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Effects of harvest ripeness and fruit storage on turbidity in cloudy apple juice

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Turbidity in varietal not-from-concentrate (NFC) cloudy apple juices can differ and gives the chance to meet different consumer expectations. If the intent is to reduce the processing steps in juice production, natural ways of adjusting the turbidity of NFC juices are preferred. Therefore, the effects of harvest ripeness and apple storage on NFC juice turbidity were investigated. Turbidity was negatively correlated with fruit firmness ($\alpha < 0.01$) and Streif Index ($\alpha < 0.01$). As ripening continued, turbidity increased in juices made from apple varieties of 'Golden Delicious' parentage ('Golden Delicious', 'Elstar', 'Evelina', and 'Gala'). Once turbidity peaked at a late stage of maturity, further apple senescence decreased turbidity. Additionally, this study showed for the first time a decrease in turbidity in juice made from 'Elstar' apples at a certain stage of ripeness (Streif Index between 0.06 and 0.07; NDVI (normalized difference vegetation index), -0.19 ± 0.32; respiration, 25.44 ± 3.07 mg CO_2/(h × kg)). These properties could not be observed in 'Fuji' juice, likely because of its different parentage. The choice of apple variety, harvest ripeness and duration of fruit storage provides simple means to adjust the turbidity of NFC apple juices.
Keywords: NFC juice, turbidity, storage, stage of ripeness, apple variety, varietal apple juice

Auswirkungen des Reifegrades während der Ernte und der Fruchtlagerung auf die Trübung in naturtrübem Apfelsaft. *Die Trübung in reinsortigem, naturtrübem Apfeldirektsaft kann variieren und bietet so die Möglichkeit, verschiedene Konsumentenerwartungen zu erfüllen. Um die Verarbeitungsschritte in der Saftproduktion zu reduzieren, werden natürliche Wege zur Regulierung der Trübung bevorzugt. Aus diesem Grund wurde der Einfluss der Reife zum Erntezeitpunkt und der Lagerung der Äpfel auf die Trübung von Direktsaft untersucht. Die Trübung korrelierte negativ mit der Fruchtfleischfestigkeit ($\alpha < 0.01$) und dem Streif Index ($\alpha < 0.01$). Bei fortschreitender Reife der Äpfel stieg die Trübung in den Säften an, die aus Apfelsorten hergestellt worden waren, die auf Grund der Züchtungslinie mit 'Golden Delicious' verwandt waren ('Golden Delicious', 'Elstar', 'Evelina', und 'Gala'). Nachdem die Trübung ihren Höhepunkt erreicht hatte, nahm sie in Folge weiterer Seneszenz der Äpfel ab. Zusätzlich zeigte diese Arbeit erstmalig einen Trübungsabfall im Saft von Äpfeln der Sorte 'Elstar' bei einem bestimmten Reifegrad (Streif-Index zwischen 0.06 und 0.07; NDVI (Normalized difference vegetation index) -0.19 ± 0.32; Respiration 25.44 ± 3.07 mg CO_2/(h × kg)). Diese Eigenschaften konnten bei Fuji-Saft nicht beobachtet werden, vermutlich wegen der unterschiedlichen Züchtungsherkunft. Die Wahl der Apfelsorte, des Reifegrades und der Lagerdauer der Früchte bietet einfache Wege, die Trübung von Apfeldirektsaft einzustellen.*
Schlagwörter: Direktsaft, Trübung, Lagerung, Reifegrad, Apfelsorte, reinsortig

Making juice is one of the most popular ways to process fruits. Next to orange juice, apple juice is the most frequently consumed fruit juice worldwide (McLellan and Padilla-Zakour, 2005). On the European apple juice market, three different product types are available: clarified apple juice made from concentrate, not-from-concentrate (NFC) juice, and NFC juice produced from a single apple cultivar. NFC juice, which is mainly offered as a cloudy product, is associated with high quality and low technological input and is therefore more expensive. It is also very popular; 63 % of consumers prefer NFC juice (Verband der deutschen Fruchtsaft-Industrie, 2008).
Although any type of apple juice generally improves antioxidant status in humans (Yuan, 2011), cloudy juice is more effective than clarified juice. It has broad

cancer prevention activity because of its high polyphenolic contents and antiradical activity (Barth et al., 2005; Garnweidner et al., 2007; Oszmianski et al., 2007). Furthermore, cloudy apple juice is characterized by higher concentrations of aromatic components, determined by sensory as well as analytic tests (Pour Nikfardjam and Koppmann, 2010). Aroma profile, sweetness, acidity and physical properties (including cloudiness, cloud particle size and cloud stability) differ depending on the apple cultivar used for juice production (Jaros et al., 2009). Thus, producing varietal apple juice meets different consumer expectations and offers a large market potential, especially for small-scale producers. Cloudy apple juice must have a bright, whitish-yellow colour and considerable turbidity (Nagel, 1992). To be precise, consumers expect a turbidity level of 300 nephelometric turbidity units (NTU) (Krapfenbauer, 2004), but the intensity of turbidity in juice varies and is often higher than this (Zimmer, 1996). High turbidity levels, however, bear the risk of conglomerate formation in juice during storage (Schnürer et al., 2013).
The turbidity level can be adjusted by centrifugation, which is frequently used to remove coarse cloud particles (McLellan and Padilla-Zakour, 2005). However, if the intent is to produce a minimally processed beverage, natural ways of adjusting the turbidity of NFC juices are preferred. In addition, only a small number of small-scale producers have a centrifuge. Therefore, the aim of this study was to control the turbidity level by using simple means that are practical for small-scale producers instead of using a centrifuge. Markowski (1998) reported that fruit storage may increase juice turbidity and stability. However, he did not document the apples' stage of ripeness; a number of changes in physical and chemical properties can be observed during maturation and ripening of apple fruit. Degradation of starch and cell walls (Tucker, 1993), increase in enzyme synthesis (Frenkel et al., 1968) and changes in CO_2 emission levels (Wills et al., 2007) are only a few examples which determine ripeness stage and may affect juice quality. Therefore, this study investigated the influence of different apple ripeness stages (achieved by different harvest dates and storage times) on juice turbidity, and several ripeness parameters were documented to show the non-linear correlation between turbidity and stage of ripeness. Additionally, some previously undocumented characteristics of selected varieties were determined.

Materials and methods

Apple varieties and study designs

Fresh apples (*Malus domestica* Borkh.) of different varieties were obtained from VermarktungsGesmbH Wachau Obst (Gföhl, Austria) and from the experimental orchard of the HBLA und BA für Wein- und Obstbau Klosterneuburg (Austria) in 2009 and 2010. In study design 1, apples of the variety 'Elstar' were harvested at two different dates: first, when they were ripe for storage, and second, when they were ripe to eat. Before processing, all apples were stored for 4 days at 4 °C; half of the second batch was stored for additional 7 days at 24 °C resulting in overripe fruit. Thus, three different stages of ripeness were achieved.
In study design 2, the varieties 'Elstar' ('Golden Delicious' x 'Ingrid Marie'), 'Gala' ('Kidds Orange' x 'Golden Delicious'), 'Evelina' ('Clivia' x 'Golden Delicious'), 'Golden Delicious' ('Grimes Golden' x 'Golden Reinette'), and 'Fuji' ('Ralls Janet' x 'Red Delicious') were used. The apples were harvested at three different stages: ripe for storage, ripe, and one week later than ripe (overripe). Each batch was divided into three, and then each sub-batch was stored for a different length of time before processing (0 months of storage/1 month of storage/2 months of storage).

Ripeness analyses

Batches of ten apples from each treatment were subjected to both non-destructive and destructive sampling methods to determine fruit quality. In study design 1, all below-mentioned analyses were conducted; in study design 2, only the Streif Index was measured.
Evaluation of apple peel chlorophyll was conducted using emission measurement by calculating the normalized difference vegetation index (NDVI) = $(I_{780} - I_{660})/(I_{780} + I_{660})$ with a hand-held photodiode array spectrophotometer (Pigment Analyzer 1101; Control in Applied Physiology, Berlin-Falkensee, Germany) (Zude et al., 2007; Solomakhin and Blanke, 2007; Kuckenberg et al., 2008). Measurements were conducted on undefined areas of apple peels, since shaded and sunlit sides did not differ significantly in preliminary tests.
Respiration rates were measured using CO_2 evolu-

tion. Analyses were performed twice using a sample of five apples each time. After determination of weight and diameter, apples were placed in a closed 12 l-tank for 10 min. CO_2 evolution was recorded (Almemo FY A600-CO2 H & Almemo 2590-3S; Ahlborn, Holzkirchen, Germany) and calculated as mg CO_2/(h × kg).

Determination of firmness was carried out using a lever-operated penetrometer (AFG 500 N; Mecmesin, Slinfold, UK). After removing the peel covering the testing areas, a 1cm^2-plunger was pushed 8 mm into the flesh on opposite sides of the apple equator.

Starch degradation level was estimated as described previously (Zude-Sasse et al., 2002). The iodine-iodide test was used on one-half of each fruit with an index from 1 to 10 referring to the Ctifl starch degradation table (Centre Technique Interprofessionnel des Fruits et Legumes, Paris, France). Total soluble solid content (SSC, °Bx) was determined from the strained samples as described previously (Krapfenbauer et al., 2006).

Streif Index, which declines during the ripening process and is commonly used to determine the optimal date for harvesting, was calculated as: fruit firmness/(starch index × soluble solids content) (Streif, 1996; Kompetenzzentrum Obstbau-Bodensee, 2012).

Preparation of cloudy apple juice

After washing, crushing (Raetz-Mill; Voran, Pichl, Austria), pressing (Belt Press; Stossier, Pörtschach, Austria) and 'high temperature short time' treatment (HTST) using a tubular heat exchanger (Fischer, Ebreichsdorf, Austria) at 85 °C for 20 s, the juice was poured in plastic tanks with a capacity of 25 l each. Then, the juice was stored at 20 °C for 24 h so that the coarse particles could settle.

Juice analyses

In study design 1 particle-size distribution was investigated using a particle-size analyser Cilas 1090 (Cilas, Orléans, France) and the corresponding programme of the analyser. Samples of 'Elstar' juice were evaluated after 12 weeks of storage.

Total pectin content was determined by using the modified International Fruit Juice Union (IFU) method by using m-phenylphenol instead of carbazole (List et al., 1985). Precipitation of pectic substances was carried out using only ethanol (α = 95 %), without addition of water. Content of dissolved protein in the juice was determined by performing the Bradford protein assay (Bradford, 1976).

Folin-Ciocalteu reagent was used for colorimetric assay of total phenols (study design 1) (Zöcklein et al., 1994). In study design 1 and 2 measurement of turbidity (NTU) in the juice was conducted with a laboratory turbidity photometer (LTP5; Dr. Lange, Düsseldorf, Germany) in a 1 cm-cuvette. Juice samples were taken without stirring from the middle of the tanks after 24 h of storage.

Statistical analyses

Experiments and analyses were performed twice. Statistical analyses were carried out using SPSS 12.0 (Statistical Package for the Social Sciences) (SPSS Statistics, Munich, Germany) and Microsoft Excel (Microsoft GmbH, Unterschleißheim, Germany).

Results and discussion

Changes of NDVI, respiration, firmness, starch degradation level, total soluble solid content, and Streif Index in study design 1 (variety 'Elstar')

The chlorophyll in the apple peel is responsible for the ground colour. The highest chlorophyll content, which indicates the least chlorophyll breakdown, is expressed by the highest NDVI. During the vegetative and postharvest periods, NDVI values decrease (Rutkowski et al., 2008). In study design 1, overripe apples had the lowest NDVI value at -0.45. In contrast, ripe-for-storage apples had the highest NDVI at 0.07. Ripe-for-storage apples and overripe apples differed significantly ($\alpha < 0.05$) from each other (Table 1).

Respiration rates were highest in ripe apples. During the cell division phase of apple fruit growth, the rate of respiration is high. Then it declines, followed by a marked increase when the fruits reach physiological maturity and the ripening process starts. This results in increased evolution of CO_2 and is known as the respiratory climacteric. Thereafter, respiration rates decline once more (Kidd and West, 1925; Zude et al., 2007).

The results of study design 1 indicated this climacteric rise; results, however, were not significant.
Fruit firmness decreased significantly with stage of ripeness, from 7.08 to 5.55 kg/cm2. Firmness and NDVI were significantly correlated (α = 0.04). Ripe-for-storage apples showed the lowest starch degradation level (α < 0.05). Ripe and overripe apples did not differ significantly in starch degradation level. Soluble solid content ranged between 12 and 15 °Bx and increased according to the stage of ripeness. Streif Index, calculated using firmness, starch degradation level and SSC decreased along with the stage of ripeness (Table 1).

storage time seemed to have an important impact on juice turbidity. However, the influence of the stage of ripeness on turbidity was not clear. Thus, study design 2 was performed.

Total soluble solid content, fruit firmness, starch degradation level, and Streif Index analyses in study design 2

Apples intended for processing are supposed to be of eating quality. Therefore, in this study, most of the measured ripeness parameters (Tables 3 to 6) differed from the recommendations found in literature, which are designed for apples intended to be placed in storage. SSC was generally low in the year of study execution because of the large amount of rain during the ripening period.
The total soluble solid content varied between 9.5 and 14.0 °Bx (Table 3). Fruit firmness ranged from 3.75 ± 0.37 to 9.49 ± 1.72 kg/cm2 (Table 4). Starch degradation levels were between 6.7 and 10. The lowest starch degradation levels were found in apples which were not stored. Storing increased the starch degradation levels. After two months of storage starch could be found only in 'Gala' apples. The other apple varieties showed a starch degradation level of 10 (Table 5). Streif Index values ranged from 0.10 to 0.03 (Table 6). Apart from 'Evelina' ripe-for-storage, 'Evelina' overripe, and 'Fuji' overripe, all apples showed decreased Streif Index values as storage time and stage of ripeness increased. 'Fuji' apples had the highest Streif Index values.

Table 1: Results of chemical and physical analyses in fresh fruit; study design 1

	Ripe for storage	Ripe	Overripe
Firmness (kg/cm^2)	7.08 ± 0.72 a	6.39 ± 0.83 b	5.55 ± 0.81 c
Starch degradation level	5.4 ± 0.97 a	7.30 ± 1.25 b	7.20 ± 0.92 b
SSC (°Bx)	12.00 ± 0.47 a	13.00 ± 0.47 b	15.00 ± 0.24 c
Streif Index	0.11 ± 0.00 a	0.07 ± 0.00 b	0.05 ± 0.00 c
NDVI	0.07 ± 0.29 a	-0.19 ± 0.32 ab	-0.45 ± 0.16 b
Respiration (CO_2 mg h*kg)	20.76 ± 3.23	25.44 ± 3.07	20.94 ± 6.56

Different letters within a row indicate significant differences at α < 0.05.

Juice analyses in study design 1

The particle size distributions of the juices made from ripe-for-storage and overripe apples are displayed in Figure 1 and 2. Due to the low amount of particles contained in the juice made from ripe apples the analysis was not possible. In the juices ‘ripe-for-storage’ and ‘overripe’ 50 % of the particles showed a diameter smaller than 0.91 µm and 0.79 µm, respectively. The peak of particle size distribution in Figure 2 is wider than the peak in Figure 1. In both juices 90 % of the particles were smaller than 2.24 µm. In conclusion, the particle size distributions in ‘ripe-for-storage’ and ‘overripe’ juice did not differ in a significant way.
Results of total phenolics, total pectin content and protein measurements are displayed in Table 2. The values differed significantly among the juices. However, no conclusions about their impact on juice turbidity could be drawn.
Turbidity also differed significantly among the juices. Juice from ripe apples showed the lowest turbidity (135.5 ± 5.5 NTU), and juice from overripe apples the highest (1185.5 ± 23.2 NTU). Juice made from ripe for storage apples was characterized by 536.0 ± 94.5 NTU.
Given that ripe and overripe apples were harvested at the same time but stored for different lengths of time,

Juice turbidity in study design 2

Generally, juice turbidity correlated negatively with fruit firmness and Streif Index, especially 'Golden Delicious' and 'Elstar' (α < 0.01). All but one of the juices were characterized by an increase in turbidity

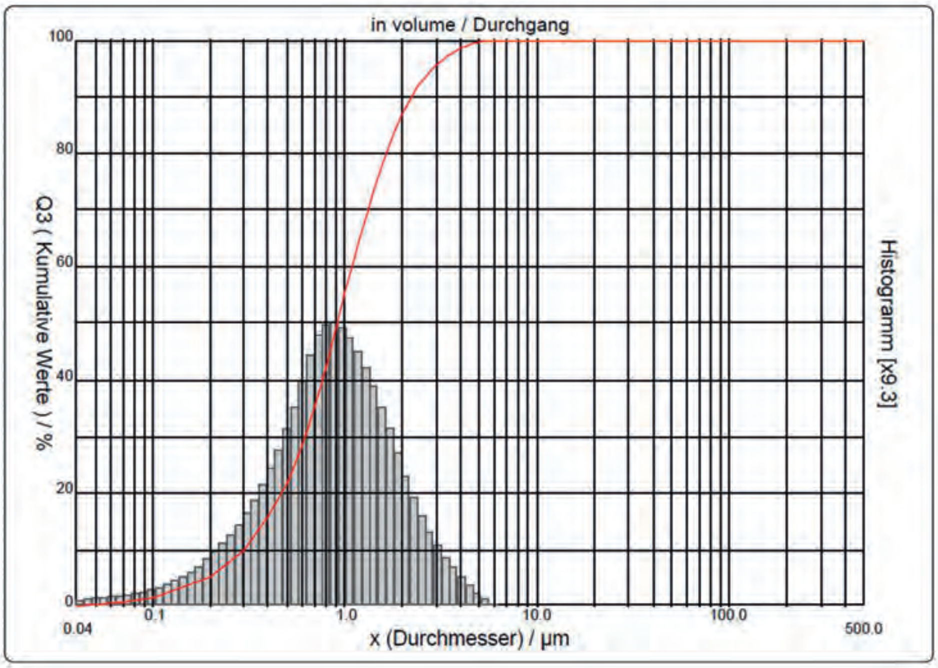

Fig. 1: Particle size distribution in the juice 'Elstar ripe-for-storage' (study design 1)

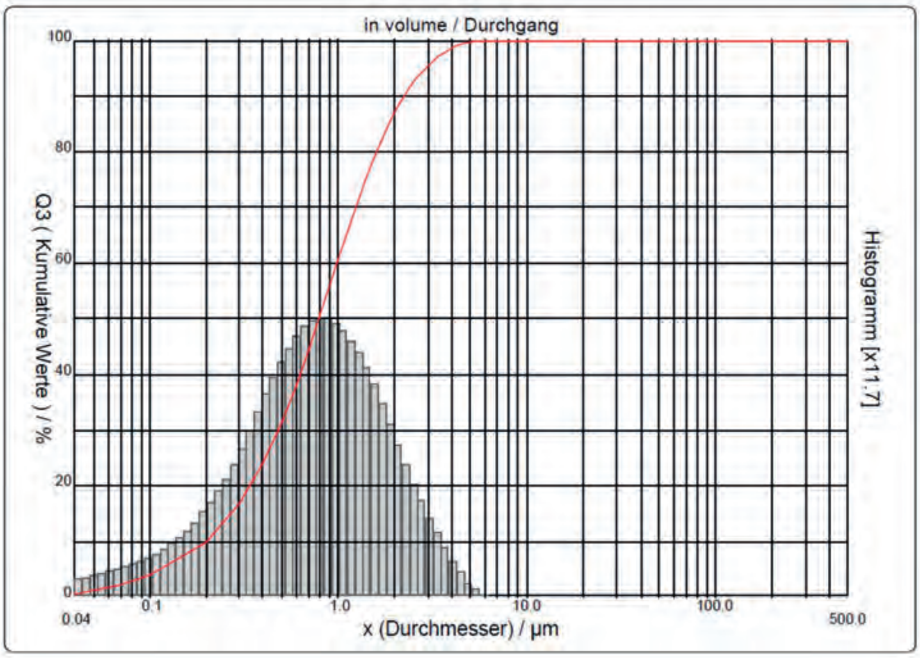

Fig. 2: Particle size distribution in the juice 'Elstar overripe' (study design 1)

Table 2: Results of chemical analyses in the juices; study design 1

	Ripe for storage	Ripe	Overripe
Protein (mg/l)	63,91 ± 4,10 a	81,15 ± 0,54 b	100,10 ± 1,81 c
Total pectin content (mg/l)	39,50 ± 2,29 a	65,02 ± 6,19 a	180,45 ± 18,32 b
Total phenolics (mg/l)	236,43 ± 6,43 a	342,80 ± 3,85 b	321,53 ± 1,63 c

Different letters within a row indicate significant differences at α < 0.05.

Table 3: Total soluble solids (°Bx) content; study design 2

Stage of ripeness	Ripe for storage			Ripe			Overripe		
Months of storage	0	1	2	0	1	2	0	1	2
Gala	9.75 ± 0.35	12.0 ± 0.0	11.5 ± 0.0	9.5 ± 0.0	12.0 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.25 ± 0.35	12.0 ± 0.0
Evelina	12.0 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	12.5 ± 0.0	12.5 ± 0.0
Golden D.	12.0 ± 0.0	13.0 ± 0.0	12.75 ± 0.35	13.0 ± 0.0	14.0 ± 0.0	13.5 ± 0.0	13.5 ± 0.0	12.5 ± 0.0	12.5 ± 0.0
Fuji	11.5 ± 0.0	11.0 ± 0.0	11.5 ± 0.0	12.0 ± 0.0	11.5 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	10.75 ± 0.35
Elstar	11.0 ± 0.0	11.0 ± 0.0	11.0 ± 0.0	11.5 ± 0.0	12.0 ± 0.0	12.0 ± 0.0	11.5 ± 0.0	11.0 ± 0.0	11.0 ± 0.0

Table 4: Fruit firmness (kg/cm²); study design 2

Stage of ripeness	Ripe for storage			Ripe			Overripe		
Months of storage	0	1	2	0	1	2	0	1	2
Gala	n.d.	6.65±2.12	5.76±0.43	n.d.	7.69±2.31	6.48±0.59	6.46±2.85	6.82±1.73	6.26±0.72
Evelina	6.38±2.20	5.29±2.47	6.24±1.08	6.62±2.33	7.19±2.32	6.80±1.68	5.34±3.10	8.20±1.92	7.19±1.28
Golden D.	7.40±2.90	4.53±1.65	4.83±0.47	6.71±2.25	5.10±2.30	5.30±0.48	5.48±3.22	4.73±0.44	5.02±0.40
Fuji	9.49±1.72	7.51±1.08	6.35±0.56	8.25±1.73	7.33±0.89	6.23±0.35	8.77±0.61	8.59±0.70	7.45±0.56
Elstar	5.81±1.90	4.35±0.28	3.75±0.37	6.15±0.70	4.02±0.80	3.80±0.37	4.14±1.00	4.27±0.77	3.55±0.65

n.d. = not determined

Table 5: Starch degradation level; study design 2

Stage of ripeness	Ripe for storage			Ripe			Overripe		
Months of storage	0	1	2	0	1	2	0	1	2
Gala	7.0 ± 1.2	9.4 ± 0.7	10.0 ± 0.0	6.7 ± 1.8	7.7 ± 0.8	9.7 ± 0.5	8.7 ± 1.2	9.9 ± 0.3	10.0 ± 0.0
Evelina	8.6 ± 0.5	9.9 ± 0.3	10.0 ± 0.0	8.6 ± 1.1	9.9 ± 0.3	10.0 ± 0.0	9.6 ± 0.5	9.9 ± 0.3	10.0 ± 0.0
Golden D.	8.1 ± 1.1	10.0 ± 0.0	10.0 ± 0.0	7.1 ± 0.6	8.4 ± 0.8	10.0 ± 0.0	8.1 ± 0.9	10.0 ± 0.0	10.0 ± 0.0
Fuji	10.0 ± 0.1	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.06	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0
Elstar	7.9 ± 0.7	10.0 ± 0.0	10.0 ± 0.0	8.5 ± 0.8	9.8 ± 0.4	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0

Table 6: Streif Index analyses; study design 2

Stage of ripeness	Ripe for storage			Ripe			Overripe		
Months of storage	0	1	2	0	1	2	0	1	2
Gala	n.d.	0.06	0.05	n.d.	0.08	0.05	0.06	0.06	0.05
Evelina	0.06	0.04	0.05	0.06	0.06	0.05	0.04	0.07	0.06
Golden D.	0.08	0.04	0.04	0.07	0.04	0.04	0.05	0.04	0.04
Fuji	0.10	0.07	0.06	0.07	0.06	0.06	0.08	0.08	0.07
Elstar	0.07	0.04	0.03	0.06	0.03	0.03	0.04	0.04	0.03

n.d. = not determined

after 1 month of apple storage. This increase might be explained by the breakdown of the middle lamella, which is a part of the fruit cell walls, resulting in cell separation and development of a mealy texture (Knee, 1993). Subsequently, the cells do not fracture during pressing but split along the middle lamella (Lapsley et al., 1992), and the released cloud particles are of smaller size resulting in good cloud stability (Pecoroni et al., 1996; Stüssi, 1998).

Juice made from 'Fuji' apples gave different results; juice made from ripe and overripe apples showed a decline in turbidity after 1 month of storage, followed by an increase in turbidity after 2 months of storage. The turbidity of juice made from other varieties either increased more slowly or declined after 2 months of apple storage (Fig. 3). This phenomenon might be explained by the different parentage of 'Fuji' ('Ralls Janet' x 'Red Delicious') compared with the other varieties used in this study, which are crosses of the variety 'Golden Delicious'. Thus, it seems that turbidity characteristics vary according to apple variety. 'Fuji' apples do not show an increase in ethylene production during ripening (Jobling and McGlasson, 1995), which may influence turbidity characteristics.

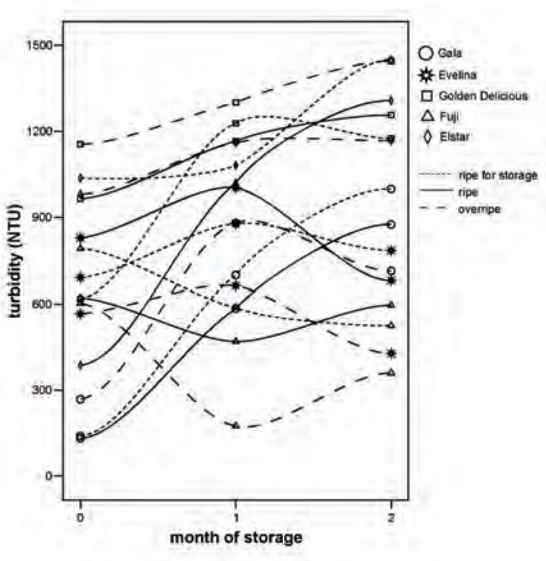

Fig. 3: Turbidity in the juices of study design 2

The ripening process of apples and the effects on juice turbidity

Our results suggest, that pre-processing storage of apples from 'Golden Delicious' parentage will result in an increase in turbidity. At a particular point of ripeness or apple senescence, turbidity of the produced juice decreases. This point has yet to be defined for each variety. In addition, results of our study indicate an abrupt decline in turbidity of 'Elstar'; this finding has not yet been described in literature. In study design 2, juice made from ripe apples was characterized by remarkably low initial turbidity (387 NTU) compared with juice made from ripe-for-storage (1037 NTU) and overripe apples (980 NTU). In study design 1, ripeness had a similar effect on juice turbidity. An abrupt decline in turbidity was also observed; turbidity in juice made from ripe apples was 540 NTU lower than in juice made from ripe-for-storage apples. It should be noted that the Streif Index was similar in both cases of turbidity decline (study design 1: 0.07; study design 2: 0.06). Overripe apples in study design 1 were stored for 7 days at 24 °C, resulting in higher turbidity than in juice made from ripe apples, even though they were harvested at the same time.

Conclusions

In conclusion, the results of the study indicate that turbidity in varietal NFC cloudy apple juice depends on apple variety and stage of ripeness, which in turn is influenced by harvest date and duration of fruit storage. Juice made from apples of 'Golden Delicious' parentage is characterized by a turbidity increase corresponding to the ripening sequence, probably initiated by increased respiration and ethylene production, followed by a turbidity decrease starting with apple senescence. Furthermore, varieties differ in their optimal harvest time for production of juice with the desired level of turbidity. In 'Elstar' apples, it was shown that the time of harvest significantly affects the turbidity of fresh juice. In order to produce minimally processed apple juices with the natural turbidity level preferred by consumers, apples must be harvested at a particular time, depending on variety. Moreover, harvesting for optimal turbidity instead of centrifuging the juice probably bears a lower risk of conglomerate formation.

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