

## Raw Materials for Processing of Sour-Sweet Slices of Red Beetroot

Karl Kaack

Aarhus University, Kirstinebjergvej 10, 5792 Aarslev, Denmark Institute for Agri Technology and Food Innovation, Denmark

---

**Summary:** *This research focuses on the possibilities for fitting raw material quality characteristics of red beetroot for industrial processing of preserved sour sweet red beetroot slices size 40-50 and 50-60 mm. The relationship between degree days and beet root was significantly associated with the yield of beetroot that may be 90 and 100 t ha<sup>-1</sup> with a high percentage of roots with a diameter from 40 to 60 mm. Optimization of beetroot length and size may increase the yield of slices during processing in order to improve the coordination between the growing of beets in the fields and the processing chain.*

*The quality characteristics color, sucrose, nitrate, oxalic acid, and mineral contents varied significantly between cultivars and the data from this research shows how to select new genetic variants that determine the most important properties of interest for the consumers. An increased level of nitrogen fertilization may decrease the purple colorant, keep the yellow colorants constant, decrease dry matter and soluble solids and increase nitrate, glutamic acid, and glutamine. Increases in harvest time may keep red colorants stable, increase yellow colorants, soluble solids sucrose, oxalic acid and water insoluble dry matter. The white precipitate in jars with beet slices may contain from 4 to 90 % calcium oxalate and the amounts of this deposit depended on salt and sugar concentration and decreased by increasing pH.*

**Keywords:** *Sowing - harvest - storage - color - texture - oxalic acid - white sediment.*

---

### INTRODUCTION

In the north western part of Europe are preserved sour sweet slices of beetroots produced in brines with sugar, vinegar and some spices may be used as a decoration and flavor ingredient upon several kinds of open rye or white wheat bread. They are often served with hot dishes together with different kinds of meats, boiled potatoes and the most important basic sensory tests carried out using hedonic scales have shown that sweetness, sourness and texture are very important quality characteristics [1] that are primarily combined with a high level of red and yellow colorants [2]. Beetroot slices are produced industrially in two sizes with diameter 40-50 or 50-60 mm that are packed in glass jars and pasteurized in brine made from sugar, vinegar and eventually some characteristic vegetable spices in water [2]. Considerations in 1973 regarding application of ball shaped or cylindrical beetroots resulted in determination of size distribution of three ball shaped cultivars 'Grosby', 'Detroit' and 'Rubia' resulted in 50, 57 and 60 % in size 40-60 mm, while the cylindrical cultivars 'Cylinder', 'Formanova' and 'Rød Valse' yielded 74, 87 and 96 % slices with diameter 50 to 60 mm showing that processing of cylindrical beets was the most fruitful. Danish producers of beet slices in 1970 found high percentages completely white and partially white beets in the raw materials of 'Formanova' and that forced them to use synthetically colorants. The concentration of betanin in these beets varied significantly from 200 mg 100 g<sup>-1</sup> in small beets to less than 100 mg 100 g<sup>-1</sup> in 'Formanova' beets weighing 250 g. These data resulted in a breeding program with successively selection of 'Formanova' in three generations. The average concentration of betanin, sucrose in the finally selected beets was 79.1 mg betanin 100 g<sup>-1</sup> and 13.2 g sucrose in 100 g<sup>-1</sup> beet. The purple color of beet slices is due to presence of red-violet betalain and yellow-orange betaxanthin pigments [3] and the sour-sweet flavor of the processed beet slices are due to a combination of the sour flavor from vinegar with the sweet flavor of sucrose [1, 2, 3, 4]. The yellow and violet to red colorants in beets may be destroyed or eluted during cooking of beets in water. A common praxis among red beet growers with application of a high rate of nitrogen fertilizer to beets may influence the yield of beets and their contents of several chemical compounds in beets. Another reason to study the effects of nitrogen is that use of a high level of nitrogen may re-

sult in a strong off-flavor of the sliced beets [5, 6]. Previous research in this area has included the importance of cultivars, soil fertility, water status, sowing and harvest time, growth temperature, fertilization and their effects on sugars, nitrogen, alcohol soluble solids and colorants [7, 8]. However, data from coherent research including the crucial stages in the processing of high quality sour-sweet slices of beetroot was not carried out taking growing, harvesting, cool storage and industrially processing methods into consideration. It was expected that research in beet growth rate may depend significantly on cultivar and temperature determined as degree days.

Therefore, the aim of this study was to study the effects of fertilization, sowing, growing and storage of raw beetroot characteristics in order to optimize the industrial processing of beetroot slices.

## **MATERIALS AND METHODS**

### *Field experiments*

Field experiments were carried out on sandy soil at the experimental field at Aarhus University according to common horticultural praxis regarding nitrogen fertilization, sowing, weed control, harvest time and cool storage. Experiment one included studies of the effects of four sowing and four harvest dates on growth rate, size distribution of the cylindrical cultivar 'Forono' in experiment one and comparison of the growth rates of 'Forono', 'Halanga', 'Rød Valse' and 'Unik' in experiment two with one sowing and four harvesting dates [9, 10]. The third field experiment included growing of three cylindrical cultivars 'Cylinder', 'Formanova', 'Rød Valse' and three ball shaped cultivars 'Crosby', 'Detroit' and 'Rubia' that was used for comparison of the average and variation in slice diameter from cylindrical and ball shaped cultivars, respectively. Field experiment four encompassed growing of the four cylindrical cultivars 'Forono', 'Rød Valse', 'Unik' and 'Halanga' in order to determine the yield and quality characteristics by sowing 12<sup>th</sup> June and determination of beetroot diameter of 40 beets in two replicates seven times during the growth period encompassing 6, 17, 31 August and 7, 14, 24, and 28 September using a caliper gauge. All beetroots from each cultivar were sorted in nine size classes with diameter from 3.5 to 8.0 cm and step length 0.5 cm using a mechanical sorting machine. Forty roots from each of the nine diameter classes of each cultivar were selected randomly and sliced using thickness 0.5 cm.

All beetroots from each cultivar were sorted in nine size classes with diameter from 3.5 to 8.0 cm and step length 0.5 cm using a mechanical sorting machine. Forty roots from each of the nine diameter classes of each cultivar were selected randomly and sliced using thickness 0.5 cm. Beet diameter was measured using a diaper. -Beetroots from 'Rød Valse' were also applied for determination of horizontal and vertical concentration of betacyanin in five beetroot samples taken downwards from the lowest leaf scar to root tip using an eight mm cylindrical cork. Each of them was cut into pieces representing the horizontal and vertical contents of betacyanin in three replicates (n = 5). Beet diameter was measured using a diaper.

Experiment three included growing of the cultivar 'Forono', with harvest 5 September, 3 October and 31 October, encompassing three sorting sizes that were processed immediately after harvest. Another series of beetroot samplings were harvested 5 September, 19 September, 3 October, 17 October, 31 October and divided into seven samplings in three replicates for cool storage at 3°C. These samples were also used for determination of the mechanical damage during peeling and cooking and the effects of various cooking and cooling temperatures.

Experiment four encompassed an experiment using circular pots in size 1 m<sup>2</sup> with sandy soil and application of 0, 32, 65, 129 and 258 kg N ha<sup>-1</sup> in four rows of replicates (n = 4).

Degree days in °C were determined using the sum of the average daily minimum ( $T_{\min}$ ) and maximum temperature in ( $T_{\max}$ ) and basis temperature 4.5°C (degree days =  $\Sigma(T_{\min} + T_{\max})/2 - (4.5)$ ) using data from the meteorological measurements at the experimental field institute in cooperation with Danish Meteorological Institute (DMI).

### *Harvest*

The harvest of beetroots included manual removal of the beetroot leaves using a sharp kitchen knife and the major amount of soil was removed gently before the beetroots were transported to the processing facilities for washing by hand in tap water (13°C), weighing and mechanical sorting by diameter into roots with diameter < 40, 40-60 and > 60 mm to determine their size distribution and

yield ( $n = 3$ ). Thereafter the beetroots from each treatment were mixed carefully and packed into four or ten kg net bags made of plastic and kept in cool storage at  $1^{\circ}\text{C}$  and 98 % relative humidity (RH) until analysis and processing ( $n = 3$ ). Samples for analysis included raw materials that were frozen and stored at  $-25^{\circ}\text{C}$ .

### *Dry matter*

Dry matter was determined by vacuum drying at 20 mm Hg for 20 hours at room temperature ( $20^{\circ}\text{C}$ ) if the dry material was used for further analytical measurements of organic compounds or in a heating cabinet at  $80^{\circ}\text{C}$  for 20 hours by determination of minerals and dry matter only ( $n = 3$ ). Water insoluble dry matter (Widm) was determined from 50 g macerated material weighed onto dried filters (S&S 520S, 185 mm), washing with boiling distilled water (Elgastat SB, BioSurplus, San Diego, USA) until complete removal of betanin and drying of the filters at  $80^{\circ}\text{C}$  for 20 hours ( $n = 3$ ).

### *Betacyanin and betaxanthin*

Betanin and betaxanthin were extracted from the macerated red beet materials using 0.05 M phosphate buffer pH 6.5 (Merck, Darmstadt, Germany) and measurement of absorbance at 476, 538 and 600 nm in filtrated samples ( $n = 3$ ) using a spectrophotometer (Shimadzu, MPS 2000, Kyoto, Japan) and the extinction coefficient 60.700 for betanin and 25.373 for betaxanthin by calculation of betanin and betaxanthin according to [11].

Samples for measurement of the betaxanthin contents in both vertical and horizontal direction of 'Rød Valse' beetroots was taken from the lowest leaf scar to the root tip using a cylindrical cork drill 6.8 mm in diameter ( $n = 4$ ).

### *White precipitate*

The amount of white precipitate in the bottom of the jars with preserved beetroot slices were isolated using centrifugation at 15,000 g (Sorvall Products, RC-SB, UK) and freeze drying (Martin Christ GmbH, Gamma 1-20, Osterode am Harz, Germany) at  $10^{-6}$  mm Hg and  $-25$  to  $25^{\circ}\text{C}$ .

### *Sugars*

Soluble solids ( $\text{g } 100 \text{ g}^{-1}$ ) were determined in filtrates from water extractions of macerated samples of red beet material using a refractometer in three replicates (Bellingham + Stanley, RF M 800, Turnbridge Wells, Kent, UK). This method and gas liquid chromatography was used in the experiments with four red beetroot cultivars and cool storage of beetroots ( $n = 3$ ).

Measurement of sucrose and glucose using gas liquid chromatography was initiated by treatment of 100 g red beet cubes using 300 g double distilled water and transfer of 5 g macerate that was diluted ten times with double distilled water. Then 1000  $\mu\text{L}$  extract were mixed with 100  $\mu\text{L}$  solution of trehalose in water (2.50 g in 100 mL) and diluted to 5 mL extracts with internal standard that were frozen to  $-25^{\circ}\text{C}$  and dried in vacuum (25 mm Hg) before addition of 500  $\mu\text{L}$  pyridine and 500  $\mu\text{L}$  Trisil to silylate the sugars. Separation and quantification of sugars were obtained using a Hewlett Packard 5840 A gas chromatograph equipped with a 2.5 m column (i.d. 1/8 inch) packed with 80/100 mesh OV-1, an HP7651 automatic sampler, FID detector at  $300^{\circ}\text{C}$ , air flow  $300 \text{ mL min}^{-1}$ , hydrogen flow  $30 \text{ mL min}^{-1}$ , oven temperature  $200$ - $295^{\circ}\text{C}$  increasing  $4^{\circ}\text{C min}^{-1}$ , attenuation  $2^8$ , slope 2, area rejection  $10^4$ , and  $10 \text{ mL min}^{-1}$  nitrogen as carrier gas.

### *Organic acids*

The gas chromatographic measurement of oxalic acid in three replicates was carried out using Pye Unicam 64 gas chromatograph combined with an integrator (Hewlett Packard 3370, Hillerød, Denmark) equipped with a stainless steel column (5 m, 1/8 inch i.d.) packed with 80/100 mesh diatomite CT, 10 p.c. CW 20M. Injection temperature  $200^{\circ}\text{C}$ , column temperature  $50^{\circ}\text{C } 5 \text{ min}$ ,  $6^{\circ}\text{C min}^{-1}$  to  $150^{\circ}\text{C}$ , carrier gas flow  $40 \text{ mL N}_2 \text{ min}^{-1}$ , FID detector at  $350^{\circ}\text{C}$ , air flow  $300 \text{ mL min}^{-1}$ , hydrogen flow  $30 \text{ mL min}^{-1}$ , slope  $0.1 \text{ mV min}^{-1}$  and injection of 10  $\mu\text{L}$  ester preparation. The standard curve was prepared using a solution of oxalic acid ( $10$ - $50 \text{ mg mL}^{-1}$ ) in methanol ( $n = 3$ ).

### *Amino acids*

Glutamin and glutamic acid determined using a Boehringer Mannheim electrode system (Vienna, Austria). Nitrate was determined using a spectrophotometric method according to [11] Nilsson and using an ion selective electrode (Boehringer Mannheim, Vienna, Austria), respectively. The number of repli-

cates for these methods was three. Calcium, magnesium and potassium were measured using atom absorption spectrometry as described previously.

*Flavor*

Eight panel members aged 20-40 evaluated the flavor of sour sweet preserved beetroot slices at five sessions using a scale from 1 to 10, was 1 was no off flavor and 10 was very intensive off flavor.

*Statistical analyses*

The statistical methods included linear and logarithmic regression, ranking, one-way and multiple ANOVA using a Statgraphic Statistical Package (Statistical Graphics, Version 4, Rockville, USA. Averages were separated using letters P < 0.05. Factor analysis included varimax rotation of normalized data and a Scree test was carried out according to [12, 13].

**RESULTS**

*Beetroot dimensions*

Percentage beetroots with diameter from 40 to 60 mm from the cylindrical cultivars ‘Cylinder’, ‘Formanova’ and ‘Rød Valse’ were 73, 87 and 96 w/w% and significantly higher than 62, 57 and 60 w/w% from ball formed cultivars ‘Crosby’, ‘Detroit’ and ‘Rubia’ in experiment 1 (P < 0.05). The beetroot weights of these six cultivars were strongly related to their volume where weight = k<sub>1</sub> + k<sub>2</sub>\*volume where k<sub>1</sub> is the constant and k<sub>2</sub> is regression coefficient for each cultivar (P < 0.05). On this background it was concluded that using cylindrical beetroots is more efficient compared to use of ball formed beetroots (Table 1). This experiment included sowing of from May 1<sup>st</sup> to 11<sup>th</sup> July and harvesting from 14<sup>th</sup> Aug to 7<sup>th</sup> Nov in experiment 1 and to 1<sup>st</sup> to July to harvest in August to October increased significantly linearly with degree days until the last harvesting in November. The constants in the regression equations were significantly different except for the first and latest harvest date for ‘Forono’ with the highest growth rates after the earliest and latest sowing and harvest dates. The slope of the regression equations belong to a relative similarity with growth rates from 0.02 to 0.04 mm degree day<sup>-1</sup>. The growth rate of the two pairs of cultivars including ‘Halanga’ and ‘Unik’ and ‘Forono’ and ‘Rød Valse’ was significantly different and that resulted in a significantly larger diameter on the first harvest dates for the first pair in comparison to the second pair. Because of the levels of significance of the obtained equations it was concluded that application of base temperature 4.5°C seems to be an optimum. ‘Halanga’ and ‘Unik’ obtained maximum diameter by harvest in the first week of August and ‘Forono’ had maximum one month later and the average diameter of ‘Rød Valse’ varied significantly over time. The percentage roots with diameter above 60 mm were significantly lowest for ‘Forono’ and the other three cultivars had similar percentage above 60 mm by harvest 31 August. Maximum diameter was obtained in the first two weeks of September (Table 2), The significantly largest and lowest amount of beetroots with diameter above 60 mm were obtained for ‘Unik’ and ‘Forono’. On the basis of these data it was concluded that the cultivar ‘Forono’ was the optimum cultivar for producing beetroot slices with diameter 40-60 mm. Growing of the cultivars ‘Halanga’ and ‘Unik’ may result in possibilities for earlier harvest. That could be an optimum choice by a low processing capacity in comparison to the use of one cultivar only. On this background it was concluded that the harvest and processing order must be ‘Unik’ before ‘Halanga’ before ‘Forono’, while ‘Rød Valse’ had the latest and least exact development in root diameter.

**Table 1.** Sowing, harvest and beet growth

Cultivar	Sowing	Harvest	Constant mm	P	Slope mm (degree day °C <sup>-1</sup> )	P
‘Forono’	1 <sup>st</sup> May	14 <sup>th</sup> Aug	7.51	ns	0.03952	***
"	20 <sup>th</sup> May	17 <sup>th</sup> Sep	10.58	**	0.02752	***
"	17 <sup>th</sup> Jun	31 <sup>st</sup> Oct	13.47	***	0.02220	**
"	11 <sup>th</sup> Jul	7 <sup>th</sup> Nov	0.50	ns	0.04186	**
‘Halanga’ and ‘Unik’	12 <sup>th</sup> Jun	10 <sup>th</sup> and 17 <sup>th</sup> Sep	17.91	**	0.03264	***
‘Forono’ and ‘Red Valse’	"	1 <sup>st</sup> Oct	14.67	**	0.03115	***
<sup>1</sup> Degeee days = Σ(T <sub>min</sub> + T <sub>max</sub> )/2 - (4.5).						

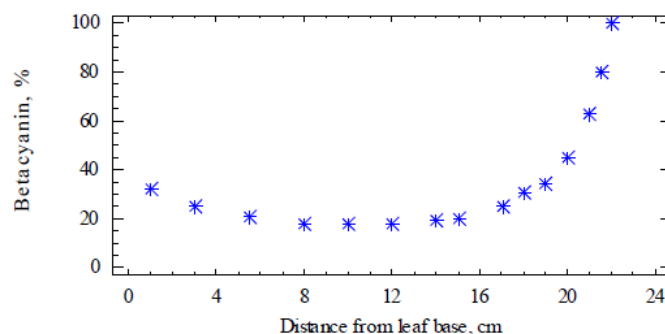


Fig 1. Vertical content of betacyanin in a beetroot (Rød Valse).

Table 2. Percentage beetroots with diameter 40-60 and > 60 mm at up to seven dates during the growth period.

Harvest	'Forono'	'Halanga'	'Rød Valse'	'Unik'	'Forono'	'Halanga'	'Rød Valse'	'Unik'
6 <sup>th</sup> Aug	53c	70a	45c	73a	3d	5c		
17 <sup>th</sup> Aug	55b	73a	60b	80a	5c	10c		
31 <sup>st</sup> Aug	65b	73a	70a	73a	10d	23c	9d	20c
7 <sup>th</sup> Sep	73a	73a	63b	58c	15f	25c	23c	35d
14 <sup>th</sup> Sep	73a		70a	52c	18e	23d	23d	48c
24 <sup>th</sup> Sep	73a		68b	18d	18	25c	25c	
28 <sup>th</sup> Sep	73a		73a					

Late sowing and harvest resulted in significant decreases in beetroot length, diameter and weight of 'Forono' (Table 3). Roots from 'Forono' and 'Rød Valse' were significantly longer than roots from 'Halanga' and 'Unik'. The diameter of roots from 'Forono' in this experiment occurred in two significantly size classes with small significant differences, while the diameter of the four cultivars in experiment two were non-significantly different. The weight of beetroots within the two cultivar groups 'Forono' and 'Rød Valse' and 'Halanga' and 'Unik', were non-significantly different, whereas the difference in weight between the two groups were significant. A decrease in beetroot length is serious because shorter roots result in fewer slices by each root.

Table 3. Length, diameter and weight of analyzed and processed beetroots.

Experiment	Cultivar	Sowing	Harvest	Length, cm	Diameter, cm	Weight, g	Roots, no
One	'Forono'	1 <sup>st</sup> May	14 <sup>th</sup> Aug	15.3a	42.6a	223a	240
"	"	20 <sup>th</sup> May	17 <sup>th</sup> Sep	13.3b	42.8a	189b	"
"	"	17 <sup>th</sup> Jun	31 <sup>st</sup> Oct	12.2c	41.0b	163c	"
"	"	1 <sup>st</sup> Jul	"	12.0c	42.2b	155d	"
Two	'Forono'	12 <sup>th</sup> Jun	1 <sup>st</sup> Oct	15.6a	49.0a	261a	50
"	'Halanga'	"	10 <sup>th</sup> Sep	11.8b	"	175b	"
"	'Rød Valse'	"	1 <sup>st</sup> Oct	15.7a	"	251a	"
"	'Unik'	"	17 <sup>th</sup> Sep	11.7b	"	170b	"

Yield of beetroots

The results from an experiment with four cylindrical beetroots showed that 'Halanga' resulted in the significantly highest total yield followed by 'Unik' and 'Forono' with equal total yields, and 'Rød Valse' with the significantly lowest yield (Table 4). 'Forono' gave almost equal amounts of size 40-60 and above 60 mm, which may be applied for processing of two slice sizes of preserved red beet slices. 'Halanga' and 'Unik' resulted in significantly higher yields of beetroots with diameter 40-60 mm in comparison to the yield of roots with a diameter above 60 mm, and the opposite was obtained for 'Rød Valse', which may be used for processing of most slices in the upper size classes (Table 4). The amount of crooked roots was significantly highest (21%) for 'Rød Valse' in comparison to 4 % among roots from 'Forono' and 'Halanga' and 7 % from 'Unik', which makes 'Rød Valse' less valuable in comparison to the other three cultivars. 'Forono' had a significantly lower percentage of roots with a diameter below 40 mm compared to 'Halanga' and 'Unik'. Based on these data it was concluded that 'Forono' may be preferred if the amount of products in the two size classes is equal, and 'Halanga' or 'Unik' may be preferred if the production requires more products with a diameter 40-60 mm than of the larger slices with a diameter above 60 mm.

**Table 4.** Yield of four cylindrical beetroots in four size classes (five sortings).

Cultivar	Sowing	Harvest	t ha <sup>-1</sup>			Total
			< 40 mm	40-60 mm	> 60 mm	
'Forono'	12 <sup>th</sup> Jun	1 <sup>st</sup> Oct	2.2b	37.8c	39.0b	79.0b
'Halanga'	"	10 <sup>th</sup> Sep	5.0a	52.7a	35.7c	93.4a
'Rød Valse'	"	1 <sup>st</sup> Oct	1.4c	26.6d	47.8a	75.8c
'Unik'	"	17 <sup>th</sup> Sep	2.6b	44.1b	32.3d	79.0b
'Forono'	1 <sup>st</sup> May	15 <sup>th</sup> Aug	1.6c	41.6b	9.3b	52.5b
"	20 <sup>th</sup> May	17 <sup>th</sup> Sep	5.9b	49.3a	13.5a	68.7a
"	17 <sup>th</sup> Jun	31 <sup>st</sup> Oct	7.8a	42.5b	3.3c	53.6b
"	1 <sup>st</sup> Jul	7 <sup>th</sup> Nov	5.6b	28.0c	1.2d	34.8c

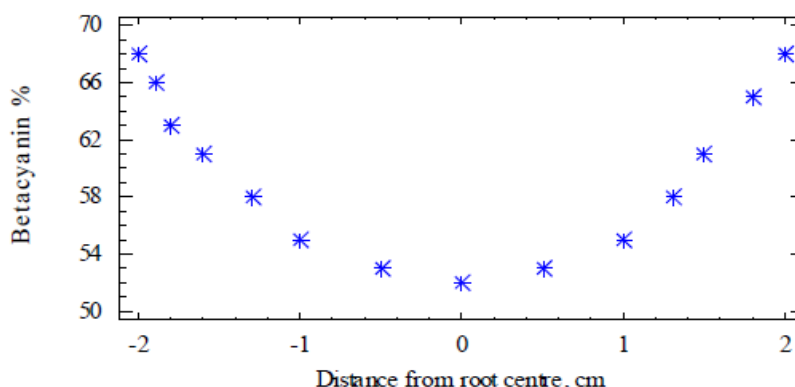


Fig 2. Horizontal concentration of betanin in a beetroot (Rød Valse)

The highest yield of roots in experiment one was obtained by sowing of 'Forono' on the 20 May and harvesting on the 17 September that also resulted in the highest yield of beets with a diameter above 60 mm (Table 4). Sowing 12<sup>th</sup> June as in experiment two resulted in the highest yield of 'Halanga' and 'Unik' in size 40-60 mm and significantly lower much lower yield of both 'Rød Valse' and 'Forono'.

#### Chemical compounds

The red pigments in beet root include betacyanin, iso- $\beta$ -cyanate and trace quantities of betacyanine [16] (Table 5) (Drdáket al., 1989) and linear regression analysis of data from experiment 1 showed that the contents of betacyanine in 'Rød Valse' decreased significantly ( $P < 0.05$ ) with beetroot weight:  $\text{mg}/100 \text{ g betacyanine} = 102.8 - 0.18 \cdot \text{weight}$  ( $R = -0.81$ ). The vertical concentration of red colorant in beet root surface decreased from 35 % at the leaf basis towards 18% at medium root height and increased then to 100% in the root tip and the horizontal distribution increased from 52% at the root center to 68 % at the root surface (Fig 4). These data shows that the highest concentration of the red betaxanthines occur in the beetroot tip and close to the beetroot peel, which may be taken into consideration peeling in order to reduce the losses of colorants by a too deep peeling. The contents of betacyanin in the three cultivars 'Cylinder', 'Formanova', and 'Rød Valse' were 52.2b, 53.1a and 49.3cmg 100g<sup>-1</sup> and the concentration in 'Crosby', 'Detroit' and 'Rubia' were 45.4b, 41.4c and 48.6a mg 100 g<sup>-1</sup>.

The contents of betanin and betaxanthin were highest in 'Forono' and lowest in 'Rød Valse', whereas soluble solids, sucrose and water insoluble dry matter were significantly highest in 'Unik', lowest in 'Rød Valse' and medium in 'Halanga' (Table 5).

**Table 5.** Chemical composition of beetroots from four cultivars ( $n = 4$ ).

Cultivar	Betanin	Vulga-xanthin	Soluble solids	Sucrose	Widm	Nitrate	Oxalic acid	Ca	Mg
	mg 100 g <sup>-1</sup>	mg 100 g <sup>-1</sup>	w/w%	g 100 g <sup>-1</sup>		mg kg <sup>-1</sup>	mg 100 g <sup>-1</sup>		
'Forono'	119a	96a	12.7b	9.7b	3.08b	760b	92a	285b	170a
'Halanga'	82c	66b	12.4b	9.5b	3.08b	813a	79b	306a	164a
'Rød Valse'	64d	44c	11.7c	9.3c	2.84c	685c	81b	253c	133b
'Unik'	97b	65b	13.0a	10.4d	3.23a	468d	69c	286c	165a

## Raw Materials for Processing of Sour-Sweet Slices of Red Beetroot

Soluble solids and sucrose were significantly highest in 'Unik', lowest in 'Rød Valse' and medium in 'Forono' and 'Halanga', whereas water insoluble dry matter was highest in 'Unik', lowest in 'Rød Valse' and medium in 'Forono' and 'Halanga' (Table 5).

Sucrose in beetroots is very important because this sugar contributes considerably to the flavor of the preserved sour sweet beetroot slices and depends on cultivar, sowing, harvest dates, and soil fertility [8]. The high concentration of oxalic acid and calcium is not acceptable because it may result in more white precipitate in the jars with preserved red beet slices. A high level of water non-soluble dry matter in 'Unik' and 'Forono' may cause an increasing cooking time. However, this may not be a solid argument to grow 'Rød Valse' with a lower concentration of oxalic acid and a very low concentration of colorants, sucrose and low concentration of magnesia.

Nitrate was significantly highest in 'Halanga' and lowest in 'Unik' while oxalic acid was highest in 'Forono' and lowest in 'Unik'. Oxalic acid was highest in 'Forono' and lowest in 'Unik' and medium in 'Halanga' and 'Rød Valse'. Among the contents of minerals were calcium highest in 'Halanga' followed by 'Forono' and low in both 'Rød Valse' and 'Unik' and magnesium was highest in 'Forono' followed by 'Unik' and 'Halanga' while it was lowest in 'Rød Valse' (Table 6). The variation in calcium and magnesium was rather small.

**Table 6.** Effects of nitrogen fertilization on the chemical composition of beetroots at harvest.

Nitrogen kg ha <sup>-1</sup>	Soluble solids g 100g <sup>-1</sup>	Betanin mg 100 g <sup>-1</sup>	Vulgaxanthin mg 100 g <sup>-1</sup>	Widm <sup>1</sup> g 100 g <sup>-1</sup>	Nitrate mg kg <sup>-1</sup>	Oxalic acid mg 100 g <sup>-1</sup>
40	12.3a	134a	86c	3.86a	1287a	156c
80	12.8a	134a	93b	3.72b	1018d	180b
120	12.3a	136a	91b	3.68b	1255b	190a
160	12.4a	128a	98a	3.71b	1193c	188a

Oxalic acid was highest in 'Forono' and lowest in 'Unik' and medium in 'Halanga' and 'Rød Valse'. Among the minerals were calcium highest in 'Halanga' followed by 'Forono' and low in both 'Rød Valse' and 'Unik'. Magnesium was highest in 'Forono' followed by 'Unik' and 'Halanga' while it was lowest in 'Rød Valse' (Table 6). Because the betaxanthin molecules contain nitrogen it was expected that the contents of this yellow colorant would increase by increasing nitrogen fertilization (Table 7). Using from 40 to 160 kg N ha<sup>-1</sup> did not affect soluble solids and betanin and the effect on nitrate was unclear, whereas betaxanthin, water insoluble solids and oxalic acid increased significantly by increasing level of applied nitrogen (Table 7). Application of higher amounts of nitrogen fertilizer up to 258 kg N ha<sup>-1</sup> resulted in significantly increasing yield of beetroots and contents of nitrate, glutamin and glutamic acid, whereas betanin, dry matter, and soluble solids decreased significant [15]. Decreases in the contents of soluble solids and colorants occurred by high rates of nitrogen fertilization and low supply of water [3]. Glutamin and glutamic acid increased also in the preserved beet slices, which are in accordance with previous research [14]. Betaxanthin increased significant up to 65 kg N ha<sup>-1</sup>, while glucose and sucrose was unaffected by increased supply of nitrogen to the soil (Table 7).

**Table 7.** Effect of nitrogen on the concentration of chemical compounds in the cultivar Hunderup 66 (n = 4).

	Relative yield	Betanin	Vulgaxanthin	Nitrate	Dry matter	Soluble solids	Glucose	Sucrose	Glutamine	Glutamic acid	Glutamine	Glutamic acid
	%	mg 100 g <sup>-1</sup>			g 100g <sup>-1</sup>							
									Raw beets		Preserved slices	
0	52e	108a	38b	729c	16.4a	14.2a	0.20b	11.5b	0.120e	0.026e	0.058e	0.072b
32	68d	97b	37b	610d	15.8b	13.3c	0.25a	12.2a	0.150d	0.035d	0.093d	0.069b
65	84c	88c	45a	399d	15.9b	13.8b	0.19b	12.3a	0.199c	0.043c	0.131c	0.076b
129	89b	76d	38b	1964b	14.5c	11.7c	0.19b	12.4a	0.367d	0.059b	0.158b	0.089a
250	100a	66e	39b	2185a	13.7d	10.4d	0.20b	9.8c	0.505a	0.062a	0.188a	0.084a

It has previously been found that heat treatment of red beet materials may result in degradation of the amino acid glutamine which furthermore may result in formation of pyrrolidone carbonic acid [15, 17, 19] that have a bitter and phenolic off flavor. Sensory evaluation of preserved sour sweet red beet slices by six trained panel members aged 20-40 did not result in finding any off flavor associated to increased application of any of the applied levels of nitrogen (data not shown).

#### *Sowing and harvest*

Data from experiment 2 showed that the contents of betacyanin was 53.0c, 56.4b and 60.8a after sowing 12<sup>th</sup> May, 6<sup>th</sup> and 15<sup>th</sup> Jun and 57.6a, 56.9a and 54.8a mg 100 g<sup>-1</sup> after harvest 3<sup>rd</sup> and 22<sup>nd</sup> Oct and 12 Nov, which means that the contents of betaxanthins increased significantly by later sowing time, whereas increasing harvest date not affect the contents of betacyanin. The change in betacyanin concentration in 'Forono' with harvest time varied slightly through the harvest period, whereas betaxanthin, soluble solids and water insoluble dry matter increased through the period (Table 8). Betanin increased significant from the first to the second storage day and varied slightly in the remaining part of the storage period, whereas betaxanthin and water non-soluble dry matter seemed to have a maximum after 32 days of storage. Dry matter, soluble solids and extrusion force increased during the growth period, whereas the contents of red pigment decreased [3].

**Table 8.** Changes in composition of 'Forono' during harvest and cool storage.

Compound	Harvest time, days						
	1	14	28	42	56		
Betanin, mg 100 g <sup>-1</sup>	105b	106b	90c	101b	115a		
Vulgaxanthin, mg 100g <sup>-1</sup>	68e	78d	83c	98b	105a		
Soluble solids, %	12.2e	11.6d	13.7c	13.6b	14.0a		
Sucrose, g 100 g <sup>-1</sup>	8.8a	9.1bc	9.7ab	9.9a	9.3a		
Oxalic acid, mg 100 g <sup>-1</sup>	105c	129a	121a	128a	127a		
Widm, g 100 g <sup>-1</sup>	2.4d	2.8c	3.0b	3.2a	3.3a		
	Storage time, days						
	2	29	32	45	56	71	82
Betanin, mg 100 g <sup>-1</sup>	103b	106a	106a	107a	106a	109a	107a
Vulgaxanthin, mg 100g <sup>-1</sup>	86d	89c	95a	89c	87c	90b	82e
Soluble solids, %	13.0a	12.8a	13.3a	13.5a	13.1a	13.1a	13.2a
Sucrose, g 100 g <sup>-1</sup>	9.7a	9.5a	9.6a	9.5a	9.1b	9.0b	8.9c
Oxalic acid, mg 100 g <sup>-1</sup>	121e	127d	150b	149b	145c	144c	158a
Widm, g 100 g <sup>-1</sup>	2.9b	3.2a	3.2a	3.0b	2.8b	2.9b	3.0b

Previous research showed that betanin decreased in the last part of the storage period. Water insoluble dry matter increased also from harvest to the first sampling day during storage and decreased during the last part of the storage period as [2]. Oxalic acid in raw beets increased significantly from first to second harvest and remained on this level to the last harvest. During storage oxalic acid increased through the first samplings and remained at this level until the last harvest where a significant increase occurred (Table 8).

In products of preserved slices of beetroot white precipitates appear at the bottom of the jars, which blemish the food and consumers are complaining the qualities because of this defect. A microscopic test showed that the white material contains crystals that were soluble in sulphuric and hydrochloric acid.

The amount of dried white precipitate in up to ten medium size jars with red beet slices varied from 17 to 60 mg in five experimental samples and from 40 to 310 mg in 14 commercial samples (Table 8). And the amount of calcium oxalate varied from 7 to 44 mg corresponding to between 4 and 76 w/w % calcium oxalate. The amount of calcium oxalate in the brine varied from 13 to 34 mg L<sup>-1</sup> in experimental samples and from 2 to 28 mg L<sup>-1</sup> in commercial samples.

The data showed significant relationship between theoretical and analytical contents of calcium oxalate and the level decreased significantly from pH 2.0 to 4.5. By addition of 0 to 2.0 g w/w % NaCl the solubility of calcium oxalate increased from 29 to 66 mg L<sup>-1</sup>. In raw beetroots soluble sodium and potassium salts and insoluble calcium salts occur [15] and the maximum amount of calcium oxalate was between 50 and 125 mg 100<sup>-1</sup> g beetroots [17, 20].



The solubility product of calcium oxalate ( $\text{CaOxH}_2\text{O}$ ) and the constant dissociation at  $25^\circ\text{C}$  found in Handbook of Chemistry and Physics [21] and the equilibrium constants ( $j$  = activity constants) may be written:  $K_0 = (\text{Ca}^{++})(\text{Ox}^-)j^2 = 2.57 \cdot 10^{-9}$  (1);  $K_1 = (\text{OxH})(\text{H}^+)/\text{OxH}_2 = 5.91 \cdot 10^{-2}$  (2),  $K_2 = (\text{Ox}^{--})(\text{H}^+)/(\text{OxH}) = 6.41 \cdot 10^{-5}$ . The amount of solved  $\text{Ca}^{++}$  may be written  $(\text{Ca}^{++}) + (\text{OxH}^-) + (\text{OxH}_2)$  and the three the three equations ( $K_0, K_1, K_2$ ) were used to estimate  $(\text{Ox}^{--}) = K_0/(j(1 + \text{H}^+)/K_2 + (\text{H}^+)(\text{H}^+)/K_1K_2$ . [22](Daniels and Alberty, 1961). Using these equations, the theoretical solubility of  $\text{OxH}_2$ ,  $\text{Ca}(\text{OxH})_2$ ,  $\text{CaOx}$  and the total amount of dissolved  $\text{CaOxH}_2\text{O}$  was calculated. On average the red table beets contained 44 mg precipitate  $100 \text{ g}^{-1}$  beet slices of which 9 mg were  $\text{CaOxH}_2\text{O}$ .

The effects of sugar (0;5;10;15:20 w/w%), NaCl (0.25; 0.5;1.0;15;20 w/w%) and acetic acid (0.0005; 0.001; 0.005; 0.01; 0.05; 0.1; 0.5 mole  $\text{L}^{-1}$ ) on the solubility of calcium oxalate were determined in a fully factorial experiment. The obtained results showed agreements between theoretical and experimental data.

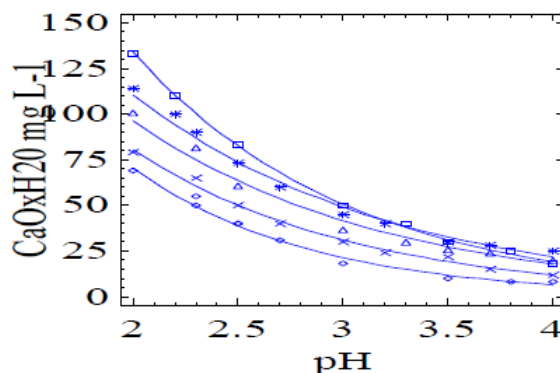


Fig 3. Solubility of calcium oxalate decreased with increased concentration of NaCl.

NaCl 0 squares  
 NaCl 0.25 stars  
 NaCl 0.5 triangles  
 NaCl 1 crosses  
 NaCl 2 diamonds

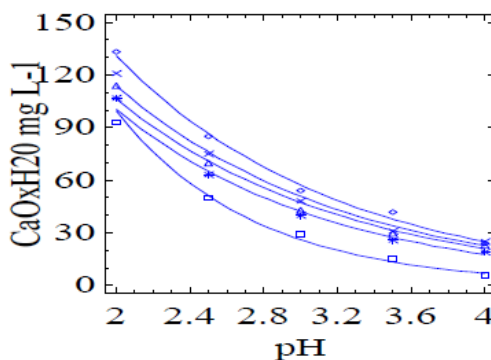


Fig 4 Solubility of calcium oxalate increases with increasing concentration of NaCl using sugar in the brine..

With sugar  
 NaCl 0 squares  
 NaCl 0.25 stars  
 NaCl 0.5 Triangles  
 NaCl 1 crosses  
 NaCl 2 diamonds

In solutions without sucrose decreased the concentration of calcium oxalate significantly with increasing pH and with increasing level of NaCl (Fig 3), whereas the concentration of calcium oxalate increased by increasing concentration of NaCl (Fig. 4). The data fitted with logarithmic functions as for example  $\text{CaOxH}_2\text{O} = \exp(\text{constant } a) - (\text{constant } b \cdot \text{pH})$  with correlation coefficients above 0.99. On the basis of these data it may be concluded that the white material in the bottom of jars with red beetroot slices mainly are calcium oxalate and that they occur because of the contents of oxalate in the beetroots and calcium in the tap water.

## DISCUSSION

The presented data regarding the effects of degree days on beetroot growth between sowing and harvest may be used for coordination of the field production and processing capacities in order to obtain optimum beetroot dimensions and diameter of the processed beetroot slices. The necessary number of sowing and harvest dates may be estimated on the basis of the cultivar, field characteristics and amount of daily slice production. Use of the information about the effects of degree days, and red beet diameter may be used to make plans for sowing and considerations regarding the optimization of harvest time to obtain the maximum number of beet slices and the most efficient exploitation of the processing facilities. Combination of degree days with cultivar, climate conditions and degree days may improve the yield for the farmer. Forecasts and forecasts regarding beetroot size may improve the possibilities for careful planning of harvest and processing capacities taking slice diameter into consideration. The most important quality characteristic is beetroot length because longer beetroots may increase the yield of slices significantly. It may be considered that application of the beetroots with significantly low and high diameter could be used for processing of other beetroot products in order to improve the occurring resources of beetroot materials.

Betacyanin in beetroots cultivars varied from 9 to 135 mg 100 g<sup>-1</sup> [18, 19, 22, 23]. The sum of vulgaxanthin I and II varied from 36-42 and 0.03-0.04 mg 100 g<sup>-1</sup>. The relationship between betanin (y) and beetroot weight (x) was estimated to be logarithmic:  $y = a \cdot e^{bx}$  or  $\log(\text{betanin}) = \log a - bx$ , where a and b are constants and that equation shows that the contents of betanin decrease significantly with beetroot size ( $P < 0.05$ ).

The major color of sour sweet beetroot slices is betacyanin making up to 75-95 % of the total amounts and the remainder red to purple color pigments is isobetacyanin and pre-betacyanin [21]. The concentration of betacyanin in raw beetroots of breeding lines varied from 27 to 135 mg betacyanin 100 g<sup>-1</sup> [18, 19, 22]. The yellow color compounds in red beetroots are betaxanthins that include betaxanthine I and II that encompassed from 36 to 42 mg 100 g<sup>-1</sup> and from 0.03 to 0.04 mg 100 g<sup>-1</sup>, respectively [23, 24, 25, 29]. Impact of thermal treatment on colour and pigment pattern of red beet [24] and shows thereby the large difference in concentrations found between cultivars [22, 23] and the considerable proportion between betacyanines and betaxanthines in red beets [19, 23]. Beet colorants are sensitive to heat [19, 22, 24, 26, 27, 28, 30, 32] and are degraded according to first order kinetics. Therefore the colorants may be decreased considerably by blanching, cooking and canning [19] (von Elbe et al., 1981). This has been of concern by canning industry [7].

Betacyanines were less stable at pH 3 than 5 and most stable between pH 4 and 5 [25] and betaxanthines were much less stable than betacyanines, whereas betaxanthines are similarly affected by pH and light as betacyanins [22].

Betacyanines and betaxanthines react with molecular oxygen [25, 30] (von Elbe et al., 1974; [30] and their stability may be improved by antioxidants [31, 33, 34] or by establishment of a nitrogen atmosphere [30, 31].

The changes in the very important constituent in beetroots including betanin, betaxanthin, soluble solids and water insoluble dry matter depended linearly on time. On average Betacyanin increased 0.03258 mg 100 g<sup>-1</sup> each day (betacyanin = 104.7 + 0.03258 days mg 100 g<sup>-1</sup>) and the decrease in betaxanthin was significantly higher (betaxanthin = 90.3 - 0.04691 days mg 100 g<sup>-1</sup>) than the increase in betacyanin. The increase in soluble solids (soluble solids = 13.0 + 0.004307 days g 100 g<sup>-1</sup>) that may be due to respiration and decreases in starches which may be included in the changes of water insoluble soluble solids (Widm = 3.1 - 0.002039 days).

Storage of betacyanin solution under low oxygen resulted in decreased pigment degradation because low oxygen levels favor partial recovery of this compound [11, 25]. The increases in betacyanin and betaxanthin with later sowing/harvesting may be due to less mature beets, which normally have higher contents of these compounds [2, 7]. Total dry matter in 'Formanova' and 'Rød Valsewere' 15.6 and 15.7 w/w% while soluble solids were 13.2 and 13.0 w/w% and insoluble solids 2.4 and 2.7 w/w%, respectively. Soluble solids and sucrose in beetroots increased with increasing plant age [3, 18]. During cool storage at 3.5°C reducing sugar decreased significantly (Table 9) as found previously [18]. Firmness of canned beet slices decreased significantly linearly during storage at 18 and 38°C [3].

**Table 9.** Yield of four old cylindrical beetroots in four size classes (n = 5).

Cultivar	Sowing	Harvest	t ha <sup>-1</sup>			
			< 40 mm	40-60 mm	> 60 mm	Total
‘Forono’	12 <sup>th</sup> Jun	1 <sup>st</sup> Oct	2.2b	37.8c	39.0b	79.0b
‘Halanga’	"	10 <sup>th</sup> Sep	5.0a	52.7a	35.7c	93.4a
‘Rød Valse’	"	1 <sup>st</sup> Oct	1.4c	26.6d	47.8a	75.8c
‘Unik’	"	17 <sup>th</sup> Sep	2.6b	44.1b	32.3d	79.0b
‘Forono’	1 <sup>st</sup> May	15th Aug	1.6c	41.6b	9.3b	52.5b
"	20 <sup>th</sup> May	17th Sep	5.9b	49.3a	13.5a	68.7a
"	17 <sup>th</sup> Jun	31st Oct	7.8a	42.5b	3.3c	53.6b
"	1 <sup>st</sup> Jul	7th Nov	5.6b	28.0c	1.2d	34.8c

Calcium and magnesium may to some degree be associated with water insoluble solids. Even trace amounts of mineral cations such as iron, copper, tin and aluminum accelerate betacyanin degradation during processing [24, 32, 33]. The solubility of calcium oxalate decreases by increasing pH from 2 to 4 both with and without sugar in the brine. Without sugar decreased the solubility of calcium oxalate with increasing contents of NaCl whereas increasing levels of NaCl caused increasing solubility of calcium oxalate by presence of sugar in the brine. These effects of increasing concentration of salts are mainly due to increased ion activity constants [21]. Data from small experiments showed that occurrence of the white material may be avoided by using 1 w/w% NaCl and pH 3.6 or by increases in acetic acid, phosphoric acid or citric acid in comparison with or without using NaCl.

#### REFERENCES

- [1] Kaack, K., 1972. Taste evaluation of fruits and vegetables (Danish J. Plant and Soil Sci. 76, 604-610).
- [2] Kaack K., 1977. Changes in contents of colorant substances in red beet during growth, storage, industrial processing of red canned beets. Danish J. Plant and Soil Sci. 81, 165-170. (1977).
- [3] Delgado-Vargas, F., Jiménez A.R., Paredes-López O., Natural pigments: Carotenoids, anthocyanins, and Betalains -characteristics, biosynthesis, processing and stability. Crit. Rev. Food Sci. Nutr. 40, 173-289.(2000).
- [4] Rao, M.A., Kenny, L.F., Shannon, S., Bourne, M.C. 1977. Firmness and thermal conductivity of red beets in storage. J. Food Process Engineering 1, 259-267.
- [5] Shallenberger, R.S., Moyer, J.C., Relationship between pyrrolidone carboxylic acid and an off-flavor in beet puree. J. Agric. Food Chem. 6, 604-606. (1958).
- [6] Markakis, P., Amon, A., The presence and origin of 2-pyrrolidonecarboxylic acid in processed grape juice and its concentrate. Food Technol. 23, 1463-1465. (1961).
- [7] Lusas, E.W., Rice, A.C., Weckel, K.G., Changes in the color of canning beets. : I. Changes during growth and processing: 2. Effects of variables in processing on the color of canned beets. 3. Effects of additives on color of canned beets. Res. Bull. 218, 1-23. University of Wisconsin - Madison. (1960).
- [8] Shannon, S., Changes in soluble solids, red pigment content, and firmness of table beet cultivars with growing time and season. J. Amer. Soc. Hort. Sci. 97, 223-228. (1972).
- [9] Kaack, K. Effects of sowing date, harvest time, and storage on raw beet quality and processing requirements. Danish J.Plant and Soil Sci 92, 316-324. (1988a).
- [10] Kaack K. Quality assessments of cylindrical red beet (*Beta vulgaris* L.) cultivars for programmed growing. Danish J. Plant and Soil Sci 92, 325-327. (1998b).
- [11] Nilsson, T., Studies into pigments in beetroot. Lantbrukshögskolans Annaler, 36, 179-210. (1970).
- [12] Überla, A. K., Faktorenanalyse. Springer, Berlin. (1971).
- [13] Sharma S. Applied Multivariate Techniques (1996).
- [14] Herrbach, K.M., Stintzing, F.C. Carle R Impact of thermal treatment on color and pigment pattern of red Beet (*Beta vulgaris* L.). J Food Sci 69, C491-C498 (2004).
- [15] Ugrinovic, K. Effect of nitrogen fertilization on quality and yield of red beet (*Beta vulgaris* L.). Acta Hort.506, 99-104. (1999).

- [16] Drdák, M., Vallová, M., Daučík, P., Greif, G., Effect of fermentation on the composition of the red beet pigments. *Z. Lebensm. Unters. Forsch.* 188, 547-550. (1989).
- [17] Mahdi, A.A., Rice A.C., Weckel, K.G., Effect of pyrrolidonecarboxylic acid on flavor of processed fruits and vegetables. *J. Agric. Food Chem.* 9, 143-146. (1961).
- [18] Davies A.C.W., Oswin, P., Rutherford, P.O., Tucker, W.G., Phelps, K., Investigations on the long term storage of red beet. *Expl. Hort.* 28, 15-30. (1976).
- [19] Luh, B.S., Antonakos, J., Daoud, H.N., Chemical and quality changes in strained carrots canned by a septic and retort processing. *Food Technol.* 23, 377-381. (1969).
- [20] Adrianse A. and Robbers J. E., Über eine modifizierte Gesamtoxalatbestimmung in Gemüsen. *Z. Lebensm. Unters. Forsch.* 141, 158-60. (1969).
- [21] Daniels, F., Alberty, R.A., *Physical Chemistry* 2nd edition. John Wiley and Sons Inc. New York. (1961).
- [22] Sapers, G. M., Hornstein J. S., Varietal differences in colorant properties and stability of red beet pigments. *Food Sci.* 44, 1245-1248. (1979).
- [23] Gasztonyi, M.N., Daood, H., Hájos, M.T., Biacs, P., Comparison of red beet (*Beta vulgaris var conditiva*) varieties on the basis of their pigment components. *J. Sci. Food Agric.* 81, 932-933. (2001). and retort processing. *Food Technol.* 23, 377-381. (1969).
- [24] Pasch J.H., von Elbe J.H., Betanine stability in buffered solutions containing organic acids, metal cations, antioxidants, or sequestrants. *J. Food Sci.* 44, 72-74 & 81. (1979).
- [25] Kujala, T.S., Loponen, J.M., Klika, K.D., Pihjala, K. J., Phenolics and betacyanin in beetroot (*Beta vulgaris* L.). Distribution and effect of cold storage on the content of total phenolics and three individual compounds. *J. Agric. Food Chem.* 48, 5338-5342. (2000).
- [26] Saguy J., Kopelman J. F., Mizrahi, S., Computer-aided determination of beet pigments. *J. Food Sci.* 43, 124-127. (1978).
- [27] Saguy, J., Thermostability of red beet pigments (Betanine and Vulgaxanthin-I). Influence of pH and temperature. *J. Food Sci.* 44, 1554-1555. (1979).
- [28] Savolainen, K., Kuusi, T., The stability properties of golden beet and red beet pigments. Influence of pH temperature and some stabilizers. *Z. Lebensm. Forsch.* 166, 19-22. (1978).
- [29] Singer, J.W., von Elbe J.W., Degradation rate of vulgaxanthine I. *J. Food Sci.* 45, 489-491. (1980).
- [30] von Elbe, J.H., Sy, S.H., Maing, I.Y., Abelman, W.H., Quantitative analysis of betacyanins in red table beets (*Beta vulgaris*). *J. Food Sci.* 37, 932-934. (1972).
- [31] von Elbe, J.H., Schwartz, S.J., Hildenbrand, B.E., Loss and generation of betacyanin pigments during processing of red beets. *J. Food Sci.* 46, 1713-1715. (1981).
- [32] Wyler, H., Vincenti, G., Mercier, M., Saguy, G., Dreiding, A. S. Zur Konstitution des Randenfarbstoffes Betanin. *Helv. Chim. Acta* 42, 1696-1698. (1959).
- [33] Czapski, J., Heat stability of betacyanins in red beet juice and in betanin solutions. *Z. Lebensm. Unters-Forsch.* 191, 275-278. (1990).
- [34] Huang, A.S., von Elbe J. H., Kinetics of the degradation and regeneration of betanine. *J. Food Sci.* 50, 1115-1120 & 1129. (1985).
- [35] Huang, A.S. and von Elbe, J. H., Effect of pH on the degradation and regeneration of betanine. *J. Food Sci.* 52, 1689-1693. (1987).
- [36] E., L., and von Elbe J. H., Degradation kinetics of betanine in solutions as influenced by oxygen. *J. Agric. Food Chem.* 30, 708-712. (1982).
- [37] Attoe E.L., von Elbe, J. H., Oxygen Involvement in betanine degradation: effect of antioxidants. *J. Food Sci.* 50, 106-110. (1985).
- [38] Han, D.; SJ Kim; S.H. Kim, Kim, D.M., Repeated regeneration of degraded red beet juice pigments in the Presence of antioxidants. *J. Food Sci.* 63, 69-72.
- [39] Attoe EL, Von Elbe JH (1984) Oxygen involvement in betanin degradation. Oxygen uptake and influence of metal ions. *Z Lebensm-Unters Forsch* 179: 232-236.
- [40] Snell, A.D. and Snell, C.T. 1949. *Colometric Methods of Analysis*. D. van Nostrand, New York.