (Rincón et al., 1999). A detailed study of the starch granule morphology will be done for a better determination of the proportion of each size and form in tuberous-rooted chervil.

3. Amylose Content: In general, amylose content is known to influence both nutritional and technological properties such as susceptibility to enzymatic hydrolysis, gelling and pasting behaviour (Slattery et al., 2000). The proportion of amylose in starch ranges from 0 in waxy maize starch to 70 % in *rugosus* pea starch (Buléon et al., 1998). The amylose content of tuberous-rooted chervil starch (Table 2) was rather low: 17 %. However, it cannot be considered significantly different from the amylose content of starch of the other tuberous crops like potato (21 %) and manioc (19 %). Therefore, if tuberous-rooted chervil would be used in the starch industry it would be for food applications.

4. X-ray Diffraction: X-ray diffraction sowed that the starch profile was similar to that of the other tuber crops such as potato or manioc, which is known as B-pattern. Starch of B-type is very resistant to amylolysis (Buléon et al., 1998).

The degree of crystallinity of tuberous-rooted chervil starch was 41 % (Table 2); this is similar to that of starch potato (Buléon et al., 1998).

The characteristics discussed here confirm the idea that *C. bulbosum* could be used as a new starch source. The starch extraction must then be realised at harvest time when the starch content is very high and the taste is non-developed. This is very important because any taste will be transferred to the native starch, which will be used in the food industry. Indeed, as we shall see in the next sections of this paper, there is a carbohydrate and taste evolution during root storage.

Carbohydrate Evolution during Storage

The carbohydrate evolution during the storage period is shown in Fig. 3.

Whatever the storage conditions there is an inverse evolution between starch and sucrose contents. In potato tubers, as in many other non-photosynthetic organs (like fruits), the source of carbon for sugar synthesis is certainly stored starch. However, the transformation way of starch in sucrose has not yet been clarified in detail. Moreover, a direct correlation between starch hydrolysis and sucrose synthesis could be not found because the energy for other processes, such as respiration, is supplied by the carbon reservoir (starch). In the present study, the correlation coefficient between starch content and sucrose content is very high (-0.96).

The starch content decreased more rapidly in the roots stored at 4°C. The starch hydrolysis in the roots stored at 16°C was the slowest. In parallel, the sucrose content increase was the fastest in the roots stored at 4°C. For the three storage conditions the carbohydrate turnover was more important within the first two weeks; it continued all along the two following months only at 4°C. In contrast, at 10°C and the more so at 16°C, the carbohydrate reserves evolved very slowly.

This phenomenon of increasing sugar content could be linked to storage at low temperature (cold sweetening). Nielsen et al. (1997) explained it as a kind of self-protection. Sugars, accumulated in many plants in cold conditions, may act as cryoprotectants. For species growing in temperate climates the low temperatures may render cryoprotectants useful for survival. While in potato tubers this process is undesirable, it is convenient in tuberous-rooted chervil: the storage time needed for the root to reach the consumable stage is greatly reduced.

Billot et al. (1989) found that the root's consumable stage is reached when its sucrose content is about 25 % of root DM. In the present study, this threshold was reached after one month of storage at 4°C and two months of storage at 10°C. In the roots stored at 16°C this sucrose level was never reached. At both temperatures (4 and 10°C), the starch content was still very high when this sucrose level was reached (Fig. 3). Gastronomically this is interesting: sucrose and starch are the main high-energy sources in the human diet. Sucrose is a very rapidly digested sugar, whereas starch digestion requires a longer time. High values of sucrose and starch in combination provide an energy source over a longer time, which is desirable for a good health.

Although the starch characteristics of *C. bulbosum* reported here are very similar to those of other tuber crops, like potato, its high starch content combined with a high dry-matter content is exceptional for a species grown in temperate climatic conditions. Moreover, once the threshold of root maturity is reached, the combination of a high rate of starch with a high rate of sucrose in a single organ, uncommon for a vegetable, reveals the great interest of tuberous-rooted chervil for the human diet.

Thus, we consider that tuberous-rooted chervil is a good option in the sustainable use of vegetable diversity, thanks to its carbohydrate reserves accumulated in the roots and its excellent gastronomic proprieties.

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Literature Cited

Ayala Garay, O.J., Briard, M., Granger, J. and Péron, J.Y. 1999. Phenologic development and carbon reserves within tuberous-rooted chervil (*Chaerophyllum bulbosum* L.). Umbellifer Improvement Newsletter 9 : 20-23.

Billot, J., Hartmann, C., Imbault, N., Joseph, C. and Péron, J.Y. 1989. Cerfeuil tubéreux aliment méconnu. Variations au cours de la conservation au froid des réserves glucidiques de la racine du cerfeuil tubéreux (*Chaerophyllum bulbosum* L.). RGF 4: 344-347.

Bois, D. 1927. Les plantes alimentaires chez tous les peuples et à travers les âges. p: 243-244. In: Paul Lechevalier (Eds). Phanérogames légumières,. Tome 1, Histoire, utilisation, culture., Paris.

Buléon, A., Colonna, P., Planchot, V. and Ball, M. 1998. Starch granules: structure and biosynthesis. Int. J. Biol. Macromol. 23: 85-112.

Gérard, C., Barron, C., Colonna, P. and Planchot, V. 2001. Amylose determination in genetically modified starches. Carbohydr. Polym. 44: 19-27.

Gérard, C., Colonna, P., Buléon, A. and Planchot, V. 2002. Order in maize mutant starches revealed by mild acid hydrolysis. Carbohydr. Polym. 48: 131-141.

Gernat, C., Radosta, S., Damaschun, G. and Schierbaum F. 1990. Supramolecular structure of legume starches revelated by X-ray scattering. Starch/Stärke 42: 175-178.

Gravoueille, J.M. 1996. Utilisation pour l'alimentation humaine. p. 451-493. In: P. Rousselle, Y. Robert and J.C. Crosnier (eds.), La pomme de terre. INRA Editions, Paris.

Massiot, P., Rouau, X. and Thibault, J.F. 1988. Characterisation of the extractable pectins and hemicelluloses of the cell wall of carrot. Carbohydr. Res. 172: 229-242.

Martin, C. and Smith, A.M. 1995. Starch biosynthesis. Plant Cell 7: 971-985.

Hill, L.M., Reimholz, R., Schröder, R., Nielsen, T.H. and Stitt, M. 1996. The onset of sucrose accumulation in cold-stored potato tuber is caused by an increased rate of sucrose synthesis and coincides with low levels of hexose-phosphates, and activation of sucrose phosphate synthase and the appearance of new form of amylase. Plant Cell Environ. 19: 1223-1237.

McCleary, B.V., Solah, V. and Gibson, T.S. 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal Sci. 20: 51-58.

Péron, J.Y. 1989. Les potentialités d'élargissement de la gamme des légumes dans la famille des Apiacées (= ombellifères): l'exemple du cerfeuil tubéreux (*Chaerophyllum bulbosum* L.) et du chervis (*Sisum sisarum* L.). Acta Hort. 242: 123-131.

Nielsen, T.M., Deiting, U. and Stitt, M. 1997. A β-amylase in potato tubers is induced by storage at low temperature. Plant Physiol. 113: 503-510.

Rincón, A.M., Padilla, F.C., Araujo, C. and Tillet, S. 1999. *Myrosma cannifolia,* chemical composition and physicochemical properties of the extracted starch. J. Sci. Food Agric. 79: 532-536.

Slattery, C.J., Kavakli, H. and Okita, T.W. 2000. Engineering for increased quantity and quality. Trends Plant Sci. 7:291-298.

Suttle, J.C. 1996. Dormancy in tuberous organs: problems and perspectives. p. 133-143.

In: G.A. Lang (ed.), Plant dormancy: physiology, biochemistry and molecular biology. CAB International, Oxon, U.K.
Wang, T.L., Bogracheva, T.Y. and Hedley, C.L. 1998. Starch: as simple as A, B, C. J. Exp. Bot. 49: 481-502.
Valetudie, J.C. 1992. Modifications structurales et physico-chimiques de tubercules amylacés tropicaux au cours de la cuisson. Relation avec la structure de leurs amidons. Ph D. Thesis, Univ. Nantes. Nantes, France. 153 p.

Tables

Table 1. Root characteristics of tuberous-rooted chervil at harvest time.

Characteristics	**Value**
Dry-matter (DM) content (% of fresh weight)	37.6 ± 1.4
Carbohydrate content (% of root DM)	
Starch	76.8 ± 0.8
Sucrose	7 ± 1.2
Glucose	0.6± 0.02

Table 2. Characterisation of tuberous-rooted chervil starch in roots at harvest time.

Characteristics	**Value**
Starch yield (% of root DM)	69 ± 4.5
Amylose content	17 % ± 0.5
Amylose/amylopectin ratio	0.20
X-ray diffraction	
Type of diffraction	B-pattern
Crystallinity level (%)	40.9
Granules	
Form	Ovoid or polyhedral
Diameter (µm)	3-22

Figures

Fig. 1. Root appearance of tuberous-rooted chervil.

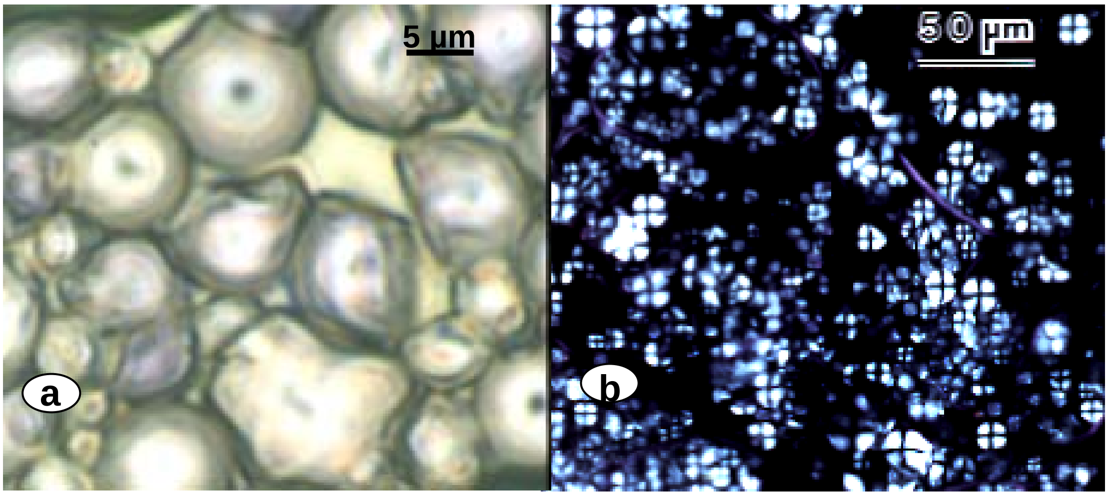

Fig. 2. Images of tuberous-rooted chervil starch granules. (**a**) Circular and polyhedral shapes of isolated starch granules. (**b**) Histological preparation of root cortical parenchyma; polarisation reveals the starch granules to be marked with a cross.

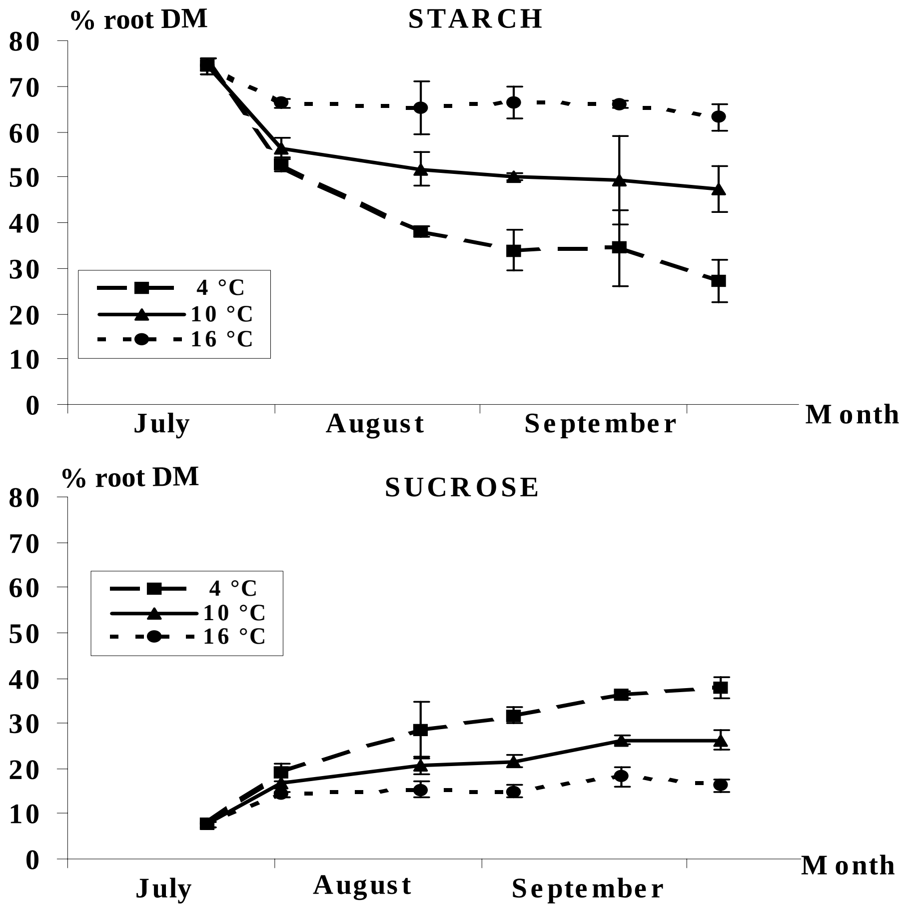

Fig. 3. Changes of the starch and sucrose levels in the tuberous-rooted chervil root during storage at three temperature conditions.