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# Improvement of Quality Characteristics of Cherry Fruit Juice Processed using Fruits from 'Stevnsbær clone 23' (*Prunus cerasus* L.)

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**Abstract:** *This research include studies regarding processing of cherry fruit juices by improvement of the cherry fruit odour by inclusion of benzaldehyde and elimination of hydrogen cyanide obtained by mechanical maceration of cherry fruit stones containing amygdalin. Maturation of fruits from 'Stevnsberry clone 23' included linear increases in acidity, soluble solids, anthocyanins, lightness and redness. Berry weight increased according to a positive multiplicative equation, while cyanide, stone weight, benzaldehyde and yellowness decreased significantly according to negative multiplicative equations, respectively. Maceration of cherry stones included maceration of the pressing residues using a small home-made mill with two circular drums that may be used for release of benzaldehyde and hydrogen cyanide. The contents of hydrogen cyanide may be eliminated by chemical reactions with sugar anomers from the cherry juice and the added sugar as described previously. The chemical reactions by elimination of hydrogen cyanide during processing of cherry fruit juice includes: Glucose + HCN → cyanohydrin + $H_2O$ → heptagluconacidamide + $H_2O$ → ammoniumheptagluconate that is a neutral eluent and may be excluded without from the juice by centrifugation.*

**Keywords:** *fruit juice processing.*

## 1. INTRODUCTION

Research in prediction of the optimum harvest time for cherries may be based on the relationship between the average flowering day 15$^{th}$ May and the number of degree days that result in accumulation of about 1400 degree days on location of each fruit growing plantation [1]. Previous research showed that berry and stone weight, acidity, soluble solids, anthocyanins, benzaldehyde, hydrogen cyanide, lightness, redness and yellowness may be used for prediction of maturity and picking time using linear equations obtained by factor analysis [2]. Mechanical damage of cherry fruits during industrial processing of juices result in release of the glucoside amygdalin that occur in the meat and seed of cherry fruits and may be hydrolysed to glucose, benzaldehyde and hydrogen cyanide in two steps [3, 4]. Such reactions may result in occurrence of more than of 0.012-1.2 mg $g^{-1}$ cyanide in the cherry fruit juices [3]. The risk by consumption of hydrogen cyanide has been described by the National Institute for Occupational Safety and Health in US by writing that exposure to 2.7 ppm hydrogen cyanide for 10 min may result in personal discomfort and 27 ppm may result in death. Because of this dilemma it will be necessary to identify a way to eliminate the developed cyanide as fast as possible during industrial processing of cherry fruit juice.

The hypothesis of this research is therefore that elimination of cyanide may be obtained during or shortly after industrial processing using the reaction of the cyanide ion CN with anomeric keto- and aldohexoser by formation of non-toxic carbonic acid nitriles as described previously [5]. The reason for these reactions is that soluted and naturally produced keto- and aldohexoser occurs in several mutarotational equilibria including sugars in the cherry juice and by addition of common sugar [6, 7, 8, 9]. The irreversible reaction of hydrogen cyanide with sugars results in formation of cyanohydrins that is combined with water by synthesis of ammoniumheptagluconate that react with more water during formation of ammoniumheptagluconate as described by previously [10].

The aim of this research was therefore to study the industrial processing of cherry fruit juice including mechanical damage of cherry stones and frozen cherry fruits.

## 2. MATERIALS AND METHODS

Fruits from ʻStevnsbær clone 23', ʻKelleris 16', ʻDubbel Gorsem Krieck', ʻWielcin K', ʻNefris' and ʻRexelle' grown at the experimental location 10° 27' (E) and 55° 18' (N) were also picked at their optimum maturity, frozen and kept at minus 25 °C until analysis and processing. The fruits from the cultivar ʻStevnsbær clone 23' used by industrial processing of juice were delivered from a local farm and processed freshly together with samples from a parallel experimental area.

The industrial processing of cherry juice included weighing, disintegration of the cherry fruit materials and a short heating to 90 °C for two minutes, followed by efficient cooling to 45°C that resulted in release of a large amount of juice. The next step included addition of a liquid containing hydrolytic enzymes (Pectolase) that promote hydrolysis of pectins by keeping the fruits at 45°C in two hours until pressing in order to obtain maximum yield of juice from the Bucher-Geyer equipment. The final part of processing encompassed centrifugation, pasteurization at 95°C, cooling to 12 °C and transported to storage in steel tanks kept at 12 °C. Samples of cherry fruits were taken from the raw material, free juice eluted during loading of the drum, after pressing, centrifugation and pasteurization in five replications within two hours. These samples with 10 kg cherry fruit materials were cooled to room temperature, frozen and stored at -25°C until analysis using the physical and chemical analyses as described below. The soft parts from the fruits were separated from the stones by sieving. Non-damaged cherry fruit seeds were obtained by gently breaking of the stones immediately before each experiment using a pair of nutcrackers.

Dry matter was determined by drying of homogenised materials at 80°C to a constant weight after 20 hours. Non-soluble dry matter was obtained by drying and weighing of the materials in filters (Sleicher & Schüll 520b, 185 mm) from measurement of the soluble solids.

Disintegration of cherry stones included also maceration of the cherry fruits using a small home-made mill with two circular drums with diameter 85 and 95 mm made of stainless steel with 24 and 85 small drills with maximum difference 0.1 mm between the two drums. The stones were added through a funnel while the drums were rotated slowly and resulting in complete disintegration in one treatment.

Processing of juice in the laboratory were carried out at room temperature using 200 g frozen fruit materials by increasing pressure up to 150 atmospheres for fifteen minutes in a home modified automatic tincture presser. The obtained samples were packed in bootless of glass and pasteurized in a water bath at 85 °C for 15 min, water cooled in another water bath at 65 °C for 5 min and then cooling for 30 min in cool tap water at 12 °C. Samples of juice were prepared using a mixture of glucose and fructose or with 40 w/w % sucrose. Processing of juice without stones included careful removal of stones manually using a sharp homemade needle very gently.

Disintegration of cherry fruits with or without stone in connection with chemical and physically analyses were carried out using four parts of berries with or without cherry stones mixed with one part cold tap water (12°C) and homogenised for two minutes at maximum speed using a Robot-Coupe blender (Model R602VV, Vincennes, Cedex, France) or a hand-held blender (Braun, Miniper Compact, MR 404, Braun Gmbh, Kronberg, Germany). Soft parts from the fruits were separated from the stones by sieving. Disintegration of cherry stone and frozen whole fruits encompassed also use of a home-made mill with two stainless steel circular drums with diameter 85 and 95 mm with 24 and 85 small drills with maximum difference 0.1 mm between the two drums. Fruits and stones were added through a funnel while the drums were rotated slowly and resulted in severe disintegration.

Dry matter was determined by drying of homogenised materials at 80°C to a constant weight after 20 hours. Non-soluble dry matter was obtained by drying and weighing of the materials in filters (Sleicher & Schüll 520b, 185 mm) from measurement of the soluble solids.

Samples of pulp from berries without stones were used for measurement of soluble solids using a refractometer (Bellingham and Stanley, RF M800, Turnbridge Wells, Kent, UK) and the levels of acidity was measured by titration of the samples to pH 8.1 using 0.1 N NaOH (Bie and Berntsen, Copenhagen).

Anthocyanin content was measured using 100 g of carefully mixed cherries that were disintegrated in buffer solutions with KCL/HCL at pH 1.0. The contents of anthocyanin were measured using absorbance at 515 nm, molar extinction coefficient of 29.600 and mole weight 445.2 of cyanidin-3-glucoside using a spectrophotometer (Shimadzu, MPS 2000, Kyoto, Japan).

Isolation of hydrogen cyanide was carried out using a micro kjeldahl distillation column according to Pregl. Registration of the contents of HCN were carried out using a cyanide selectrode F 1042 CN with reference electrode K401, pH meter 63, buffer stabilizer S3566 pH 10.2 (Radiometer, Copenhagen, Denmark) and a 0.010 N standard solution of potassium cyanide. This standard solution was used by preparation of solutions with molarity $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ of potassium cyanide at 20°C. Hydrogen cyanide were measured in disintegrated and filtrated samples acidified with 1 mL concentrated sulphuric acid and transferred to 10 ml 1N NaOH by micro-distillation according to Pregl. After dilution to 50 mL were the content of HCN determined using a cyanide electrode F1042 CN, a reference electrode K 401 with pH meter 63 and buffer stabilizer S 3566 with pH 10.2 (Radiometer, Copenhagen, Denmark). A $10^{-2}$ molar standard solution of KCN was prepared using distilled water and titration with 0.01 N $AgNO_3$ in order to obtain a precise normality. And thereafter may the occurrence of hydrogen cyanide in a solution with known molarity be applied by preparation of solutions with molarity $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ of potassium cyanide using 10 ml $10^{-2}$ molar KCN and 50 ml ion stabilizer by each of these steps. The obtained regression equation may be used by measurement of the cyanide ion in the plant materials and processed foods using other relevant standard solutions prepared as described above.

Non-damaged cherry fruit seeds were obtained by gently breaking of the stones immediately before each experiment using a pair of nutcrackers in order to use the seeds by processing of cherry fruit juice.

Aroma compounds were measured using a Hewlett-Packard 7675A sampler with a sample size of 10 ml, involving a purge gas level of $N_2$ 37.5 mL, a five-minute pre-purge, 30-minute purge, pre-column filled with tenax-GC, temperature 20°C, one-minute elution time and a cleaning temperature of 250°C. A Hewlett-Packard 5890 series II Plus gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a split/splitless injector (200°C) and a flame ionisation detector (FID) operating at 230°C were applied by separation and quantification of the aroma compounds. The contents of benzaldehyde was measured using a Hewlett Packard HP 58OA GC-column 5 m steel i.d. 1/8 inch, carrier: diatomite, C-W, 80-100 mesh, 10% Ucon-LB-1715, carrier gas 15 $mL^{-1}$ $min^{-1}$, column temperature 50-200°C, 4°C $min^{-1}$, °C $min^{-1}$, paper velocity 3 mm $min^{-1}$ attenuator 2 exp. 10, slope sensitivity, internal standard cyclohexanone with a retention time of 25.5 minutes

Sampling of benzaldehyde was obtained using a Hewlett-Packard 7675A sampler with a sample 10 ml, involving a purge gas level of $N_2$ 37.5 ml, a five-minute pre-purge, 30-minute purge, pre-column with tenax-GC at 20°C, one-minute elution time and a cleaning temperature 250°C. A Hewlett-Packard 5890 series II Plus gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a split and splitless injector at (200°C) and a flame ionisation detector (FID) operating at 230°C. The contents of aroma compounds were separated using a Hewlett Packard HP 58OA GC-column 5 m steel i.d. 1/8 inch, carrier diatomite, C-W, 80-100 mesh, 10% Ucon-L-1715, carrier gas 15 $ml^{-1}$ $min^{-1}$, column temperature 50-200°C, 4°C $min^{-1}$, paper velocity 3 mm $min^{-1}$ attenuator 2 exp. 10, slope sensitivity, internal standard cyclohexanone with a retention time 25.5 min. minutes.

## 3. SENSORY EVALUATION

Sensory evaluation of cherry juice, jam and yoghurt jam were carried out by a sensory panel with four men and six women aged 28-36 years and very motivated in sensory evaluation of fresh and processed foods. They were previously tested for skills in the nasal tastes firmness, bitterness, sourness, sweetness and saltiness. In this research could the members of the panel use experiences from previous training of in sensory evaluation of at least six different raw or processed fruits and at least six raw or processed vegetable foods, previously. In this research were judges specifically trained in evaluation of the characteristic cherry odour, sweetness, sourness, benzaldehyde and fruity esters using a 10 point scale with 1 for the lowest and 10 for the highest intensities of these quality characteristics.

## 4. STATISTICAL ANALYSES

Statistical analysis was carried out using analysis of variance and regression analysis. Averages were separated through a multiple range test significance were $P < 0.05$. Averages were separated using letters.

## 5. RESULTS AND DISCUSSION

Determination of benzaldehyde and cyanide fifteen times during maturation of fruits from ʻStevnsbær clone 23' during 50 days of maturation showed that the contents of benzaldehyde (B) decreased from 15 to 3 mg $kg^{-1}$ and the contents of cyanide C decreased from 5 to 1.5 mg $kg^{-1}$, respectively. Interpretation of these data by regression analysis showed that the contents of both compounds decreased with time according to logarithmic equations, while the proportion between these compounds increased linearly with time:

ln (B) = -0.0345days + 2.7532 (r = 0.95)

ln (C) = 0.00469days +1.5392 (r = 0.98)

ln B/C = 0.0124days (r = 0.97)

The maturation rate of the fruits from ʻStevnsberry clone 23' included linear increases in acidity, soluble solids, anthocyanins, lightness and redness. Berry weight increased according to a positive multiplicative equation, while cyanide, stone weight, benzaldehyde and yellowness decreased significantly according to negative multiplicative equations, respectively.

**Table 1.** *Pick Maturation of cherry fruits (n= 3).*

| Days before picking | Equations | r |
|---|---|---|
| Acidity, g 100 $g^{-1}$ | 1.87 +0.01day | 0.81 |
| Soluble solids, g 100 $g^{-1}$ | 14.58 + 0.143day | 0.95 |
| Anthocyanin, mg 100 $g^{-1}$ | 91.16 + 1.92day | 0.93 |
| Lightness (L) | 14.06 + 0.31day | 0.96 |
| Redness (a) | 15.48 + 0.22day | 0.96 |
| Berry weight, g | $0.97day^{\wedge 0.230}$ | 0.99 |
| Cyanide, mg $kg^{-1}$ | $24.49days^{\wedge -0.71}$ | -0.93 |
| Stone, g 100 $g^{-1}$ | 12.73day^-0.16 | -0.98 |
| Benzaldehyde | 8.94day^-0.29 | -0.97 |
| Yellowness (b) | 12.73day^-0.07 | 0.70 |

Acidity, soluble solids, anthocyanin, lightness (L) and redness (a) increased significantly linearly during cherry fruit maturation. Berry weight increased according to a positive multiplicative equation. Soluble solids were correlated significantly with sucrose in the product, whereas inverted sugar were inversely associated to soluble solids from the cherries (Table 2). Acidity and anthocyanin were significantly associated with soluble solids.

**Table 2.** *Quality characteristics of samples from industrial processing and from a parallel field experiment using fruits from 'Stevnsbær clone 23' (n = 5).*

| Materials | Acidity g 100 $g^{-1}$ | Soluble solids g 100 $g^{-1}$ | Antho-cyanin mg 100 $g^{-1}$ | Benz-aldehyde mg $kg^{-1}$ | Cyanide mg $kg^{-1}$ | Stone g 100 $g^{-1}$ |
|---|---|---|---|---|---|---|
| | Industrial processing | | | | | |
| Raw fruit | 1.89b | 20.8b | 204.3a | 92.8a | 33.9a | 12.1a |
| Fruit meat | 2.45a | 21.5a | | | | |
| Free juice | 1.66c | 21.8a | 193.4b | 15.7d | 6.2b | |
| After pressing | 1.63c | 19.0c | 150.8d | 19.8b | 6.0b | |
| After centrifugation | 1.63c | 22.1a | 180.9c | 17.3c | 6.6b | |
| After pasteurization | 1.66c | 21.7a | 178.4c | 17.8c | 6.0b | |
| Average for juices | 1.65c | 21.2a | 175.9d | 17.7 | 6.b2 | |
| Stone | Parallel field experiment | | | | | |
| Without stone | 1.75a | 22.0a | 248.8a | 5.6b | 2.7b | 2.9b |
| With stone | 1,78a | 20.3a | 226.5b | 157.5a | 46.3a | 12.9a |

Raw fruits used by industrial processing had the significantly highest contents of acidity, anthocyanin, benzaldehyde and cyanide, while soluble solids were non-significantly different in free juice, centrifuged and pasteurization, respectively. The significantly highest content of anthocyanin was found in the raw fruits followed by free juice, after centrifugation, pasteurisation and significantly lowest in the juice after pressing.

A significantly high content of cyanide was found in the raw fruit and the significant lowest content was found in the samples taken during processing. The contents of soluble solids decreased to 19.0 g 100 $g^{-1}$ in the juice and were not significantly different in the remaining samples of juices. Soluble solids were non-significantly different in free juice after centrifugation and pasteurization, while the contents of acids in raw fruit and juice after pressing were significantly different.

An experiment with increasing temperature from 30 to 80 ºC in macerated cherry fruit materials in 90 min resulted in the first place to an increase of benzaldehyde from 52 to 60 mg $l^{-1}$ and thereafter decreased benzaldehyde from 60 to 43 with average 50 mg $l^{-1}$ (Table 3). Therefore it was concluded that the optimum temperature by enzyme treatment may be 40ºC. Similar treatments and storage of other samples of macerated cherry fruits at 40 and 60ºC for 15-90 min resulted on average in 35 mg $l^{-1}$ benzaldehyde. The maximum treatments resulted in loss of 9 mg $l^{-1}$ and increases to 28 mg $l^{-1}$, respectively. The presented data resulted on average in disappearance of 17 mg 100 $g^{-1}$ and an increase to 28 mg 100 $g^{-1}$ benzaldehyde, respectively. Increasing the time for enzyme treatment to 80 min did not affect the level of benzaldehyde significantly.

Cherry fruit meat, seed and stone shells may contain 0.15-0.6, 3.1-26.3 and 2.5-20.5 mg hydrogen cyanide 100 $g^{-1}$ [3] and cherry fruit juices may contain from 0.2 to 2.6 mg hydrogen cyanide 100 $ml^{-1}$ [12]. The contents of HCN in cherry juices varied from 2.1 to 14.7 mg $l^{-1}$, without stone was the content of HCN 5.3-5.4 mg $l^{-1}$ and with stone were the contents 11.5-25.9 [13, 14] reported that the contents of HCN in cherry juice varied from 2-22 mg $l^{-1}$. Other measurements showed that the contents of hydrogen cyanide in cherry juice varied between 0.10 and 0.12 mg 100 $mg^{-1}$ without enzyme treatment and from 0.56-0.92 mg 100 $ml^{-1}$ after enzyme treatment [15]. Other research data showed that hydrogen cyanide in cherry juices varied from 2.1 to 14.7 mg $l^{-1}$, without stone was the content 5.3-5.4 mg $l^{-1}$ and with stone were the contents 11.5-25.9 mg $l^{-1}$ [13, 14]. The content of bound cyanide in cherry juices was estimated to be 2.2 mg $l^{-1}$ hydrogen cyanide [4, 14]. On the basis of these data it was concluded that the contents of cyanide in cherry fruit juices may vary significantly in dependence of cultivar, mechanical damage of cherry meat and stone and the possibilities for keeping a low content of cyanide seem therefore to be possible.

**Table 3.** *Contents of benzaldehyde in completely macerated cherry fruit juice.*

| Temperature - | Benzaldehyde mg $l^{-1}$ | Time min | Benzaldehyde mg $l^{-1}$ | |
|---|---|---|---|---|
| 30 | 52c | 15 | 19d | 28d |
| 40 | 60a | 30 | 27c | 32c |
| 50 | 57b | 45 | 33b | 37b |
| 60 | 45d | 60 | 40a | 39a |
| 70 | 44d | 90 | 40a | 38a |
| 80 | 43d | 120 | 41a | 41a |

The contents of anthocyanin, amygdalin, acids and soluble solids were in accordance with the known ranges for sour cherries and for some seed cultivars [16, 20, 21]. Soluble solids and acidity in 'Stevnsbær clone 23' corresponds with data from analysis of sour cherries published previously [21]. The contents of soluble solids in ten sour and duke cherry cultivars varied from 15.0 to 18.9 g 100 $g^{-1}$ and citric acid varied from 0.7 to 1.6 g 100 $g^{-1}$ [20]. The contents of the glucoside amygdalin in cherry fruit meat and seed of cherry fruits [3, 4] are hydrolysed to glucose, hydrogen cyanide and benzaldehyde [4, 17]. And that may result in significantly higher contents of cyanide and benzaldehyde in the cherry fruit juices (Table 4).

**Table 4.** *Composition of cherry cultivars (n= 10)*

| Cultivar | Seed g $kg^{-1}$ | Benzaldehyde mg $kg^{-1}$ seed | Juice mg 100 $g^{-1}$ |
|---|---|---|---|
| 'Dubbel G. Krieck' | 22.3c | 2553e | 63e |
| 'Kelleris 16' | 27.5b | 3543b | 108b |
| 'Nefris' | 18.8d | 3491b | 73d |
| 'Rexella' | 18.0d | 3067c | 61e |
| 'Wielcin K' | 27.0b | 2781d | 83c |
| 'Stevnsbær clone 23' | 31.4a | 3720a | 130a |

The contents of benzaldehyde in cherry fruit juice processed from the six cultivars were 130 mg 100 $g^{-1}$ for 'Stevnsbær clone 23', 108 for 'Kelleris 16', 73c for 'Nefris' and 83c for 'Wielcin K', while the lowest values enclosed 'Rexella' 61d and 'Duel G. Krieck' with 63d). Anthocyanin was at maximum for 'Wielcin K' 135.7a, and 'Rexella'114.1, medium for 'Dubbel G. Krieck' 97.0 and 'Nefris' 96.8. The last pair in this content was 'Stevnsbær clone 23' with 70.4 mg 100 $g^{-1}$and 'Kelleris 16', with 25.7 mg 100 $g^{-1}$.

Enzyme treatment did not result in release of all the residues in the pressing residue because they are associated strongly to a diversity of various compounds. The content of bound cyanide in cherry juices was estimated to be 2.2 mg $l^{-1}$ hydrogen cyanide [15] and cherry seed contained 2.7-3.9 mg $g^{-1}$ amygdalin [4]. Results from measurement of cyanide and benzaldehyde in 'Stevnsberry clone 23' applied in this research showed that the pressing residues contained 5.50 mg $kg^{-1}$ cyanide and 21.8 mg $kg^{-1}$ benzaldehyde.

Maceration of 120 g pressing residue in 880 g water and used for determination of acidity, soluble solids, anthocyanin, benzaldehyde and the proportion B/CN without and with stone decomposition. The content of cherry seeds where 2.68 and 3.89 g mg $g^{-1}$[4]. Inclusion of the pressing residues with stone into the pressing materials resulted in significantly higher contents of benzaldehyde and cyanide. As another example were 120 g pressing residue macerated in 880 g water and used for determination of acidity, soluble solids, anthocyanin, benzaldehyde and the proportion B/CN without and with stone decomposition. The content in cherry seeds where 2.68 and 3.89 g mg $g^{-1}$[4]. Inclusion of pressing residues with stone into the pressing materials resulted in significantly higher contents of benzaldehyde and cyanide.

The contents of anthocyanin in the cherry fruit juice were significantly higher and the contents of benzaldehyde and hydrogen cyanide were significantly lower in juices processed without stone, while application of stones resulted in significantly higher contents of benzaldehyde and hydrogen cyanide (Table 5). Processing of cherry fruit juice without stone and with stone resulted on average in 1.76 g 100 $g^{-1}$ acidity, 20.3 g 100 $g^{-1}$ soluble solids, 237.3 mg 100 $g^{-1}$ benzaldehyde. The yield of benzaldehyde without and with stone were 5.6 and 157.5 mg $l^{-1}$ benzaldehyde and the yield of hydrogen cyanide were 2.7 and 46.3 mg $l^{-1}$, respectively

**Table 5.** *Average content of acidity, soluble solids, anthocyanin and hydrogen cyanide (n= 3).*

| Materials | Acidity g 100 $g^{-1}$ | Brix g 100 $g^{-1}$ | Anthocyanin mg 100 $g^{-1}$ | Benzaldehyde mg $kg^{-1}$ | HCN mg $l^{-1}$ |
|---|---|---|---|---|---|
| Without stone | 1.75a | 20.2a | 248.8a | 5.6b | 2.7b |
| With stone | 1.78a | 20.3a | 226.5b | 157.5a | 46.3a |

A test regarding enzyme activities was carried out using 50 g cherry fruit meat, 750 g water and 0.70 g seeds by increasing temperature from 30 to 100 °C in eight steps. That resulted in release of benzaldehyde up to 60 °C, whereas the contents of cyanide decreased to 141a, 79b, 68c, 21d and 11e mg $l^{-1}$ when the temperature increased from 60-100°C. These data shows that enzyme activity in cherry seeds may be particularly killed by temperature above 60 °C (Table 6). Temperatures below 50 °C did not result in hydrolysis of amygdalin, while increasing temperature above 60 °C resulted in significantly decreases in the content of benzaldehyde from 3.47 to 0.28 g $l^{-1}$ because of enzyme inactivation by heating.

Using one litre buffer solution with 13.61 g $KH_2PO_4$, 21.01g citric acid, adjusted to pH 3.31 using 69 ml 1 N NaOH, 50 g glucose, 50 g fructose and supplied with 60 g cherry fruit seeds and macerated. The obtained liquid was transferred to 100 ml glass bottles and pasteurized for 0, 10, 40 and 80 min and cooled using tap water (12 °C). Analysis using gas chromatography showed that increasing pasteurization time up to 40 min resulted in a significantly higher content of benzaldehyde in non-sweetened juice by comparison to sweetened juice (Table Time). The increases in benzaldehyde by time may be due to enzyme catalysed degradation of amygdalin and the significantly lower content of benzaldehyde in sweetened juice may be due a combination of cyanide with the small content of anomeric sugars. Benzaldehyde may be reduced to benzoic acid by chemical destruction [11].

The contents of cyanide and benzaldehyde increased significantly with increasing percentage crushed stone. Increases in storage time for both non-sweetened and sweetened samples resulted also in significantly decreases of both cyanide and benzaldehyde. Storage of the macerated materials up to 80 min after processing did not affect the contents of these chemical constituents. The contents of

cyanide and benzaldehyde in juices processed without and with sweetening using sucrose as sweetener showed that stepwise increases in maceration resulted in increasing contents of hydrogen cyanide and benzaldehyde (Table 6).

A test was carried out at room temperature using 50 g cherry fruit meat, 750 g water and 0.70 g seeds and keeping the sample at 30 to 100 °C in eight steps. That resulted in release of 158 mg $l^{-1}$ benzaldehyde, whereas the contents of cyanide decreased to 141, 79, 68, 21 and 11 mg $l^{-1}$ when the temperature was increased from 60 to 100°C. This is a well know example on the relations between temperature and enzyme activity. The effect of temperature on release of benzaldehyde showed that temperature up to 50°C not resulted in hydrolysis of amygdalin and that increasing temperature above 60°C resulted in significantly decreases in the content of benzaldehyde from 3.47 to 0.11 g $l^{-1}$. The reason is that the hydrolytic enzymes in the seed are inactivated and that the content of benzaldehyde decreased significantly from 3.47 g $l^{-1}$ at 60°C to 0.28 g $l^{-1}$ at 100 C°.

Increasing the time for enzyme treatment to 80 min did not affect the level of benzaldehyde significantly, whereas sweetening and addition of sugar resulted in a significantly lower content of benzaldehyde in cherry fruit juices because of elimination of hydrogen cyanide by production of juice. These data confirm that hydrogen cyanide may be eliminated using more sugar and it may be theorized that the content of benzaldehyde also may decrease by degradation to benzoic acid [11]. The contents of benzaldehyde were significantly lower in sweetened juice by comparison to non-sweetened juice.

**Table 6.** *Effect of sweetening on juice composition (n = 4).*

| Juice | Time, min | | | | Average |
|---|---|---|---|---|---|
| mg $L^{-1}$ | 0 | 10 | 40 | 80 | |
| Non-sweetened | 91.4ap | 87.7aq | 93.2aq | 91.5ap | 90.9a |
| Sweetened juice | 60.0bq | 58.7br | 62.2bp | 52.6b | 60.9b |

Using one litre buffer solution with 13.61 g $KH_2PO_4$, 2 1.01g citric acid, adjusted to pH 3.31 using 69 ml 1 N NaOH, 50 g glucose and fructose and supply of 60 g cherry fruit seeds were macerated intensively. The obtained liquid were transferred to 100 ml glass bottles and pasteurized for 0, 10, 20, 30, 50 and 70 min and cooled using tap water (12 °C). Analysis using gas chromatography showed that all samples on average contained 69.4 mg benzaldehyde and that shows possibilities for processing of similar contents of benzaldehyde because pasteurization at temperatures op to 70 min not affect the level of benzaldehyde. Heating of juice without sugar and with sugar for 0, 10, 40 and 80 min at 85ºC showed again that heating time not affected the content of benzaldehyde, whereas the average content of benzaldehyde were 90.0 and 60.9 mg $l^{-1}$ in non-sweetened and sweetened juices resulted in a significant reduction in the content of benzaldehyde during storage with 33 % sucrose. The content of cyanide and benzaldehyde increased significantly with increasing percentage crushed stone. Increases in storage time for both non-sweetened and sweetened samples resulted in significantly decreases of both cyanide and benzaldehyde (Table 7). Storage of the processed juice for 15 days resulted in non-significantly different contents of cyanide and benzaldehyde in sweetened samples stored for one day. Further storage of sweetened samples for 15 days resulted in the significantly lowest contents of cyanide and benzaldehyde by all levels of maceration of pressing juices. Data from processing of juice with increasing macerated stone, sweetening and storage time shows that the contents of cyanide decreased to an acceptable level of hydrogen cyanide. The contents of hydrogen cyanide were lower in comparison to the levels of this compound found previously by several researchers as described above.

**Table 7.** *Effects of percentage macerated cherry stone on the contents of cyanide and benzaldehyde (n= 3)*

| Juice | Days | Chrushed stone % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 25 | 50 | 75 | 100 | 0 | 25 | 50 | 75 | 100 |
| | | Cyanide, mg $l^{-1}$ | | | | | Benzaldehyde, mg $l^{-1}$ | | | | |
| Non-sweeted | 1 | 1.3epq | 5.9dpr | 9.2cap | 10.6p | 12.7ap | 7.1e | 33.3d | 64.2c | 72.3p | 79.5ap |
| " | 15 | 0.7eq | 2.7dqr | 4.3cq | 5.1q | 6.5aq | 4.4e | 25.7d | 41.6c | 49.6q | 59.2aq |
| Sweetened | 1 | 0.5dq | 2.8dqr | 4.1cq | 5.3q | 6.3aq | 6.4e | 24.2d | 35.2c | 41.5r | 47.8ar |
| " | 15 | 0.4cr | 1.2crq | 1.8ccr | 2.1r | 2.5ar | 2.1f | 18.5g | 39.2h | 40.8i | 41.2j |

The contents of benzaldehyde that contributes to the characteristic cherry flavour component as in marzipan occurred in a very high concentration in these juices compared to the other cherry aroma compounds, encompassing hexanal, (E)-2-hexenal, (E)-2-hexen-1-ol, and benzyl alcohol [19, 22, 23]. The average content of benzaldehyde measured by gas chromatography in this research was 3.9 mg 100 $g^{-1}$ in 'Nefris' (339), 4.0, 'Dubbel G. Krieck', 5.0 in 'Rexelle', 6.1 in 'Wielcin K', 6.7 in 'Stevnsbær clone 23' and 7.0 in 'Kelleris 16' (LSD = 0.6).

Infrared absorption spectra of the crystalline sugars including aldoses and ketoses occur in one form only, while each of these occurs in several mutarotational equilibria of aldoses and ketoses [6]. This knowledge was confirmed using NMR polarimetry measurements [8], NMR spectrometry [9] and confirmed for presence of several forms of sugars in aqueous solutions [24]. Occurrence of a non-reversible reaction between ketoses and aldoses as descried by [5] and presence of several anomeric ketoses and glucoses was descried afterwards [7, 8, 24, 25] have resulted in a method for elimination of hydrogen cyanide from liquids such as cherry fruit juice as a part of ammonium heptagluconat descried by [10]. The chemical reactions may be initiated by supply of a glucose anomers being a cyanohydrins that absorb water being heptagluconacidamide that absorb more water in order to formation of ammoniumheptagluconate being a neutral compound that easily may be excluded from the cell without special requirements [10]. The chemical reactions encompassed: Glucose + HCN → cyanohydrin + $H_2O$ → heptagluconacidamide + $H_2O$ → ammoniumheptagluconate that is a neutral eluent that is excluded from the cells without any special requirements [10].

Infrared absorption spectra of the crystalline sugars aldoses and ketoses occur in one form only, while each of these occurs in several mutarotational equilibria of aldoses and ketoses such as D-glucose and D-fructose in water [6]. This knowledge may also be confirmed using NMR polar metric measurements [8] and NMR spectrometry [9] and several forms of ketoses and aldoses may occur in aqueous solutions [21].

During industrial processing of cherry fruit juice was acidity in the raw fruits 1.89 g 100 $g^{-1}$ which was significantly higher than in the obtained juices with non-different contents of acidity (Table 7). The contents of soluble solids and anthocyanine in raw cherry fruit juices after pressing were significantly low and may occur because of a delay in release of soluble compounds during pressing.

The contents of acidity and soluble solids were non-significantly different, whereas the content of anthocyanine decreased and both benzaldehyde and hydrogen cyanide increased significantly using maceration of cherry fruit stone and seed if the juices were sweetened using common sugar. Occurrence of residues with or without sweetening using a mixture of glucose and fructose shows that in water solutions occur in several anomers [6] and they react with the cyanide ion and are eliminated according to [10]. In an experiment with complete disintegration of the pressing residue with or without stone were the contents of cyanide and benzaldehyde similar, whereas the contents of benzaldehyde and cyanide increased significantly using pressing residue with stone. Increasing disintegration with or without sweetening using a mixture of glucose and fructose caused significantly decreases in cyanide and benzaldehyde during the storage time after one and fifteen days. Sweetening caused significantly decreases already after one day of storage (Table cyan). The contents of cyanide decreased according to the [10] equation and the degradation of benzaldehyde that may be due to degradation of this compound to benzoic acid [11]. The content of cyanide and benzaldehyde in non-sweetened samples of cherry juice decreased significantly by storage of juice samples for fifteen days (Table 7). Sweetening using 50/50 mixture glucose and fructose decreased the contents of both compounds significantly the first day after processing. However, sweetening with sugar by processing decreased the contents of cyanide to the same level, whereas the level of benzaldehyde, were decreased significantly more using 75 and 100 percent decomposition. Inclusion of pressing residues with stone into the pressing materials resulted in significantly higher contents of benzaldehyde and cyanide in the juices and the cherry fruit meat had a significant level of the enzyme activities involved in release of benzaldehyde and hydrogen cyanide from the cherry fruit mashes. Increasing processing time up to 80 min did not affect the level of benzaldehyde significantly, whereas addition of sugar resulted in a significantly lower content of benzaldehyde in cherry fruit juices (Table 7).

Using one litre buffer solution with 13.61 g $KH_2PO_4$, 21.01g citric acid, adjusted to pH 3.31 using 69 ml 1 N NaOH, 50 g glucose 50 g fructose and 60 g cherry fruit seeds were macerated. The obtained liquids were transferred to 100 ml glass bottles and pasteurized in 0, 10, 20, 30, 50 and 70 min and cooled using tap water (12 °C). Analysis by gas chromatography showed that all samples on average

contained 69.4 mg 100 $g^{-1}$ benzaldehyde. Heating of juice without sugar and with sugar for 0, 10, 40 and 80 min at 85°C showed that heating time not affected the content of benzaldehyde significantly at these conditions. Increasing levels of macerated cherry stone resulted in higher contents of cyanide and benzaldehyde after storage for one day. However, increases in storage time from one to fifteen days for both non sweetened and sweetened juices resulted in significantly decreases in the content of cyanide and benzaldehyde. Non-sweetened juices after 15 days were not significantly different from sweetened juices after one day of storage for both cyanide and benzaldehyde. The dependence between cyanide and benzaldehyde in a liquid prepared by maceration of 122 g pressing residue from 'Stevnsbær clone 23' in 880 g water showed that cyanide and benzaldehyde were released according to two log equations.

$$\ln C_{CN}^{-1} = -0.29 + 0.69 \ln (\text{stone \%}) \; 100 \; R^2 = 98$$

$$\ln C_{ans} = 1.80 + 0.71 \ln (\text{stone \%}) \; 100 \; R^2 = 98$$

## 6. CONCLUSION

The contents of cyanide in cherry fruit juice may be reduced using anomers according to Krøller and Krulls equation presented in this text.

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