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The effect of mechanical vibrations during transport under model conditions on the shelf-life, quality and physico-chemical parameters of four apple cultivars

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Abstract: The study assessed the changes in the quality and physical and chemical parameters of apples of four cultivars ('Gala', 'Idared', 'Topaz', 'Red Prince') subjected to mechanical vibrations during transport under model conditions and after storage (shelf-life). Quality changes in apples were evaluated based on skin and flesh colour, total soluble solids, dry matter, firmness, titratable acidity, pH value, total polyphenol content and antioxidant capacity. The applied vibrations at a frequency of 28 Hz caused changes in the above parameters, which were visible also after storage and depended on cultivar, but did not show any clear trend or direction. Skin colour varied whereas flesh colour remained stable. Vibrations resulted in a decrease in firmness. The greatest stability of quality parameters, the highest content of bioactive compounds and the highest antioxidant capacity were observed for 'Red Prince' and 'Topaz' apples – this refers to the control and treated samples before and after storage. However, total polyphenol content and antioxidant capacity increased in all studied cultivars as a result of vibrations and storage, which suggests that 28 Hz mechanical vibrations and short-term cold storage did not reduce the health promoting potential of the apples.

Keywords: fruit transport; mechanical damage, physiological disorders, fruit maturity, colour, firmness

1. Introduction

Apples are among the most popular fruit species in the world. The worldwide annual production of apples is 86 million tonnes [1]. Because apples are typically grown in temperate climate and require a winter dormancy period, they are traded internationally and are often transported over long distances. In terms of their share in international trade, they are, after bananas and grapes, the third most frequently transported fruit [2]. The sensitivity of apple flowers to spring frosts, which are typical of the temperate climate zone, results from time to time in lower yields even in countries with high production potential. This also contributes to a high trade volume of this commodity [3]. Apples harvested either too early or too late are easily damaged and susceptible to fungal diseases and physiological disorders [4]. In the case of apples too mature at harvest, the increase in vulnerability to mechanical damage is due to the loosening of the flesh structure and the resulting formation of intercellular spaces [5].

Poland is one of the leading producers and exporters of apples and concentrated apple juice in Europe and worldwide [6]. Dessert apples are grown for local consumption and for export, mainly to the EU countries. Within Europe, they are transported mainly by road.

Transport is a key stage in the distribution of fresh food products. At the same time, however, as shown by a number of studies [7–10], transport and transshipment may expose fresh products to serious damage [11]. Fruit is a special case of transport-sensitive cargo. If fruit intended for direct consumption is transported over a distance of tens to several thousand km, it is exposed to mechanical damage and chemical and physiological changes caused by mechanical vibrations, the effect of which depends on the time and temperature of transport, the type of packaging, the number of packages (layers) laid out, the type of bodywork and the road quality [12–15]. Mechanical vibrations generate continuous impact and friction of neighboring fruit, leading to bruises on its surface. This, in turn, may cause spoilage of the load as a result of rotting fruit. Even minor mechanical injuries may lead to fungal diseases [16]. Studies show that 35% of bruises occur during the harvest and transport of apples [17]. Such damage may result in the rejection of a batch of apples during quality control. The damage level is typically between 10 % and 25 % [18] but may sometimes result in up to 50 % of losses [19]. During simulated transport, up to 80% of apples may be damaged depending on body type, packaging and position [20]. As consumers demand high-quality fruit, any damage to apples can disqualify this product from further distribution and thus generate substantial financial losses [11,16].

Apples are characterized by valuable nutritional and taste qualities, and, thanks to advanced storage technology, they are available for direct sale and consumption all year round. In addition to their attractive appearance and taste, apples are rich in saccharide, macro- and micronutrients, vitamins, dietary fibre, including soluble pectins and non-nutrients such as polyphenols. In terms of the content of polyphenols, secondary plant metabolites, apples outperform other common fruit species and contain on average approximately 2000 mg/100 g d.m. of polyphenolic compounds, especially phenolic acids and flavan-3-ols [21,22]. The polyphenol content of the fruit depends on the cultivar and growing conditions [23]. Their level affects the taste and susceptibility to enzymatic browning, especially during transport or processing [24]. Polyphenols as antioxidant compounds affect the health-promoting properties of fruit, including apples. Hence, apples are a recommended diet component as they help prevent many diseases, including cardiovascular, cancer or hypercholesterolemia [25,26]. Apples have been shown to have a high antioxidant activity in tests with an ABTS cationogen of 24–124 $\mu\text{mol Trolox}/100 \text{ g d.m.}$, on average about 70 $\mu\text{mol Trolox}/100 \text{ g d.m.}$ [22,23]. The high nutritional and health-promoting value of apples and their sensory qualities associated with chemical composition, including polyphenol content, may vary during harvest, transport, storage or processing [27].

This paper describes a study carried out to assess changes in the quality and physico-chemical parameters of selected four cultivars of apples as a result of mechanical vibrations during transport under model conditions directly after transport and after storage (shelf life).

2. Materials and Methods

2.1. *Plants and Growth Conditions*

The study was conducted on four popular apple cultivars: 'Red Prince' (coloured mutant of 'Jonagold'), 'Gala', 'Topaz' and 'Idared'. Fruit was collected in 2019 from a commercial orchard located about 50 km south of Poznan (52°07'28.3"N 16°58'57.6"E). The orchard was of full-bearing age.

The harvest took place on dates determined as the optimum harvest dates (OHD) using the starch test [28] and the sum of active temperatures (growing degree units) method proposed by Łysiak [29].

After the harvest, apples intended for storage were graded in order to eliminate those not meeting the highest commercial quality standard applicable in OECD countries [30]. According to those standards, apples of superior quality ('extra'), have to be intact, sound, clean and free of any damage, have a diameter of over 60 mm, and a cultivar-specific colour. After harvest, apples were transported to cold storage and stored at 1°C until all cultivars were collected. The experiment was carried out using 4 boxes of 15 kg of each apple cultivar. The orchard was protected and maintained in line with growing practices recommended for commercial orchards.

2.2. Test Stand

The impact of transport on the quality and parameters of apples was assessed under model conditions using a vibration simulator placed in a refrigerated vehicle body with adjustable temperature [31]. A schematic diagram of the vibration simulation stand is shown in Figure 1. The vibration simulator consisted of two basic components: 1) a vibration generating system made up of an inverter-controlled motor, flexible suspension and vibro-insulation, and 2) an integrated parameter control system. The operation of the vibration simulation stand and the parameters of individual actuators were controlled by monitoring mechanical quantities (vibration acceleration) in the PC-controlled feedback loop.

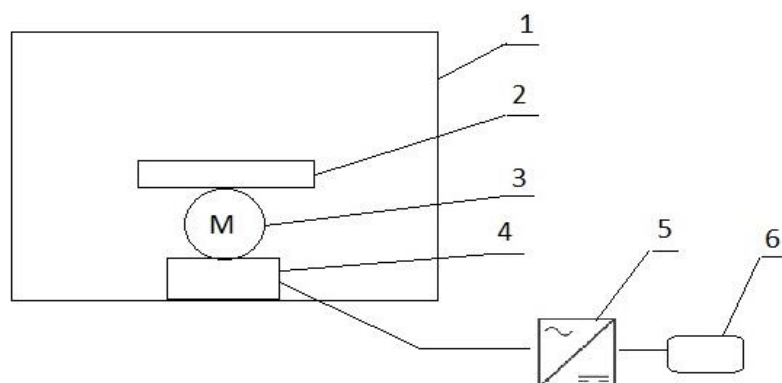


Figure 1. Diagram of the vibration simulation stand: 1 – stationary vehicle body with adjustable temperature, 2 – produce container with fixing elements, 3 – 0.09kW motor; 2800 rpm ; 230/400V; 0.58/0.33A; IMB3, 4 – suspension and vibro-insulation, 5 – Omron MX2-AB 002-E inverter (SJ200-002NFEF2), 6 – computer and software [31].

2.3. The Model System of Experiments

Figure 2 shows the experiment workflow. Apples of each cultivar were divided into two batches, one of which was subject to mechanical vibrations (transport under model conditions) and the other was a control batch. Apples from both batches were placed in plastic boxes of about 7 kg each. Boxes with the experimental batch were first vibrated at a frequency of 28 Hz for 6h at a temperature 6°C (day 0) and then both experimental and control batches were stored in cold-storage conditions comparable to those applied in trade and distribution (6°C) for 14 days to assess the shelf-life of the apples. Vibration frequency was chosen based on frequencies measured during real-life transport of food by refrigerated vehicle [32]. The frequency of 28 Hz is within a lower range of the determined predominant frequency values. Apples (both the vibrated and control batches)

were analysed in terms of their physical and chemical properties on the date of applying vibrations and after the 14-day storage period.

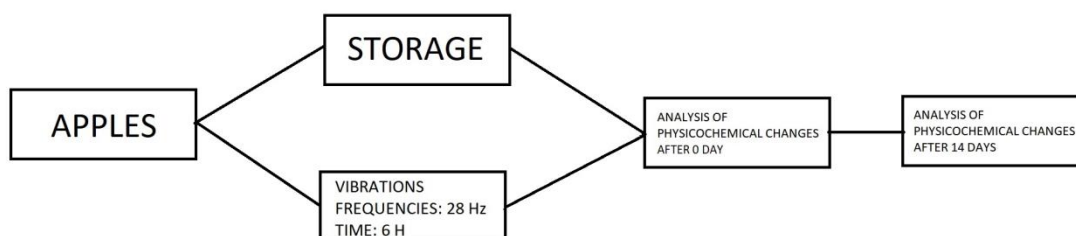


Figure 2. Experiment workflow diagram.

2.4. Analytical Methods

Three samples of 6 apples each were randomly selected from the experimental batch of each cultivar for the analysis of physical and chemical parameters. The analysis consisted of the measurement of firmness and skin and flesh colour, and after homogenizing the samples (coreless fruit with skin) – of the measurement of pH, titratable acidity, total soluble solids and dry matter. The homogenized samples were also used to prepare methanol extracts (5g of sample and 50 mL of 80% methanol (v/v)) which were analysed for total polyphenol content and antioxidant capacity. After 14 days, the apples were additionally assessed in terms of physiological properties.

2.4.1. Colour Measurement

Colour was measured according to the CIE L*a*b* colour space (CIE 1977) by a Konica Minolta CR-400 colorimeter (Konica Minolta, Tokyo, Japan) and Color SpectraMagic NX Lite software, in reflected light, using a D65 illuminant and a two-degree observer angle, to determine the L*, a*, b*, C* and h parameters as well as ΔE . The CIE L*a*b* parameters are described (arranged) in a three-dimensional space in which L* denotes the lightness and assumes values from 0 (black) to 100 (white), a* defines the share of the red (+) and green (-) colour, b* defines the share of the yellow (+) and blue (-) colour, C* indicates the chroma and h* stands for the colour hue (angle) and is expressed in degrees (°) to reflect: 0° - red/violet hue, 90° - yellow hue, 180° - blue/green hue, 270° - blue hue, 360° - coincides with 0° [34, 35]. The colour of the skin (side without blush) and flesh in the fruit cross-sectional area were measured in 30 repetitions per each sample and the result was expressed as average value. To compare the colour changes resulting from the shaking (vibrations) and storing of fruit, a total colour difference factor ΔE was calculated according to the formula $\{\Delta E = [(\Delta L^*)^2] + [(\Delta a^*)^2] + [(\Delta b^*)^2] / 2\}$ [35]. The input parameters for ΔE were the L⁰, a⁰, b⁰ values of the control samples on the first day of the experiment.

2.4.2. Firmness Measurement

Firmness was measured using a Fruit Tester 327 EFFEGI FT327 penetrometer (Facchini srl, Alfonsine (Ra), Italy), mounted on stand. The maximum penetration force of a probe of 8 mm in length and 11 mm in diameter, applied on a small area with skin removed, on two opposite sides of

the fruit, was recorded. Firmness was measured in 10 repetitions per sample. The results were presented as an average from 30 measurements and expressed in N.

2.4.3. Measurement of Total Soluble Solids

Total soluble solids (TSS) were determined using an ATAGO PAL-1 digital refractometer with automatic temperature compensation (Atago, Tokyo, Japan). The results were shown as an average from nine repetitions per sample and expressed as percentage values.

2.4.4. Measurement of Dry Matter Content

Dry matter content was determined using the gravimetric method in line with ISO 1026-1982 (E). Samples of about 5 g were dried at 105°C to obtain solid matter. The results were presented as an average from nine repetitions per sample and expressed as percentage values.

2.4.5. Measurement of pH Value

pH value was measured using a Hanna HI 221 pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA) according to the manufacturer's manual. The result was presented as an average from nine repetitions per sample.

2.4.6. Measurement of Titratable Acidity

Titrate acidity (TA) was determined using the potentiometric method in line with European Standard EN 12147: 1996. The results were presented as an average from nine repetitions per sample and expresses as malic acid content (g/L).

2.4.7. Measurement of Total Polyphenol Content

Total polyphenol content (TPC) was determined in methanol extracts by means of spectrophotometric method with Folin-Ciocalteu's reagent [39]. The analysis was carried out on solutions containing 0.7 ml of sample (methanol extract), 0.3 ml of water, 5 ml 0.2N of Folin-Ciocalteu's reagent, 4 ml of sodium carbonate solution (75 g/L) incubated in the dark for two hours. TPC was measured using a Helios Epsilon spectrophotometer (Thermo Fisher Scientific Waltham, Massachusetts, USA) at a wavelength of 765 nm against blank reagent. The results were presented as an average from nine repetitions per sample and expressed as chlorogenic acid equivalents (mg/100 g d.m.).

2.4.8. Measurement of Antioxidant Capacity

Antioxidant capacity of methanol extracts was determined by means of spectrophotometric method using a cationic radical (ABTS+) [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] [Re et al. 1999]. The ABTS+ cation was generated by mixing 7 mM ABTS and 2.45 mM K₂S₂O₈ (potassium persulfate or potassium peroxydisulfate) solutions at a ratio of 1:0.5. The ABTS+ cationic radical solution and methanol extract samples were diluted using Phosphate Buffer Solution (PBS) pH 7.4. Absorbance was measured at a wavelength of 734 nm on samples incubated for 6 min at 30°C against PBS as reference assay. Antioxidant capacity was determined based on the percentage reduction of absorbance of the ABTS+ cationic radical solution by the sample as compared to the reducing power of Trolox (6-hydroxy-2,5,7,8-tetramethylchromate-2-carboxylic acid). The measurements were conducted using a Helios Alpha spectrophotometer (Thermo Electron Corporation USA) equipped with a water bath for thermostating of samples. The results were expressed as an average from nine repetitions per sample and expressed in $\mu\text{mol Trolox/g d.m.}$

2.4.9. Physiological Assessment

Fruit was harvested in line with commercial quality standards and was free from diseases and disorders. After 14 days, the apples were assessed for the presence of physiological disorders, fungal diseases and bruising. The results were expressed as a percentage share of infected/damaged fruit in the total number of evaluated fruit.

2.4.10 Statistical Analysis

The results were analysed by one-way and two-way ANOVA using Statistica 13.3 (StatSoft) software. Significance of differences between average values was determined by Fisher's least significant difference test with significance threshold $p=0.05$. The correlation between total phenolic content and antioxidant capacity in the samples was calculated using the Pearson correlation coefficient.

3. Results and Discussion

3.1. Colour

Tables 1 and 2 show the average colour parameters of the skin on the side without blush and of the flesh in the cross-sectional area of control and treated apples.

Table 1. Average colour parameters of the skin on the side without blush of control and treated apples

Cultivar	Treatment	L*	a*	b*	C*	h	ΔE
'Gala'	Ctr 0 ¹	68.5±4.13 ^{a, b}	7.55±6.18 ^b	33.1±1.83 ^b	34.5±1.27 ^b	77.2±10.56 ^b	
	Vbr 0 ²	69.2±1.62 ^{ab}	4.39±2.78 ^a	41.3±2.22 ^c	31.4±2.09 ^a	78.5±4.15 ^b	8.82
	Ctr 14 ³	66.9±4.38 ^a	7.86±8.11 ^b	28.0±5.32 ^a	30.4±3.15 ^a	73.1±17.49 ^a	5.37
	Vbr 14 ⁴	69.8±5.46 ^{ab}	5.31±6.53 ^a	29.2±4.11 ^a	30.1±3.36 ^a	79.0±13.83 ^b	4.63
'Idared'	Ctr 0	73.5±3.35 ^b	-5.25±4.17 ^a	34.9±2.60 ^b	35.5±2.77 ^b	98.2±6.88 ^b	
	Vbr 0	69.4±3.65 ^a	2.91±5.52 ^b	30.3±2.28 ^a	31.0±1.38 ^a	84.1±10.99 ^a	10.22
	Ctr 14	71.8±5.56 ^{a, b}	1.01±5.53 ^b	30.3±2.33 ^a	30.9±1.28 ^a	87.7±11.30 ^a	7.93
	Vbr 14	71.6±3.10 ^{a, b}	-2.79±2.49 ^a	36.9±1.84 ^c	37.1±1.91 ^c	94.2±3.83 ^b	3.69
'Topaz'	Ctr 0	65.4±2.94 ^a	13.86±5.11 ^b	35.2±3.47 ^a	38.3±1.68 ^a	68.2±8.93 ^a	
	Vbr 0	65.4±5.66 ^a	13.22±1.12 ^{ab}	36.7±5.24 ^{ab}	40.5±1.75 ^b	69.7±16.16 ^{ab}	1.64
	Ctr 14	66.5±2.72 ^a	12.32±4.49 ^{ab}	37.2±3.34 ^{ab}	39.5±1.95 ^{a, b}	71.4±7.65 ^{ab}	2.76
	Vbr 14	66.9±3.12 ^a	8.98±4.62 ^a	38.5±4.51 ^b	40.0±3.10 ^b	76.4±8.48 ^b	6.10
'Red Prince'	Ctr 0	62.1±7.47 ^a	15.22±11.74 ^a	29.4±6.05 ^a	35.4±2.85 ^b	62.9±20.79 ^a	
	Vbr 0	60.6±2.90 ^a	12.73±7.49 ^a	26.1±2.91 ^a	30.0±2.88 ^a	64.7±14.36 ^a	4.34
	Ctr 14	62.5±7.77 ^a	12.82±12.57 ^a	28.6±5.39 ^a	34.0±2.64 ^b	66.4±22.87 ^a	2.57
	Vbr 14	61.6±5.21 ^a	9.30±5.38 ^a	28.6±4.22 ^a	30.6±3.84 ^a	71.9±10.84 ^a	5.99

Explanation: average values marked with the same letter are not statistically different at $p = 0.05$ within one cultivar. 1 – Ctr 0, evaluation after harvest, day 0; 2 – Vbr 0, evaluation after vibration, day 0; 3 – Ctr 14, evaluation after 14-day storage; 4 – Vbr 14, evaluation after vibration and 14-day storage.

The lightness (L^*) of the evaluated apples varied depending on the cultivar and ranged from 60.57 ('Red Prince') and 73.53 ('Idared'). Parameter a^* assumed values from -5.25 ('Idared') to 15.22 ('Red Prince'). Parameter b^* varied from 26.14 ('Red Prince') to 41.31 ('Gala'). Parameter C^* ranged from 30.0 ('Red Prince') to 40.55 ('Topaz'). Hue angle (h) showed values from 62.89 ('Red Prince') to 98.18 ('Idared'). The total colour difference factor (ΔE) calculated in relation to control sample 0 within a given cultivar was most often $\Delta E > 3$, which means that the colour differences were noticeable to an average observer. The analysis of the values of individual colour parameters, the ΔE factor and the significance of statistical differences between average values (results of Fisher's least significant difference test) revealed that they did not follow any clear trend of variations and resulted above all from the variability of the studied biological material rather than from vibrations or storage. However, the skin colour parameters suggest that 'Topaz' and 'Red Prince' apples had darker and redder skin (higher a^* values and lower L^* and h^* values) compared to 'Gala' and 'Idared' apples. When assessing skin colour during the ripening of 'Ligol' and 'Jonagored' apples Łysiak et al. [40] noted similar L^* and C^* values, whereas the share of red (a^*) was lower and the share of yellow (b^*) was higher than those identified in this study, which is connected with the maturity stage. Apples analysed in the current work were fully ripe and were kept in cold storage after harvest till the start of the analyses. Łysiak et al. [40] observed that changes in parameter a^* in ripening apples were key for determining the harvest date. The colour parameter values obtained in this study show the highest colour similarity between 'Idared' and 'Ligol' apples [40].

Table 2. Average colour parameter values of flesh in the cross-sectional area of control and treated apples.

Cultivar	Treatment	L^*	a^*	b^*	C^*	h	ΔE
'Gala'	Ctr 0	77.1±1.13 ^a	-2.33±0.55 ^b	23.1±1.65 ^{bc}	23.3±1.67 ^{b c}	95.7±1.22 ^a	
	Vbr 0	77.1±0.47 ^a	-2.82±0.86 ^{ab}	22.2±1.17 ^a	22.4±1.09 ^a	97.3±2.49 ^{bc}	1.11
	Ctr 14	78.0±0.87 ^b	-3.01±0.86 ^a	22.3±0.81 ^{ab}	22.5±0.84 ^{ab}	97.6±2.12 ^c	1.42
	Vbr 14	76.9±2.05 ^a	-2.51±1.16 ^{ab}	23.8±1.45 ^c	23.9±1.40 ^c	96.1±2.81 ^{ab}	0.73
'Idared'	Ctr 0	79.3±1.04 ^a	-3.07±0.25 ^{ab}	15.7±0.72 ^b	16.1±0.75 ^b	101.2±0.51 ^b	
	Vbr 0	79.5±1.64 ^a	-2.79±0.27 ^c	14.2±1.49 ^a	14.4±1.50 ^a	101.2±0.71 ^b	1.62
	Ctr 14	79.0±0.65 ^a	-2.95±0.50 ^{bc}	15.8±1.39 ^b	16.0±1.45 ^b	100.5±1.01 ^a	0.30
	Vbr 14	79.2±0.55 ^a	-3.21±0.21 ^a	16.6±0.92 ^c	16.9±0.94 ^c	101.0±0.35 ^{ab}	0.83
'Topaz'	Ctr 0	74.9±1.62 ^{ab}	-0.06±0.58 ^b	25.5±1.44 ^a	25.5±1.45 ^a	88.7±1.26 ^a	

	Vbr 0	74.5±1.47 ^a	-1.12±0.35 ^a	24.9±1.77 ^a	24.9±1.77 ^a	92.6±0.87 ^c	1.27
	Ctr 14	75.4±0.87 ^b	-0.09±0.63 ^b	25.2±1.40 ^a	25.2±1.40 ^a	90.2±1.43 ^b	0.57
	Vbr 14	75.5±0.64 ^b	-0.12±0.30 ^b	25.4±1.13 ^a	25.4±1.13 ^a	90.3±0.68 ^b	0.60
	Ctr 0	75.0±1.19 ^a	-1.89±0.51 ^b	24.1±1.77 ^a	24.2±1.74 ^a	94.6±1.41 ^a	
'Red	Vbr 0	75.8±0.68 ^b	-1.75±0.54 ^b	24.4±1.54 ^a	24.4±1.57 ^a	94.1±1.06 ^a	0.84
Prince'	Ctr 14	75.7±0.68 ^b	-2.43±0.46 ^a	23.6±1.47 ^a	23.7±1.44 ^a	95.9±1.38 ^b	1.03
	Vbr 14	76.1±1.09 ^b	-2.11±0.79 ^a	25.8±2.02 ^b	26.0±2.04 ^b	96.9±2.27 ^b	2.02

Explanation: average values marked with the same letter are not statistically different at $p = 0.05$ within one cultivar. 1 – Ctr 0, evaluation after harvest, day 0; 2 – Vbr 0, evaluation after vibration, day 0; 3 – Ctr 14, evaluation after 14-day storage; 4 – Vbr 14, evaluation after vibration and 14-day storage.

Initially, the studied cultivars did not significantly differ in terms of lightness (L^*) of flesh, which ranged from 74.47 ('Topaz') to 79.49 ('Idared'). However, it increased in the treated samples of all cultivars except 'Idared'. Parameter a^* assumed values between -3.21 ('Idared') and -0.06 ('Topaz'), parameter b^* – between 14.17 ('Idared') and 25.80 ('Red Prince'), parameter C^* – between 14.44 ('Idared') and 26.01 ('Red Prince'), and hue angle – between 88.70 ('Topaz') and 101.15 ('Idared'). Colour difference ΔE within one cultivar was small and ranged from 0.3 to 2.0, which means that there were no significant differences in flesh colour for an average observer. The analysis of the values of the colour parameters, the ΔE factor and the significance of differences between average values (results of Fisher's least significant difference test) did not reveal any patterns in the change of apple flesh colour. Although most of those changes were statistically significant, the low ΔE suggests that flesh colour was relative stable within one cultivar. The flesh colour parameter values obtained in this study are comparable with those described in the literature for fresh (non-treated) and sulfite-pretreated apple slices [35]. The presented flesh colour parameter values allow the conclusion that the flesh colour of 'Idared' apples was lighter and closer to the creamy-white colour than that of the other studied cultivars, which was darker and yellower, as can be inferred from the lower L^* and h^* values and higher a^* , b^* and C^* values obtained for 'Gala', 'Topaz' and 'Red Prince'.

3.2. Firmness

Table 3 shows the average firmness measurement results.

Table 3. Average firmness values of control apples, vibrated apples and apples after 14 days of storage.

Cultivar	Firmness [N]			
	Control 0	Vibrations 0	Control 14	Vibrations 14
'Gala'	58.6 ±2.90 ^b	53.0±2.70 ^a	52.3±3.72 ^a	48.4±5.34 ^a

'Idared'	45.3±5.06 ^b	41.5±2.24 ^{a, b}	41.7±2.41 ^{a, b}	39.5±2.81 ^a
'Topaz'	52.8±1.80 ^b	50.1±2.50 ^{a, b}	49.6±3.64 ^{a, b}	47.6±2.20 ^a
'Red Prince'	53.2±1.63 ^a	52.9±1.72 ^a	51.4±1.97 ^a	49.5±6.10 ^a

Explanation: average values marked with the same letter are not statistically different at $p = 0.05$ within one cultivar.

The applied vibrations and storage reduced firmness but the changes were not always statistically significant. The lowest firmness value (41.5) was observed for 'Idared' 14 days after the apples were subject to vibrations, whereas the highest firmness (58.6) was recorded for 'Gala' apples from control sample 0. The biggest statistically significant decrease in the firmness of 'Gala' apples was noted directly after vibrations and after 14-day storage of both vibrated and control samples. As for 'Idared' and 'Topaz', their firmness declined statistically significantly relative to non-vibrated apples examined on day 0 only in the vibrated sample after 14 days of storage. 'Red Prince' apples had the most stable firmness, whereas the firmness decrease was relatively the highest (by 8-10%) in 'Gala' apples. No statistically significant differences were observed for 'Red Prince'.

The range of firmness values obtained for the four cultivars analysed in this study is lower than that mentioned in the literature for 'Jonagold' apples (65-70N) [23]. In both studies firmness decreased during storage. The above-mentioned study reported a decline in the firmness of 'Jonagold' apples by 13-15% after 6 months of ULO storage at 0.5°C, whereas in this study (standard cold storage at 6°C) firmness decreased by 3-10% in the control and by 5-8% in the vibrated samples. The decrease in firmness during storage is a natural process commonly described by scholars [41-43]. No significant impact of mechanical vibrations on the firmness of the treated samples compared to control was observed. Probably, the 14-day period set for the assessment of shelf-life was too short for any significant firmness differences between control and vibrated samples to occur. From among the tested samples, 'Gala' apples proved to be the most vulnerable to changes in firmness caused by storage and vibrations. Also other authors confirm that vibrations reduce the firmness of apples [44].

3.3. Total Soluble Solids, Dry Matter, Titratable Acidity and pH Value

Table 4 shows total soluble solids (TSS), dry matter content, titratable acidity (TA) and pH value of control and treated apples.

	Storage time	Sample	Cultivar			
			Gala	Idared	Topaz	Red Prince
TSS	0	control	13.41±0.57 ^a	12.81±0.84 ^a	15.89±0.70 ^b	14.32±0.42 ^a
		vibrations	14.40±0.69 ^{b, c}	13.32±0.44 ^a	14.49±1.02 ^a	14.40±0.36 ^a
	14	control	14.72±0.35 ^c	13.11±0.37 ^a	15.53±0.52 ^{a, b}	14.14±0.60 ^a
		vibrations	13.81±0.52 ^{ab}	13.29±0.48 ^a	14.88±0.47 ^a	14.49±0.22 ^a

Dry matter	0	control	13.85±0.74 ^a	13.65±1.10 ^a	15.96±0.46 ^a	14.93±0.91 ^a
		vibrations	14.41±0.66 ^a	14.15±0.73 ^a	15.22±0.66 ^a	15.04±0.55 ^a
	14	control	15.48±0.51 ^a	14.10±0.42 ^a	16.37±0.46 ^a	14.13±1.41 ^a
		vibrations	15.37±1.19 ^a	14.49±0.78 ^a	15.51±0.84 ^a	15.05±0.20 ^a
pH value	0	control	4.10±0.11 ^b	3.78±0.14 ^c	3.54±0.12 ^b	3.80±0.05 ^{bc}
		vibrations	3.92±0.05 ^a	3.39±0.05 ^a	3.40±0.06 ^b	3.94±0.08 ^c
	14	control	3.87±0.06 ^a	3.59±0.06 ^b	3.04±0.07 ^a	3.79±0.11 ^b
		vibrations	4.06±0.06 ^b	3.72±0.05 ^{bc}	3.46±0.09 ^b	3.59±0.06 ^a
TA	0	control	2.59±0.15 ^{ab}	3.13±0.20 ^a	6.34±0.08 ^{ab}	3.17±0.28 ^a
		vibrations	2.72±0.08 ^b	4.33±0.08 ^c	6.61±0.08 ^b	3.08±0.13 ^a
	14	control	2.77±0.15 ^b	3.71±0.08 ^b	6.81±0.20 ^b	3.39±0.08 ^a
		vibrations	2.37±0.08 ^a	3.84±0.08 ^b	6.21±0.08 ^a	4.24±0.08 ^b

Explanation: average values marked with the same letter are not statistically different at $p = 0.05$ within one cultivar.

The content of total soluble solids was 12.81 – 15.89 Brix, depending on cultivar and treatment (table 4). The lowest level of total soluble solids was noted in ‘Idared’ before vibrations and storage. Apples of this cultivar are probably characterized by a relatively low TSS content, which has also been shown in other studies [45]. The highest TSS level was measured in ‘Topaz’ apples from the control sample (i.e. before vibration and storage). Vibrations caused a TSS increase in ‘Gala’ apples and a TSS decrease in ‘Topaz’ apples, both from samples not subject to storage. In turn, among the samples kept in cold storage for 14 days only ‘Gala’ apples showed a decrease in TSS. No statistically significant changes in TSS were observed in the remaining cultivars. The TSS values were consistent with the literature on the subject [22]. The level of total soluble solids is a good indicator of sugar concentration in fruit, which is strongly affected by storage [45]. In this study, vibrations mostly had no influence on the TSS content in apples [46]; nevertheless, this parameter may drop as a result of vibrations or storage [47].

Dry matter in the studied samples was 13.65 – 16.37 % and varied depending on cultivar and treatment (table 4). The lowest level of dry matter was found in ‘Idared’ apples before vibrations and storage, whereas the highest level was observed in ‘Topaz’ apples before vibrations and after 14 days of storage. The dry matter values matched those reported in the literature [48]. Vibrations did not cause any significant changes in the dry matter content, irrespective of cultivar.

pH value varied between 3.04 and 4.10 depending on cultivar and treatment (table 4). The lowest pH value was noted for the ‘Topaz’ control sample following 14-day storage, while the highest for the ‘Gala’ sample before vibration and storage. Vibrations resulted in a pH decrease in the non-stored samples of all cultivars except ‘Red Prince’ and in a pH increase in the stored samples

of all cultivars, again except 'Red Prince', which showed a reverse direction of changes. The measured pH values were in agreement with the literature [22].

Titrateable acidity ranged between 2.37 and 6.81 [g/L], depending on cultivar and treatment (table 4). The lowest titrateable acidity was observed in 'Gala' after vibrations and 14 days of storage, whereas the highest in 'Topaz' before vibrations and after 14 days of storage. Vibrations caused a slight increase in TA in non-stored apples except 'Red Prince', but the changes were mostly statistically insignificant. TA increased, in most cases statistically insignificantly, in the control samples of all cultivars after storage. In turn, there was a significant TA decrease in vibrated samples after 14 days of storage. The decrease in TA during storage of fruit was also described by other scholars [49].

The analysis of the above-described fruit parameters after harvest, during transport and storage should also take into account the indirect influence of ethylene, which is synthesized in fruit during respiration and ripening, especially in stress conditions such as mechanical vibrations induced by a moving vehicle. Ethylene initiates various physiological and biochemical processes, mainly respiration, and thus indirectly affects transpiration and disintegration of spare substances. It may influence those processes still on the tree, but this influence is most pronounced during harvest and storage. In climacteric fruit, such as apples, ethylene production increases considerably, even several dozen times, a few days preceding the optimum harvest date and a few days after harvest [50]. To reduce ethylene production, ripening processes and fruit decay after harvest, the ethylene perception inhibitor 1-methylcyclopropene (1-MCP) is applied at the beginning of storage [51].

3.4. Polyphenol Content and Antioxidant Capacity

Table 5 shows polyphenol content measured in chlorogenic acid equivalents [mg/100 g d.m.], and table 6 presents the antioxidant capacity [$\mu\text{mol Trolox/g d.m.}$] of control and vibrated apples depending on cultivar and storage time.

Tab.5. Total polyphenol content (TPC) in control and treated apples depending on cultivar and storage time.

	Storage time	Sample	Cultivar			
			Gala	Idared	Topaz	Red Prince
TPC	0	control	479 \pm 56.0 ^a	517.1 \pm 33.4 ^a	763.8 \pm 39.3 ^a	596.6 \pm 67.8 ^a
		vibrations	486 \pm 9.7 ^{ab}	549.8 \pm 21.9 ^b	824.7 \pm 85.0 ^b	620.7 \pm 56.4 ^{ab}
	14	control	517 \pm 11.7 ^{bc}	563.1 \pm 36.6 ^b	802.5 \pm 30.2 ^{ab}	664.9 \pm 21.5 ^{bc}
		vibrations	540 \pm 36.8 ^c	564.2 \pm 20.8 ^b	840.3 \pm 23.2 ^b	683.5 \pm 20.1 ^c

Explanation: average values marked with the same letter are not statistically different at $p = 0.05$ within one cultivar and one parameter

Tab.6. Antioxidant capacity of control and treated apples depending on cultivar and storage time.

	Storage time	Sample	Cultivar			
			Gala	Idared	Topaz	Red Prince
Antioxidant capacity	0	control	32,6 ± 3,8 ^a	35,2 ± 2,3 ^a	51,9 ± 2,7 ^a	40,6 ± 4,6 ^a
		vibrations	33,4 ± 0,7 ^a	37,7 ± 1,5 ^{bc}	56,6 ± 5,8 ^b	42,6 ± 3,8 ^{ab}
	14	control	34,2 ± 0,8 ^a	37,2 ± 2,4 ^b	52,9 ± 2,0 ^a	43,9 ± 1,4 ^b
		vibrations	37,7 ± 2,6 ^b	39,4 ± 1,45 ^c	58,7 ± 1,6 ^b	47,7 ± 1,4 ^c

Explanation: average values marked with the same letter are not statistically different at $p = 0.05$ within one cultivar and one parameter

TPC was between 479 and 802 mg/100 g d.m. depending on cultivar and treatment (table 5). The lowest TPC was noted in 'Gala' apples before vibrations and storage, whereas the highest in 'Topaz' after vibrations and storage. Apples that were subject to vibrations had a higher TPC than control apples. The same was found after 14-day storage. In most cases those differences were statistically significant. A two-way ANOVA showed that TPC significantly depended on the storage time, and in the case of 'Topaz' – on vibrations.

Antioxidant capacity varied depending on whether apples were vibrated and stored (table 6) and ranged between 32.6 and 58.7 $\mu\text{mol Trolox/g d.m.}$ depending on cultivar. Similarly as in the case of TPC, the lowest antioxidant capacity was observed in the 'Gala' control sample on day 0, and the highest in the 'Topaz' sample after vibrations and storage. Vibrated samples showed higher antioxidant capacity than control samples – both on day 0 and after 14 days of storage. All samples had higher antioxidant capacity after than before storage. Differences between the average values were significant in most of the samples. Changes in antioxidant capacity were significantly affected by both vibrations and storage, except 'Topaz' apples, in which case this parameter significantly depended on vibrations only.

Mechanical vibrations generated during transport make fresh fruit more likely to undergo physical, chemical and biological changes during trade and storage. Transport shocks and vibrations result directly in visual damage such as abrasion and bruising, which facilitates microbial invasion and multiplication thus accelerating fruit decay [27, 52]. Biochemical changes induced by stress conditions during transport may also include an increase in the content of polyphenols, which are secondary metabolites playing defensive functions in plants, e.g. preventing microbial access and development [53]. The content of polyphenolic compounds in apples also depends on the agricultural and climatic conditions during growth as well as on storage conditions [54].

The TPC obtained in this work is comparable to the data provided by previous studies: 221-476 mg/100g d.m. in 'Jonagold' [23], 564-748 mg/100 g d.m. in 'Golden Delicious' [55] and 790-1330 mg/100 g d.m. in 'Papierowka' and 'Gold Milenium' [26]. The literature reports both increase and decrease in TPC in fruit after harvest. Polyphenol content was observed to have increased in 'Auksis' apples cold-stored in normal and controlled atmosphere (ultra-low oxygen), including after applying 1-MCP [54]. The same was noted during the storage of e.g. 'Golden Delicious', 'Pinova' and 'Topaz' apples and the proposed explanation was that the increase may have been due to, among

other things, the activity of ethylene, which stimulates L-phenylalanine ammonia lyase (PAL), one of key enzymes responsible for the synthesis of polyphenols [53]. During long-term storage in controlled atmosphere of 'Golden Delicious' apples, polyphenol content was found to have increased in between 1st and 3rd month and to have decreased afterwards [55].

Antioxidant capacity of plant material is significantly correlated with the content of bioactive compounds with antioxidant properties, such as polyphenols or vitamins [53, 56-58]. This is also confirmed by this study, in which the correlation coefficient between TPC and antioxidant capacity was between 0.94 and 0.97 depending on cultivar, thus demonstrating a high positive correlation. The antioxidant capacity values observed in this study are comparable with those presented in the literature [22,23]. Studies also show that an increase in polyphenol content during storage is accompanied by an increase in antioxidant capacity [23, 53,59]. At the same time, it has been noted that keeping apples in room temperature, i.e. in conditions similar to consumers' homes, leads to a drop in their polyphenol content and thus also antioxidant capacity [53].

Biochemical changes take place not only when the fruit ripens on the plant but also after harvest, when such changes are triggered by physiological processes which depend on external conditions. During transport and storage of apples, as a result of metabolic processes, respiration and transpiration, changes occur in the content and profile of bioactive compounds, including polyphenols. Those compounds are crucial to the plant's resistance to pathogens, so their concentration rises in response to mechanical damage and microbial infections in fruit after harvest [53]. Harvest and transport trigger a physiological reaction of fruit to stress conditions, which is expressed by, among other things, an increase in polyphenol content and antioxidant activity during distribution and storage.

3.5. Changes Caused by Diseases and Disorders

Postharvest diseases of pome fruit result in substantial economic losses during storage worldwide every year [60]. In the study, the occurrence of fungal diseases, physiological disorders and visible physical damage of fruit flesh depended on cultivar and treatment. The apples were thoroughly selected for the experiment and of top quality, therefore virtually all apples were healthy during a preliminary assessment, and any diseases, disorders or damage were identified only in isolated cases (bitter pit in 'Red Prince' and bull's eye rot in 'Idared') and were most probably due to grading errors. Apples vibrated and assessed on the same day did not have any fungal diseases or physiological disorders; only bruising was identified, probably as an effect of treatment. After 14-day storage, both vibrated and control apples had fungal diseases and physiological disorders, which surely resulted from ripening. Many fungal diseases and physiological disorders appear on fruit as soon as it becomes mature for consumption [4].

The lowest percentage of losses was observed for 'Idared', a cultivar considered as having good storability even in a normal atmosphere cold room [61], i.e. in conditions similar to those applied in this study. The highest percentage of losses caused by diseases and disorders (11.5%) and bruising (8.0%) was identified in 'Gala' apples after 14 days following vibrations, which is quite understandable given the fact that 'Gala' also suffered the biggest drop in firmness in the same period (Table 3). 'Topaz' is sensitive to *Pezizula sp.* infections leading to bull's eye rot [62] – this was also confirmed by our study because 'Topaz' had the highest bull's eye rot incidence rate after 14 days of storage. Jonagold and its mutants are characterized by excellent storability in controlled

atmosphere [63], but are far less storable in normal atmosphere. The same will apply if apples suffer physical damage and are then stored at a relatively high temperature. The diagram shows that the treatment-induced losses in apples amounted to about 16% and were by over 10% higher than the losses identified in fruit not subject to simulated transport vibrations.

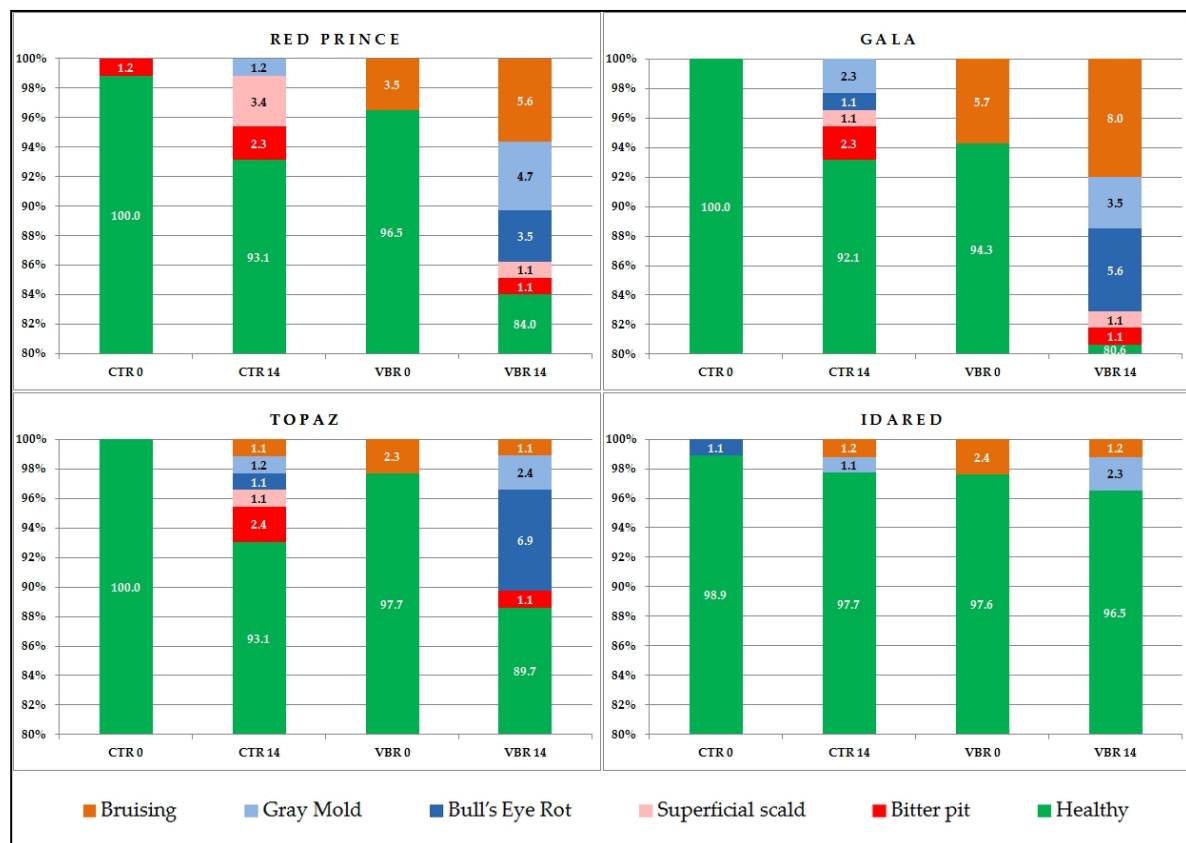


Figure 3. Influence of treatment on the incidence of physiological disorders, fungal diseases and bruising.

1 – Ctr 0, evaluation after harvest, day 0; 2 – Vbr 0, evaluation after vibration, day 0; 3 – Ctr 14, evaluation after 14-day storage; 4 – Vbr 14, evaluation after vibration and 14-day storage.

4. Conclusions

In the study on four apple cultivars, designed under model transport conditions, vibration frequency of 28 Hz resulted in the changes in some fruit parameters which occur also during storage. Those changes varied between the apple cultivars, but they often showed no clear trend or direction or were statistically insignificant. Apples subject to vibrations and storage differed in terms of colour parameters; however, there was no visible pattern of those differences, which was probably due to natural variations in raw material. Flesh colour was relatively stable across the samples, as reflected in small ΔE values. Vibrations caused a decrease in firmness, the biggest for 'Gala' apples and the smallest for 'Red Prince'. At the same time, however, 'Red Prince' showed the biggest change in total soluble solids after vibrations, whereas those changes were either significantly smaller or insignificant in the other cultivars. 'Topaz' and 'Red Prince' were the most resistant to vibrations. Mechanical vibrations and storage led to an increase in the polyphenol content and antioxidant capacity of all studied cultivars, especially 'Topaz' and 'Red Prince'. In sum, 'Red Prince' had the

most stable basic quality parameters and the highest polyphenol content and antioxidant activity – this refers to the control and treated samples before and after storage, whereas ‘Gala’ ranked lowest along these criteria. Mechanical vibrations at a frequency of 28Hz and short-term storage did not reduce the antioxidant activity and thus the health promoting properties of the analysed cultivars. ‘Gala’, besides ‘Red Prince’, also proved to be the most vulnerable to diseases and damage caused by simulated transport, whereas ‘Idared’ best withstood those conditions.

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