Is There a Relation between Changes in Osmolarity of Cherry Fruit Flesh or Skin and Fruit Cracking Susceptibility?

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ABSTRACT. Rain-induced fruit cracking is a limiting factor for sweet cherry (*Prunus avium* L.) growers in many production areas. Although many studies have concerned this complex phenomenon, the basic mechanisms involved in fruit cracking remain unclear. We re-examined the relations between osmotic potential and cracking susceptibility in cherry fruit by comparing the osmotic contribution of the major metabolites separately in flesh and skin, in four cultivars (with different levels of susceptibility to cracking) at four stages of development. Several differences were observed between flesh and skin revealing compositional gradients in the fruit tissues. Acidity and malate concentrations were higher in flesh than in skin for all stages. The absolute value of osmotic potential was higher but the contribution of the sum of sugars to osmotic potential was lower in flesh than in skin. As determined using fruit immersion test, 'Fermina' and 'Regina' were less susceptible to fruit cracking than 'Lapins' and 'Brooks'. At commercial maturity when fruit susceptibility to cracking was highest, no clear difference appeared between 'Brooks' and 'Lapins' compared to 'Regina' and 'Fermina' for flesh or skin osmolarity and for the contribution of the major sugars or organic acids to skin and flesh osmotic potential.

Fruit cracking and splitting is a serious economic problem in several crops, including tomato, apple, pear, citrus, pomegranate, grape, and cherry (Opara et al., 1997). For sweet cherry growers in many production areas, rain-induced fruit cracking is a limiting factor. In spite of many studies, the basic mechanisms involved in the complex phenomenon of fruit cracking remain unclear. The physiological and cytological mechanisms involved include fruit water budget, fruit morphology, and fruit tissue structure (cuticle, cell walls, cell sizes) (Christensen, 1996; Opara et al., 1997; Sekse, 1998). These mechanisms are complex and often interrelated.

Concerning fruit water budget, osmotic potential of the cherry fruit has been considered to be a critical factor of cracking mechanisms in the past (Sekse, 1995, 1998). Osmotic potential is a component of the fruit water potential that is the driving force for water uptake. Data on the osmotic and turgor components of the cherry fruit water potential are rare (Andersen and Richardson, 1982). Cherry fruit water potential varies greatly (–900 to –2500 kPa) during the harvest period depending on the cultivar

and diurnal measurement time (Tvergyak and Richardson, 1979). However, at a given water supply, osmotic potential determines the fruit turgor, which can be related to skin failure (Considine and Kriedemann, 1972). Cherry fruit cracking has been related to increased internal turgor resulting from absorption of water into the fruit (Christensen, 1996; Sekse, 1998). The water uptake threshold at which fruit cracked was a major factor explaining differences in cracking resistance among some cultivars (Lane et al., 2000).

The major organic contributors to osmotic pressure in cherry flesh at maturity are glucose and fructose (4% to 9% fresh weight) and malate (0.5% to 0.9 % fresh weight) (Girard and Kopp, 1998). Verner (1937) described a sugar concentration gradient from the base to the apex of the fruit, which could be related to cracking areas. Later studies (Christensen, 1972a) found little correlation between refractometer reading and cracking index.

Varietal differences for cracking susceptibility are considerable (Christensen, 2000; Roser, 1996). Therefore, the development of high-quality crack-resistant cultivars remains a major objective in several breeding programs. The selection criteria still involve observation for several years in the orchard and fruit immersion tests such as the one developed by Christensen (1972b). The correlation between orchard and in vitro tests is not perfect. Sensitivity to fruit cracking also depends on the stage of development of the fruit. Severe cracking is not observed until the third growth phase (Christensen, 1973a). Sensitivity to cracking increases as the fruit ripens and decreases in some cultivars after normal harvest time (Christensen, 1973b). These changes

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in susceptibility to cracking must be taken into account for the development of selection criteria.

The objectives of the present study were to re-examine the relations between osmotic potential and cracking susceptibility in cherry fruit by comparing the osmotic contribution of the major metabolites separately in flesh and skin. Since water uptake into the fruit occurs from the other parts of the tree through the pedicel and also over the fruit surface (Sekse, 1998), we hypothesized that osmotic potential of the skin might be more related to *in vitro* cracking susceptibility than osmotic potential of the flesh. We worked on four cultivars, with different levels of susceptibility to cracking, at four stages of development.

Materials and Methods

PLANT MATERIAL. Fruits were harvested at the 'Unité Expérimentale Arboricole' (Toulence near Langon, France) during May and June 2002 from four sweet cherry cultivars: 'Brooks', 'Lapins', 'Regina', and 'Fermina'. 'Brooks', obtained in California (Hansche et al., 1988), and 'Lapins', obtained in Canada (Lane and Schmid, 1984), have fruit with good firmness, and sweet taste but are susceptible to rain-induced fruit cracking. However, 'Lapins' is less susceptible to cracking (Lane et al., 2000; Ystaas and Froynes, 1998) than 'Brooks' (Hansche et al., 1988). 'Regina', obtained in Germany is appreciated in northern countries due to its delayed maturity date and resistance to fruit cracking (Roser, 1996; Ystaas and Froynes, 1998). 'Fermina' (for which an application for Plant Breeder's right has been submitted to the European Community Plant Variety Office), obtained at INRA France in 1991, has large fruits and is more resistant to fruit cracking than 'Lapins' (J. Clavierie, unpublished data from orchard observation and immersion tests). The cultivars were grafted on 'SL 64' rootstock and the trees were 7 to 11 years old. Cultural practices and pest control were the usual for cherry. Fruit samples were harvested at four development stages: green (≈ 1 week before pink stage), pink (one-fourth to one-third of the fruit surface covered with pink color), commercial and physiological maturity (determined by color using a CR-200 Minolta chromameter). For each stage and genotype, fruits were harvested in the morning. Twenty fruits were harvested for determination of individual fruit fresh weight and juice collection, and 25 other fruits for determination of fruit cracking index.

DETERMINATION OF CRACKING INDEX. Fruit cracking index was determined after immersion in distilled water of 25 fruits for each genotype and development stage and observation of cracked fruits (Christensen, 1972b).

JUICE MEASUREMENTS. After determination of fruit weight, the fruit were grouped into five samples of four fruit each. For each fruit sample, the flesh (mesocarp) was separated from the skin [epidermis and hypodermal layers (Tukey and Oran Young, 1939) for a total thickness of $1 \text{ mm} \pm 0.2$] using a 3.5-cm surgical blade. Cross-contamination of skin and flesh was limited by working fast. Flesh and skin samples were immediately frozen in liquid N in a 10-mL syringe containing a small paper filter at the needle and then stored at -20°C until analysis. After thawing for 20 min at 4°C , the juice was extracted by hand pressing, centrifuged at $3800 g_n$ at 4°C and the resulting supernatant was stored in ice before analysis within 1 h. The acidity of the juice supernatant was determined by measuring the pH of a $150\text{-}\mu\text{L}$ sample using a pH meter (Schott Geräte, Germany) equipped with a microelectrode. Titratable acidity was determined by titration of a $100\text{-}\mu\text{L}$ sample of juice (diluted 1:5 by volume

with distilled water) with $0.1 \text{ mol}\cdot\text{L}^{-1}$ NaOH to a pH of 8.3. The osmotic potential of the juice supernatant was determined on $50 \mu\text{L}$ with a microosmometer (Roebbing, Germany). Two hundred microliters of the juice supernatant was used for determination of soluble sugar and organic acid concentrations.

DETERMINATION OF SUGARS AND ORGANIC ACIDS. Two hundred microliters of juice was fixed with ethanol at 80°C as described previously (Moing et al., 1997). Extracts were dried under vacuum, dissolved in pure water, and used for analysis of soluble sugars and organic acids. After purification of the neutral fraction, soluble sugars were analyzed by anion-exchange high-performance liquid chromatography (HPLC) with amperometry detection (CarboPac PA1 column; Dionex Corp., Sunnyvale, Calif.), as described previously (Moing et al., 1997), for some samples to identify major and minor soluble sugars in a preliminary experiment. Then, all samples were analyzed using HPLC with isocratic conditions and refractometry detection (Moing et al., 1998a). Organic acids were analyzed without sample purification, using anion-exchange HPLC with conductivity detection (Moing et al., 1998a, 1998b). Malic, quinic, citric, and isocitric acids were quantified. The total organic acids is the sum of these acids.

Sugars and organic acids were quantified with Millenium software from Waters (Milford, Mass.). HPLC peaks were identified by co-chromatography with commercial standards, their areas were calculated, and calibration was carried out with known quantities of sugar, and organic acid from Sigma (St Quentin Fallavier, France). Sugar and organic acid concentrations were used to calculate their contribution to juice osmolarity according to the Van't Hoff relation.

STATISTICAL ANALYSIS. For fruit and biochemical variables, tissues were compared using variance analysis and mean separation with Student *t* test. Genotypes were compared using variance analysis and mean separation with Tukey's test. Pearson correlation coefficient was calculated between cracking index and mean skin or flesh osmolarity for each stage and cultivar.

Results

FRUIT DEVELOPMENT AND CRACKING INDEX. Full bloom ranged from 24 Mar. for 'Lapins', 25 Mar. for 'Brooks', 27 Mar. for 'Fermina', to 4 Apr. for 'Regina'. The fruit development stages were controlled using measurements of color L a b parameters (data not shown). The green stage date ranged from 52 d after tree full bloom (DAB) for 'Brook', 55 DAB for 'Fermina', 56 DAB for 'Regina', to 61 DAB for 'Lapins'. Commercial maturity date ranged from 30 May (66 DAB) for 'Brooks', 4 June (69 DAB) for 'Fermina', 10 June (78 DAB) for 'Lapins', to 12 June (69 DAB) for 'Regina'. At commercial maturity, 'Regina' had a significantly lower fruit weight compared to the other cultivars (Fig. 1). 'Regina' and 'Fermina' fruit size continued to increase between commercial and physiological maturity (*t* test, $P < 0.05$). At physiological maturity (6 d after commercial maturity), all the cultivars had a fresh weight higher than 8.6 g and 'Fermina' had a significantly highest fruit fresh weight in comparison to the other cultivars.

The highest fruit cracking index after immersion (Fig. 2) was observed for 'Brooks' at maturity stages. Moreover, 'Brooks' was the only cultivar where cracking of some fruits was observed at the green stage. 'Regina' and 'Fermina' had a cracking index lower than 20 at commercial maturity. 'Lapins' cracking indexes were intermediary between that of 'Brooks' and 'Regina' at maturity stages. For 'Brooks', 'Lapins', and 'Fermina', the highest cracking

Fig. 1. Changes in fruit fresh weight during fruit development of 'Brooks', 'Lapins', 'Regina' and 'Fermina' cultivars. Mean of five samples. Vertical bars represent standard deviation. For each development stage, letters indicate mean separation among cultivars according to Tukey's test, $P < 0.05$. Mat C, commercial maturity; Mat P, physiological maturity.

Fig. 2. Changes in cracking index during fruit development of 'Brooks', 'Lapins', 'Regina', and 'Fermina' cultivars. Cracking index was calculated using immersion of 25 fruits. Mat C, commercial maturity; Mat P, physiological maturity.

index was observed at commercial maturity. For 'Regina' significant cracking was observed only at physiological maturity.

JUICE CHARACTERISTICS. A significant difference between skin and flesh (variance analysis not shown) was detected for juice pH and titratable acidity at all stages (Fig 3, mean acidity was significantly higher and mean pH was significantly lower in flesh compared to skin), and for juice osmotic potential at maturity stages (Fig 4, mean osmotic potential was significantly lower in flesh than in skin). The juice titratable acidity in flesh (Fig 3A) was significantly higher in 'Fermina' than in other cultivars for

all stages. The juice titratable acidity in skin (Fig 3B) was significantly higher in 'Fermina' than in other cultivars for maturity stages.

The juice osmotic potential in flesh (Fig 4A) decreased between green and pink stages, and thereafter changes with time depended on the cultivar. Little significant differences were observed among cultivars except at pink stage when 'Brooks' had a flesh osmotic potential significantly higher than the other cultivars. The juice osmotic potential in skin (Fig 4B) decreased between green and commercial maturity stages and then remained stable at physiological maturity. Little significant differences were observed among cultivars except at pink stage when 'Brooks' and 'Fermina' had a skin osmotic potential significantly higher than the two other cultivars. For each stage, the cultivar pattern for osmotic potential was similar in flesh and skin except for commercial maturity stage.

For all stages and cultivars considered together, no significant correlation was observed between cracking index and osmolarity in skin ($R = 0.40$) or flesh ($R = 0.37$) ($n = 16$, data not shown).

CARBOHYDRATES. For all genotypes and stages, a preliminary experiment (data not shown) indicated that the major soluble sugars in flesh or skin juice were glucose and fructose (96 to 140 $\text{g}\cdot\text{L}^{-1}$ each), together representing 84% to 87 % of total soluble sugars. Sorbitol represented $\approx 9\%$ of total soluble sugars in flesh or skin juice at pink and green stages, and 12% to 15% at maturity stages. Sucrose represented $<0.5\%$ of total soluble sugars. Xylose, inositol, rhamnose, and arabinose were also detected, each at a concentration lower than 0.4 $\text{g}\cdot\text{L}^{-1}$. Mannitol was not detected. The data presented below are derived from the isocratic-refractometric HPLC system where the minor sugars are not detected (only inositol is detected but not separated from fructose) since the sum of sorbitol, glucose, and fructose represented $\approx 99\%$ of total soluble sugars in the preliminary experiment. The mean total soluble carbohydrate concentration (glucose, fructose, sorbitol, and inositol) ranged from 82 to 189 $\text{g}\cdot\text{L}^{-1}$ in flesh, and 84 to 174 $\text{g}\cdot\text{L}^{-1}$ in skin, depending on the stage and cultivar.

ORGANIC ACIDS. The major organic acid in flesh and skin was malate, representing more than 89% of the sum of organic acids. For all stages and cultivars, mean concentrations in flesh ranged between 5.2 and 10.7 $\text{g}\cdot\text{L}^{-1}$ for malate, 0.19 and 0.54 $\text{g}\cdot\text{L}^{-1}$ for quinate, 0.04 and 0.23 $\text{g}\cdot\text{L}^{-1}$ for citrate, and 0.01 to 0.05 $\text{g}\cdot\text{L}^{-1}$ for isocitrate. Mean concentrations in skin ranged between 3.2 and 6.4 $\text{g}\cdot\text{L}^{-1}$ for malate, 0.21 and 0.42 $\text{g}\cdot\text{L}^{-1}$ for quinate, 0.04 and 0.16 $\text{g}\cdot\text{L}^{-1}$ for citrate, and 0.01 to 0.04 $\text{g}\cdot\text{L}^{-1}$ for isocitrate. For each cultivar and stage, malate concentration was significantly higher in flesh compared to skin (paired t test $P < 0.05$).

OSMOTIC CONTRIBUTIONS OF CARBOHYDRATES AND ORGANIC ACIDS. In flesh (Table 1), the mean contribution of the sum of sugars to osmotic potential ranged from 55% to 79%, and that of the sum of organic acids from 4% to 8%. In skin (Table 2), the mean contribution of the sum of sugars to osmotic potential ranged from 61% to 84%, and that of the sum of organic acids

Fig. 3. Changes in juice pH (A) and titratable acidity (B) in flesh and skin during fruit development of 'Brooks', 'Lapins', 'Regina', and 'Fermina' cultivars. Mean of five samples. Vertical bars represent standard deviation. For each development stage, letters indicate mean separation among cultivars according to Tukey's test, $P < 0.05$. Mat C, commercial maturity; Mat P, physiological maturity.

from 2% to 6%. The contribution of the sum of sugars increased and that of organic acids decreased in skin and flesh from green stage to physiological maturity.

The contribution of each sugar followed a distinct pattern. In flesh (Table 1), glucose was the major contributor (29% to 37% of osmotic potential) with little or no change in different cultivars and stages. Fructose contribution increased from green (22% to 26%) to pink stage (25% to 30%) and then remained stable. Sorbitol contribution increased from pink stage (3% to 6%) to physiological maturity (9% to 10%). Similarly, in skin (Table 2), glucose was the major contributor (35% to 45% of osmotic potential) with little or no change with cultivars and stages. Fructose contribution increased from green (19% to 22%) to pink stage (27% to 30%) and then remained stable. Sorbitol contribution increased from pink stage (3% to 6%) to physiological maturity (10% to 13%). However, the glucose to fructose ratio was higher in skin (1.2:2.1) compared to flesh (1.1:1.5).

The contribution of malate to osmotic potential followed that of the sum of organic acids. Malate contribution decreased from green stage to physiological maturity in flesh (Table 1, 5% to 8% to 4% to 5%) and in skin (Table 2, 4% to 6% to 2% to 4%).

Fig. 4. Changes in osmotic potential in flesh (A) and in skin (B) during fruit development of 'Brooks', 'Lapins', 'Regina', and 'Fermina' cultivars. Mean of five samples. Vertical bars represent standard deviation. For each development stage, letters indicate mean separation among cultivars according to Tukey's test, $P < 0.05$. Mat C, commercial maturity; Mat P, physiological maturity.

However, malate contribution was higher in flesh than in skin. Little significant differences among cultivars were observed for the contribution of the major sugars and organic acids to flesh or skin osmotic potential (Tables 1 and 2).

Discussion

CONSISTENCY WITH PREVIOUS STUDIES OF BIOCHEMICAL FRUIT COMPOSITION IN CHERRY. The percentages and the concentrations of the major soluble sugars observed in all cultivars were in general agreement with published data (Girard and Kopp, 1998; Neubeller and Stösser, 1977). The glucose and fructose concentrations and their ratio were similar but sorbitol concentration was lower in the present experiment than the "mannitol+sorbitol" concentration published by Girard and Kopp (1998). Moreover, mannitol was not detected in our experiment. Among the minor sugars detected, rhamnose, arabinose, and xylose present as cell wall bound sugars in pericarp (Kondo and Danjo, 2001) may indicate cell wall turnover.

The concentrations of malate and citrate were in agreement with previous data (Girard and Kopp, 1998; Looney et al., 1996).

Table 1. Osmotic potential (data from Fig. 4A) and calculated percentage of the osmotic potential contributed by glucose, fructose, the sum of soluble sugars (sum SS), malate, the sum of organic acids (sum OA) in juice collected from flesh of ‘Brooks’, ‘Lapins’, ‘Regina’, and ‘Fermina’ fruit at four different stages.

Genotype	Stage	Osmotic potential	Contribution to osmotic potential (%)					
		(MPa)	Glucose	Fructose	Sorbitol	Sum SS	Malate	Sum OA
Brooks	Green	−1.77 b ^z	36.9 a	25.5 a	3.4 c	66.1 a	5.4 c	5.7 c
Lapins		−1.96 ab	32.6 b	21.5 b	6.2 a	60.4 b	7.6 a	8.1 a
Regina		−1.91 b	31.4 bc	22.2 b	4.1 b	57.9 bc	6.6 b	7.0 b
Fermina		−2.20 a	29.1 c	22.1 b	3.2 c	54.6 c	8.1 a	8.3 a
Brooks	Pink	−1.88 b	35.1 a	29.5 ab	4.6 b	69.3 a	5.2 b	5.4 b
Lapins		−2.82 a	33.2 a	29.2 ab	6.6 a	69.3 a	4.7 b	4.9 b
Regina		−2.79 a	34.5 a	30.3 a	6.5 a	71.5 a	5.1 b	5.3 b
Fermina		−2.55 a	28.4 b	25.2 b	4.6 b	58.9 b	6.2 a	6.4 a
Brooks	Commercial Maturity	−2.98 ab	35.7 a	32.2 a	7.3 ab	75.3 a	3.7 b	3.8 b
Lapins		−2.90 ab	32.3 b	29.2 b	8.0 a	69.5 ab	3.8 b	4.0 b
Regina		−2.74 b	33.1 ab	29.5 b	6.6 b	69.2 ab	3.9 b	4.0 b
Fermina		−3.56 a	31.8 b	30.0 ab	6.4 b	68.4 b	5.5 a	5.5 a
Brooks	Physiological Maturity	−2.91 b	37.0 a	33.1 a	8.6 ab	78.8 a	3.8 b	3.9 b
Lapins		−3.42 a	34.5 ab	30.5 bc	9.6 a	74.7 ab	3.8 b	4.0 b
Regina		−3.21 ab	33.2 b	28.5 c	10.0 a	71.7 b	3.9 b	4.0 b
Fermina		−3.25 a	33.7 b	31.6 ab	7.4 b	72.8 ab	5.0 a	5.1 a

^zMean of five samples. Mean separation within stage according to Tukey’s test, $P < 0.05$.

Table 2. Osmotic potential (data from Fig. 4B) and calculated percentage of the osmotic potential contributed by glucose, fructose, the sum of soluble sugars (sum SS), malate, the sum of organic acids (sum OA) in juice collected from skin of ‘Brooks’, ‘Lapins’, ‘Regina’, and ‘Fermina’ fruit at four different stages.

Genotype	Stage	Osmotic potential	Contribution to osmotic potential (%)					
		(MPa)	Glucose	Fructose	Sorbitol	Sum SS	Malate	Sum OA
Brooks	Green	−1.63 b ^z	44.5 a	21.8 a	3.1 c	69.4 a	4.1 c	4.4 b
Lapins		−1.90 ab	38.4 b	18.6 b	6.1 a	63.2 ab	4.7 bc	5.1 ab
Regina		−1.85 ab	38.2 b	18.7 b	3.7 b	60.8 b	4.9 b	5.3 a
Fermina		−2.03 a	37.9 b	20.2 ab	3.9 b	62.1 b	5.7 a	5.9 a
Brooks	Pink	−2.07 b	40.5 a	27.6 b	4.7 c	72.9 bc	3.6 b	3.8 b
Lapins		−2.64 a	38.8 ab	30.1 a	9.2 a	78.3 a	3.0 c	3.2 c
Regina		−2.70 a	36.0 c	26.8 b	7.0 b	69.9 c	3.1 c	3.3 c
Fermina		−1.93 b	37.9 b	29.7 a	7.2 b	74.9 b	4.4 a	4.6 a
Brooks	Commercial Maturity	−2.46 b	38.1 a	29.7 a	8.7 b	76.7 a	2.7 b	2.8 b
Lapins		−3.02 a	35.2 a	28.5 a	10.5 a	74.2 a	2.3 c	2.4 c
Regina		−2.63 ab	37.3 a	28.8 a	8.2 b	74.4 a	3.0 b	3.2 b
Fermina		−2.51 b	35.2 a	29.9 a	8.6 b	73.8 a	4.2 a	4.3 a
Brooks	Physiological Maturity	−2.43 b	41.1 a	32.4 a	10.1 b	83.7 a	2.8 b	3.0 b
Lapins		−3.03 a	37.1 b	27.8 c	12.6 a	77.5 b	1.9 d	2.1 d
Regina		−2.67 ab	39.0 ab	28.0 bc	12.9 a	80.0 ab	2.4 c	2.5 c
Fermina		−2.57 b	35.5 b	30.3 ab	9.6 b	75.4 b	3.9 a	4.0 a

^zMean of five samples. Mean separation within stage according to Tukey’s test, $P < 0.05$.

Ascorbic acid could not be quantified in our experiment due to degradation during extraction and separation. The concentration of quinic acid was higher and that of citric acid was lower than in Oen and Vestrheim (1985).

DIFFERENCES AMONG STAGES. According to the immersion test, the highest susceptibility to cracking was observed at commercial maturity for ‘Brooks’, ‘Lapins’, and ‘Fermina’, considered to be very susceptible, moderately susceptible, and resistant to cracking, respectively (Hansche et al., 1988; Lane and Schmid, 1984; Roser, 1996). Between green stage and commercial maturity, fruit size approximately doubled and osmotic potential increased by $\approx 30\%$ in skin and 40% in flesh. As emphasized previously (Looney et al., 1996), the pattern of cherry fruit sizing is characterized by an

accumulation of $\approx 25\%$ of final fruit weight during the last week before harvest, with steady softening during the month preceding harvest. The mean fruit fresh weight was stable between commercial and physiological maturity for ‘Brooks’ and ‘Lapins’, but increased for ‘Regina’ and ‘Fermina’. It may be interesting to verify if this late increase in fruit fresh weight, resulting mainly from cell enlargement, is accompanied by a late cessation of cell divisions in exocarp as shown for some other cultivars with low susceptibility to cracking (Yamaguchi et al., 2003).

DIFFERENCES BETWEEN FLESH AND SKIN. Several differences were observed between flesh and skin revealing compositional gradients in the fruit tissues. Acidity and malate concentrations were higher in flesh than in skin for all stages and cultivars. The

tissue specificity of malic acid concentration has already been demonstrated in grape berries (Gutiérrez-Granda and Morrison, 1992) and may result from compartmentation of metabolism (Famiani et al., 2000).

The absolute value of osmotic potential was higher in cherry flesh than in skin, contrary to data obtained in pear cactus fruit (Nobel et al., 1994). The contribution of the sum of sugars to osmotic potential was lower in flesh than in skin. The glucose : fructose ratio was higher in skin than in flesh.

For facility purposes, skin was determined as the outer part of the fruit with a 1-mm $\pm$ 0.2 thickness. We did not verify if the thickness of epidermal and hypodermal cell layers differed between stages and cultivars. However, the same biochemical differences between flesh and skin were observed for all cultivars and stages. Due to the respective fresh weight of skin and flesh, the contamination of flesh by juice issued from the skin should be negligible. Possible contamination of the skin by some juice coming from the flesh cannot be excluded. However, as we observed lower osmotic pressure and lower acidity in skin, this possible contamination must be low.

RELATIONSHIPS BETWEEN OSMOTIC POTENTIAL AND FRUIT CRACKING. The absolute value of osmotic potential in flesh or skin of mature cherry fruit ranged between 2.4 and 3.6 MPa in the present study. The flesh values are in good agreement with those measured on fruit sap of 'Napoleon' cultivar (Andersen and Richardson, 1982). The cracking indexes observed in 'Brooks', 'Lapins', and 'Regina' were in general agreement with published data (Hansche et al., 1988; Lane et al., 2000; Roser, 1996; Ystaas and Froynes, 1998). 'Fermina' was less susceptible to fruit cracking, as determined using fruit immersion test (Christensen, 1972b), than 'Lapins'. At commercial maturity when fruit susceptibility to cracking after immersion was highest, no clear difference appeared between 'Brooks' and 'Lapins' compared to 'Regina' and 'Fermina' for flesh or skin osmotic potential and for contribution of the major sugar or organic acids to skin and flesh osmotic potential. Osmotic potential of the skin did not appear to be more related to in vitro cracking susceptibility than osmotic potential of the flesh. Therefore, global osmotic potentials of skin and flesh are not controlling factors for fruit cracking. However, the osmotic potential measured in skin or flesh results from osmotic potential in intracellular and intercellular (apoplast) spaces. Osmotic potential of intercellular spaces in skin may be important for water uptake through the fruit surface (Christensen, 1972a; Sekse, 1998). Differences among cultivars for susceptibility to cracking may result from a combination of changes in inner fruit composition as studied in the present experiment, epidermal cell plasticity (Yamaguchi et al., 2002), pedicel/fruit junction permeability to water (Beyer et al., 2002), and fruit surface characteristics, such as cuticle conductance to water (Beyer and Knoche, 2002; Knoche et al., 2000, 2001; Peschel et al., 2003) as investigated recently.

In conclusion, several differences were observed between flesh and skin revealing compositional gradients in the cherry fruit tissues. No clear difference appeared between the two susceptible compared to the two resistant cultivars for flesh or skin osmotic potential and for the contribution of the major sugar or organic acids to skin and flesh osmotic potential. Osmotic potential of the skin was not related to in vitro cracking susceptibility. These data confirm the complexity of the physiological mechanisms underlying susceptibility to cracking.

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