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Abstract

Plant growth is affected by a lot of resources, namely, water supply, sunlight, nutrients in the soil etc. This study investigates changes in potato starch composition derived from tubers grown in Finland and Sweden. In this project you will see the comparison between nine different varieties of potatoes (Asterix, Bintje, Desiree, Kuras, Maria, Maris Piper, Quadras, Rosmunda and Saturna) that were grown in Finland and those grown in Sweden. The hypothesis that growing conditions have an effect on amylose content and total starch content of the same variety was put to test among the sets of tubers. Starch powder was extracted using free-drying method from each set received from each source (Sweden and Finland) and compared. Analysis of amylose content and total starch content was done using Megazyme kits on extracted starch powder. The average calculated for amylose contents between sets of Kuras and Maria varied the most and averages calculated for total starch content varied the most between sets of Desiree and Kuras. The results indicate that growing conditions do have an effect on tubers, in specific, Desiree, Kuras and Maria.

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Introduction

The major carbohydrate present in tubers and root crops is starch accounting for 16-24% of their weight and remaining being water and trace quantities of proteins and lipids (<4%) (Hoover, 2001). Some examples of root and tubers grown for edible purposes are: potato (*Solanum tuberosum*), sweet potato (*Ipomea batatas*), cassava (*Manihot esculenta*), etc. Starch properties are affected by the cultivars and environmental factors (production area, soil, climate etc.) and based on the same, the properties vary significantly(Noda *et al.* (2004); Jansen *et al.*, 2001; Christensen & Madsen, 1996; Cottrell *et al.*, 1995; Hizukuri, 1969; Geddes *et al.*, 1965) . However, little is known about starch composition of many of the world's potato cultivars (Food and Agriculture Organisation of the United Nations, 2010). Starch comprises of two components namely amylose and amylopectin. While the amylose fraction is linear in structure, amylopectin is highly branched(Zobel, 1988). Amounts of these two components influence properties like swelling capacity(Sandhu *et al.*, 2005; Hermansson & Svegmark, 1996), water solubility(Sandhu *et al.*, 2005), water binding capacity (Sandhu *et al.*, 2005)and mechanical properties of starch films(Rindlav-Westling *et al.*, 1998) along with microscopic properties.

In this study, two sets of a potato cultivar were sourced from Sweden and Finland. Using freeze drying technique, the starch from each of sets of tubers was extracted and amylose content was calculated. The study compared the contents to their counterparts sourced from another region. The same was done for total starch content using the method derived from Åman et al (Theander & Åman, 1982) and compared.

Keywords- Solanum tuberosum, Ipomea batatas, Manihot esculenta, amylose, amylopectin

Objective

The objective of the experiment is to compare nine different cultivars of potato tubers sourced from provinces in Sweden and Finland and their total starch and amylose contents.

Materials and Method

Materials

There were nine different varieties of tubers used in the study- Asterix, Bintje, Desiree, Kuras, Maria, Maris Piper, Quadras, Rosmunda and Saturna. There were two sets of each variety, each of which were grown in a randomly allocated plot from each other in the growing area. There were two extra sets with the Asterix variety derived from Finland with differing growing plots. All tubers from the original regions had uniform growing conditions like irrigation and sunlight despite being randomly allocated within the growing plot. Table 1 shows the sample abbreviation of the tubers based on the sets they were classed into.

Table 1 Sample abbreviation of potato tuber variety based on their origin

	FinnishTubers	**Swedish Tubers**
Variety	**Name of set**	**Name of Set**
Asterix (A)	Aa	A1
	Ab	A2
	Ac	-
	Ad	-
Bintje (B)	Ba	B1
	Bb	B2
Desiree (D)	Da	D1
	Db	D2
Kuras (K)	Ka	K1
	Kb	K2
Maria (M)	Ma	M1
	Mb	M2
Maris Piper (MP)	MPa	MP1
	MPb	MP2
Quadras (Q)	Qa	Q1
	Qb	Q2
Rosmunda (R)	Ra	R1
	Rb	R2
Saturna (S)	Sa	S1
	Sb	S2

Methods

Freeze drying

Two tubers from each sample set were randomly chosen for starch isolation using freeze drying. All tubers were processed to make starch powder using a freeze drier. The tubers were washed, peeled and grated to flakes using a kitchen cheese grater. A representative from each of the sample set was allowed to rest in plastic containers in a freezer maintained at -10℃ for a period of 12 hours. The remainder of the tubers were discarded. The plastic containers were then transferred to a freeze drier for a period of 48 hours. The samples were inspected to relieve all its crystalline lustre before taking it out of the freeze drier to ensure removal of moisture. The freeze dried flakes were powdered using a mortar and pestle. This was the final dry matter extract from the selected tubers.

Starch isolation

The freeze dried starch powders were filtered using distilled water and filter paper using a hydraulic aspirator to remove proteins and fibres. The process was repeated till the Millipore water leaving the filter ran clear. The starch residue in the filter was suspended in distilled water and allowed to stand in beakers till the supernatant was clear of milky suspensions. The supernatant was then discarded and the beakers were transferred into an oven maintained at 40 C to stay overnight. The dried cakes were then powdered using a mortar and pestle to obtain the starch powder. The powder was then sieved through a 150µm mesh to ensure uniform particles. Dry matter content of the resulting starch powder for each of the sample tuber was calculated.

Total Amylose analysis

The extraction of amylose was carried out using a solution of 0.06M urea in 90% of dimethyl sulfoxide (UDMSO extraction solution). A sample solution was prepared using 20-50mg of starch powder along with 6ml of UDMSO extraction solution in individual test tubes and capped. These tubes were immediately mixed and put into a boiling water bath for 30 minutes with occasional mixing. From this sample solution, 100µl was pipetted to another tube and mixed with 200µl of absolute ethanol. Another 700µl of absolute ethanol was incremented and mixed. After capping the tubes, the solutions were allowed to stand for 15 minutes. Then, the tubes were centrifuged at 2000*G for 15 minutes. The tubes were then decanted and 2 ml of 95% ethanol solution was added to aid pellet formation. The tubes were centrifuged again at 2000*G for 15 minutes and decanted. To dissolve the amylose, 100µl of UDMSO was added to the tubes, capped and dissolved thoroughly in a boiling water bath for 15 minutes. After the solution was cooled down to room temperature, 5ml of 0.5% Trichloroacetic acid (TCA) was mixed into this solution. 50µl of Iodine solution

(prepared by adding 1.27g of Iodine crystals and 3g of Potassium Iodide per litre of distilled water) was added to each of the tubes. The tubes were then transferred to a 25℃ water bath for 30 minutes before reading the absorbances at 620nm using a spectrophotometer with water as the reference. Two blank samples were prepared using only the UDMSO solution. Amylose contents were calculated using a standard curve derived from varying amylose standard concentrations using the same wavelength and the spectrophotometer.

Starch content analysis

Starch contents were determined using the method derived from Åman et al (1994) with modifications to glucose oxidase reaction and final reagent volume. First, lipids were removed by ethanol extraction using 40 mg of starch sample dissolved in 15 ml of 80% ethanol, placed in a boiling water bath for 30 minutes, centrifuged for 10 minutes at 900*G and the pellet was washed twice with 80% ethanol before decanting the solvent. Using 25ml of acetate buffer (0.1M, pH5.0) and 50µl termamyl (α-amylase) from Megazyme was added to the sample. The tubes were then placed in a boiling water bath for 30 minutes with occasional mixing. After cooling to 40℃, 100µl of amyloglucosidase from Megazyme (diluted 1:9 with 0.1 M acetate buffer by volume) was added and tubes were put into a 60℃ shaking water bath overnight. The tubes were then centrifuged at 900*G for 10 minutes and 40 µl of supernatant was diluted with distilled water to 1960 µl and 3ml of GOPOD reagent was added. The samples were then placed in a 50℃ water bath for 20 minutes before reading the absorbance at 510nm in Shimadzu UV spectrophotometer. Glucose concentration in the samples were determined from a standard curve with solutions ranging from 0.025 to 0.1 mg/ml. Starch content was calculated using the following equation.

$$\%\text{starch (dry mass)} = [(\text{glucose})(\text{mg/ml})X\ 25.15\ X\ 0.9\ X\ 25]/\text{sample weight (mg)}$$

Results

The results are divided in three different parts: Starch contents, Amylose contents and total starch contents respectively. The averages for each potato variety from each of the sources are calculated and compared.

Dry matter content

Table 2 Starch content in same variety of tubers but grown in two different regions

Finnish Tuber	g/100g	Average	Swedish Tuber	g/100g	Average
Aa	20	19.5	A1	18	18.5
Ab	21		A2	19	
Ac	21		-		
Ad	16		-		
Ba	24	26.5	B1	17	17.5
Bb	29		B2	18	
Da	21	21.5	D1	15	15.5
Db	22		D2	16	
Ka	29	27.5	K1	16	15
Kb	26		K2	14	
Ma	23	22	M1	17	16
Mb	21		M2	15	
MPa	14	16.5	MP1	13	16.5
MPb	19		MP2	14	
Qa	26	24	Q1	14	15
Qb	22		Q2	16	
Ra	25	19.5	R1	17	16
Rb	14		R2	15	
Sa	33	30	S1	15	15.5
Sb	27		S2	16	

Taking into consideration that the tubers of a variety grown in a given area as one set, the results are compared to each other. For example, all Asterix tubers grown in a metre square on the growing area is one set and Asterix tubers from another randomly allocated 1 square metre is another set.

Among Finnish tuber sets, the highest recorded extracted starch (g/100g) was in a set of Saturna tubers (33 g/100g) and the least in one set of Rosmunda tubers (14 g/100g). After the average was calculated for every tuber variety by including all tubers from different sets, Saturna had the highest (30 g/100g)and Maris Piper was the least (16.5 g/100).

Among Swedish tuber sets, the highest recorded extracted starch was recorded in a set of Asterix tubers (19 g/100g) and the least in a set of Maris Piper tubers (13 g/100g). Calculated averages for each variety including all sets of tubers show Asterix with the highest starch yield (18.5 g/100g) and both Kuras and Quadras to be the least (15 g/100g).

In comparison, all varieties from Finland showed a higher starch content to their Swedish counterparts. The increase for each variety was calculated to be Saturna (93.6%), Kuras (83.3%), Bintje (51.4%), Desiree (38.7%), Maria (37.5%), Rosmunda (21.8%), Asterix (5.4%) and Maris Piper (0%).

Amylose contents

Table 3 Amylose content in the same cultivar of tubers but grown in two different regions

Tuber	g/100g	Co.Var(%)	Variety Avg g/100g	Tuber	g/100g	Co.Var(%)	Variety Avg g/100g	STD.DEV
Aa	30.80	2	35.01	**A1**	31.73	3	32.14	2.03
Ab	30.35	6		**A2**	32.55	4		
Ac	41.97	2		**-**	-	-		
Ad	36.93	2		**-**	-	-		
Ba	31.82	2	31.71	**B1**	29.46	4	30.73	0.69
Bb	31.59	2		**B2**	31.99	0		
Da	39.04	0	37.89	**D1**	43.09	6	37.01	0.62
Db	36.73	1		**D2**	30.93	2		
Ka	34.85	0	32.23	**K1**	28.16	6	26.74	3.88
Kb	29.60	0		**K2**	25.32	2		
Ma	31.30	2	31.56	**M1**	37.74	2	39.43	5.56
Mb	31.82	5		**M2**	41.12	4		
MPa	31.22	1	31.52	**MP1**	29.44	3	28.66	2.03
MPb	31.82	2		**MP2**	27.87	3		
Qa	34.04	3	35.82	**Q1**	34.39	1	36.20	0.27
Qb	37.60	4		**Q2**	38.00	4		
Ra	35.44	3	35.49	**R1**	34.69	1	35.18	0.22
Rb	35.54	4		**R2**	35.66	6		
Sa	29.88	1	31.89	**S1**	31.06	2	30.23	1.17
Sb	33.89	3		**S2**	29.39	1		

Among the Finnish tuber sets, the highest average amylose content was recorded in a set derived of Asterix (42 g/100g) and the least from a set of Kuras (29.6 g/100g). Averages calculated with sets of the same variety of tubers show Desiree (37.9 g/100g) with the highest amylose content and Maris Piper (31.5 g/100g) with the least.

Among the Swedish tubers, the highest average amylose content was recorded with Desiree tubers (43.1 g/100g) and the least with Kuras (25.3 g/100g). Average amylose content calculated with all sets of same variety of tubers show Maria (39.4 g/100g) to be the highest and Kuras (26.g g/100g) to be the least.

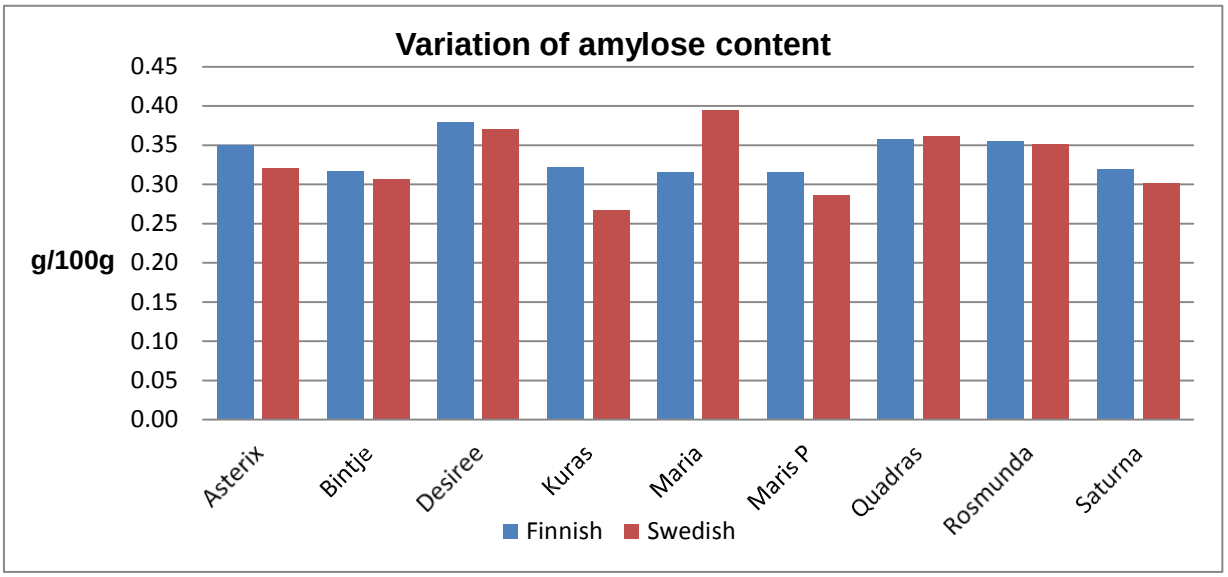

Figure 1 Amylose content variation between tubers of same cultivar but grown in two different regions

Asterix, Bintje, Desiree, Kuras, Maris Piper, Rosmunda and Saturna obtained from Finland had higher average amylose content compared to the Swedish tubers. The order of decrease: Kuras (17.7%), Maris Piper (8.9%), Asterix (8%), Saturna (5%), Bintje (2.8%), Desiree (2.4%) and Rosmunda (<1%). However, with Maria and Quras showed an increase in amylose contents by 24.7% and 1.1% respectively.

Total starch contents

Table 4 Total starch content in tubers of same cultivar but grown in two different regions

Tuber	g/100g	Co.Var	Variety Avg g/100g	Tuber	g/100g	Co.Var	Variety Avg g/100g	STD.DEV
Aa	60.9	0.24	67.10	**A1**	65.36	0.1	68.42	0.94
Ab	70.56	0.11		**A2**	71.48	0.09		
Ac	70.68	0.02		**-**	-	-		
Ad	66.25	0.17		**-**	-	-		
Ba	69.18	0.08	67.34	**B1**	67.15	0.07	67.76	0.30
Bb	65.49	0.16		**B2**	68.37	0.04		
Da	70.01	0.15	67.52	**D1**	59.48	0.14	61.61	4.18
Db	65.03	0.16		**D2**	63.74	0.05		
Ka	72.88	0.18	70.21	**K1**	54.36	0.24	54.94	10.79
Kb	67.53	0.14		**K2**	55.52	0.22		
Ma	64.94	0.12	65.12	**M1**	65.94	0.05	66.33	0.85
Mb	65.3	0.12		**M2**	66.71	0.02		
MPa	61.23	0.18	67.79	**MP1**	66.6	0.07	66.13	1.18
MPb	74.35	0.11		**MP2**	65.65	0.06		
Qa	70.97	0.11	71.25	**Q1**	66.52	0.03	67.11	2.93
Qb	71.53	0.08		**Q2**	67.7	0.04		
Ra	69.57	0.09	67.48	**R1**	67.29	0.18	66.51	0.69
Rb	65.39	0.13		**R2**	65.73	0.01		
Sa	56.83	0.04	63.45	**S1**	62.33	0.03	66.74	2.33
Sb	70.06	0.07		**S2**	71.15	0.26		

Among the Finnish tubers, the highest recorded total starch content was recorded with Maris Piper (74.4 g/100g) and the least with Saturna (56.8 g/100g). When the average was calculated with all sets of the same variety of tubers, Quadras (71.3 g/100g) was with the highest recorded total starch and Saturna (63.4 g/100g) with the least.

Among the Swedish samples, the highest recorded total starch was recorded with Asterix (71.5 g/100g) and the least with Kuras (54.4g/100g). Average calculated with sets of the same variety of tubers show Asterix (68.4 g/100g) with the highest recorded total starch and Kuras (55 g/100g) with least.

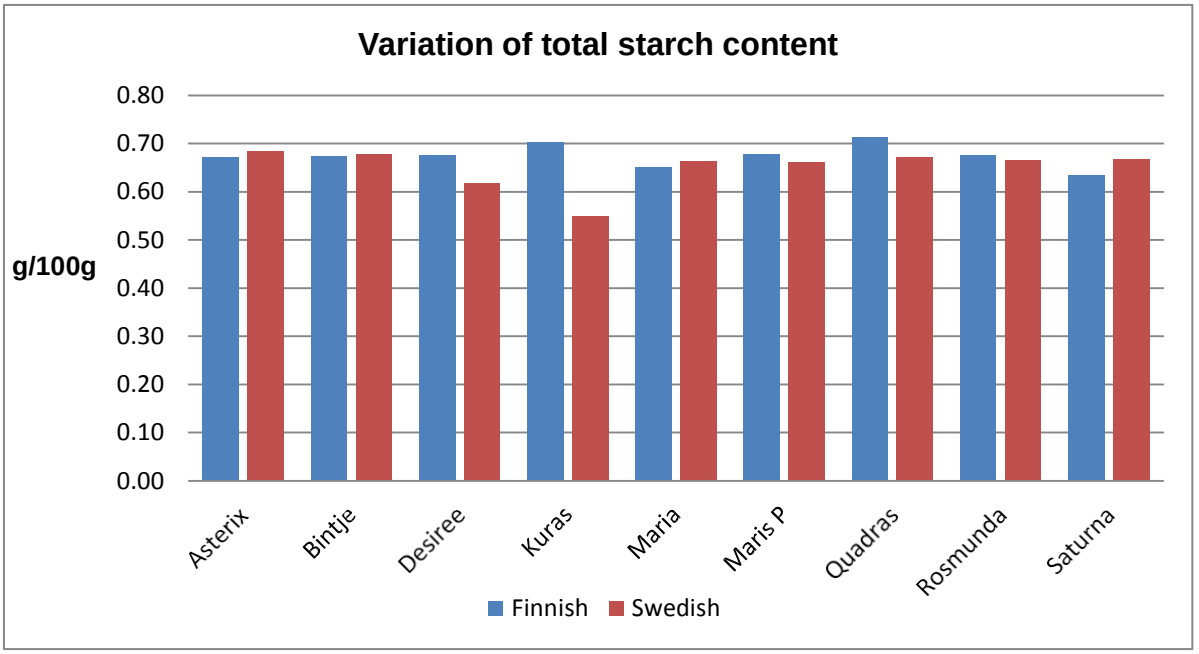

Figure 2: Average total starch content variation between same variety but different source

In comparison to Swedish varieties, five varieties sourced from Finland showed a decrease in total starch contents: Kuras (21.7%), Desiree (8.7%), Quadras (5.9%), Maris Piper (2.4%) and Rosmunda (1.5%). The remaining three varieties showed an increase in total starch content from Sweden when compared to Finnish tuber: Saturna (5.4%), Asterix (2.1%) and Bintje (<1%).

Conclusion

The starch contents were higher in the Finnish samples than the Swedish samples which indicates growing conditions and topography of the region affects the composition of potato tubers (Cottrell *et al.*, 1995). The repeatability of the starch isolation within individual sets could be inconsistent considering extraction method used; for example, some samples needed more washes than the others until the starch sediment formed itself.

Analysis of starches derived from tubers grown in same conditions have varied from 25.2 % to 29.1% in a study by Alvani et.al with 10 commercial varieties (Alvani *et al.*, 2011), the normal range falls in between 20-30%.The large variation within Amylose content values in this study can be due to the precision of amylose estimation method used (UDMSO method). The starches were not defatted prior to solution in UDMSO. A defatting method

using n-propanol for starch could be used for future reference (Hoover & Ratnayake, 2001). Starch granules also contain proteins, lipids and minerals which influence the behaviour of starch and amylose determination(Morrison & Coventry, 1985).This affects the formation of stable amylose-polyiodide colour complexes and hence the precision of the method(Morrison & Laignelet, 1983). Long chain amylopectin branches in potato starch can bind to iodine and have shown to overestimate amylose content (Hoover & Ratnayake, 2001). Furthermore, experiments using iodine binding procedure using wavelengths at 620nm and 510 nm have shown to increase the precision of the method(Zhu *et al.*, 2008). This can be used for future reference. Due to time constraints, microscopic properties of amylose were not investigated in this study. The outer most surface of the starch granule plays a vital role in starch processing and storage by affecting penetration of water and other compounds into the interior. In particular, it may influence gelatinisation, starch degradation by acid or enzyme, wetting ability etc.(Szymońska & Krok, 2003) Structrural comparison of the amylose within the varieties used could be investigated in future.

The recorded total starch content for all varieties reflected the purity of the extracted dry starch flour. The purity could be due to handling error and inconsistent sedimentation of starch observed during the extraction. A new starch method developed by Albalasmeh et. al using Sulphuric acid- UV spectroscopy has shown to improve time management in starch content determination and cost effectiveness (Albalasmeh *et al.*, 2013) in comparison to the method used in this study. The methodology could be improved by using Megazyme kits specific to detecting enzyme resistant starch for future reference.

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