Effect of light conditions on physico-chemical properties of pineapple juice with addition of small pineapple pieces during storage

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Summary

Antioxidant status, ascorbic acid concentration, 5-hydroxymethyl-2-furaldehyde (HMF) formation and total colour changes in a pineapple juice with the addition of small pieces of pineapple stored up to 26 weeks at 7 °C at different light conditions (darkness, day light) were characterized. Ascorbic acid losses reached after 26 weeks approximately 23% (day light) and 37% (darkness) of the original concentration. These trends were confirmed also by comparison of kinetic constant values. The impact of light and time on HMF formation was negligible, when maximum concentration of HMF at a level of 2 $mg \cdot l^{-1}$ was found. Slight decrease of total polyphenolic compounds concentration without respect on storing conditions was noticed, with minimum after 8 weeks of storage. Decomposition of sample components and the formation of low-molecular weight phenolic fractions are supposed. Gradual decrease of antioxidant capacity of the juice was also noticed, more apparent for the samples stored at day light with fluctuations caused by samples heterogenity and metal ions content. Juice yellowness was most significantly influenced by the processing and storage under given conditions, change of which was the most intensive during 4th week of the storage. Long-term storage for up to 26 weeks resulted in a significantly increased yellowness for both storage variants.

Keywords

pineapple juice with pieces; storage; electron paramagnetic resonance; ultraviolet–visible spectroscopy; ascorbic acid; 5-hydroxymethyl-2-furaldehyde; polyphenols; colour

Pineapple (*Ananas cosmosus*) belongs to tropical and sub-tropical fruits native to Central and South America, which have, in general, unique flavours, being also excellent sources of vitamins, minerals, phytonutrients and also polyphenols revealing thus also a substantial antioxidant activity. As known, pineapple is one of the lowest-caloric fruits and a valuable source of ascorbic acid. Ripped and matured pineapple flesh contains on average about 85% water, 0.7% citric acid, and saccharides at a level of approximately 14 °Brix, with pH 3.4. Dominant saccharides are saccharose (6.5–7%), glucose (1.7%) and fructose (2.2%). It is also a rich source of various organic and inorganic acids, such as malic, oxalic and phosphoric acid. Fresh pineapple juice contains suspended solids (112–162 $g \cdot kg^{-1}$), fructose (17.2–47.5 $g \cdot l^{-1}$), glucose (12.1–45.2 $g \cdot l^{-1}$) and saccharose (24.5–97.3 $g \cdot l^{-1}$), with total acidity (reported as citric acid) 4.6–12.1 $g \cdot l^{-1}$.

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As mentioned above, pineapple is a rich source of vitamins, especially of ascorbic acid, content of which may vary between 360 mg·kg^{-1} and 560 mg·kg^{-1} of fruit, depending on pineapple variety [1]. Its content may differ even within the same species [2]. Ascorbic acid is the most unstable vitamin, being also extremely heat-sensitive and oxygen-labile nutrient [3], readily oxidized by many non-enzymatic processes. Maintaining its original concentration in fruit juices during their processing or storage is, in general, complicated by many factors contributing to its decomposition (e.g., pH, oxygen presence, metal ions, reducing agents, light conditions, storage temperature, enzyme activity) [4, 5]. In bottled juice, it is mostly decomposed due to the progressive oxidation induced by residual oxygen in the head space, aerobic decomposition, and/or the effect of light [6].

Complex characterization of the properties of fruit juices comprises also the characterization of their antioxidant status, or their changes during the processing and/or storage. Although the information on antioxidant and radical-scavenging properties of many kinds of fruits and vegetables are published, the information on pineapple, or pineapple juice, is limited. Also, the information on composition changes (in polyphenols and/or organic acids) and subsequently, changes in antioxidant activity upon the various treatments, storing conditions or sterilization techniques are practically missing. Only scarce relevant information on antioxidant properties of some fruits and vegetables in shots and their changes upon the storage conditions at different concentrations evaluated either by electron paramagnetic resonance (EPR) or ultraviolet–visible (UV-VIS) spectroscopy are available [7]. In this context, no studies have dealt with the storage quality of thermally pasteurized pineapple juice with small pieces of fruit in terms of their antioxidant activity changes, changes in total polyphenols or colour characteristics induced by storage at different thermal and light conditions, although these changes can be supposed (taking into account quite well described composition of other fruit juices and limited stability of their components) [8–12].

Besides the vitamins and antioxidant content, for the consumer safety it is important to monitor also either the concentration of compounds with potential toxicity, or their formation during the declared shelf life of any food products including fruit juices. One of the most frequently monitored compounds in fruit juice is 5-hydroxymethyl-2-furaldehyde (HMF). In general, it is not naturally present in foodstuffs but it is being formed by the degradation of hexoses undergoing the transformation to fructose, which is prone to form HMF, in particular in the presence of organic acids. Usually, transformation of fructose into HMF is accelerated by elevated temperature and certain type of acids in the solution. The lower is the pK_a value (negative logarithm of the acid dissociation constant) of the present acid and the higher the temperature, the higher is the yield of HMF formed from fructose [13]. By metabolic processes, HMF may be transformed to allylic sulphuric acid esters, which are supposed to be the ultimate metabolite in toxification of the parent compound in vivo, i.e. HMF as non-toxic compound transforms in the presence of specific chemical substances into a toxic compound. Strong mutagenicity of sulphuric acid esters was confirmed [14]. In spite of fact that the toxic effect of HMF on human health has not been confirmed, HMF concentration is already regulated in honey [15] and it is considered also as one of the qualitative parameters for fruit juices [16, 17]; for apple juice, its limit was set to 20 mg·l^{-1} and for orange juice to 10 mg·l^{-1} [18].

New trends in pineapple juice market have led to the development of a new type of product, which is based on the addition of fruit pulp/fibres [19] or enrichment of juice by discrete fruit pieces to satisfy current consumer demands. As clearly follows from the above-presented data, the information on stability of flesh pineapple juice and its stability upon different storage conditions are limited. Moreover, there is hardly any information about the changes in physico-chemical parameters of pineapple juices, in particular of those with addition of small pineapple pieces.

Therefore, the aim of this work was to study the changes in ascorbic acid concentration, total polyphenolic compounds concentration, antioxidant activity and related colour changes and the process of HMF formation in the juice with small pieces of fruit during the storage at 7 °C under the different light conditions, i.e. stored in darkness in tight bottles or under the conditions that simulated the day light exposure. High-performance liquid chromatography (HPLC) was used for HMF determination [17, 20] and also for the study of changes in ascorbic acid concentration. Antioxidant status as well as changes in the concentration of polyphenols or colour changes were monitored by EPR and UV-VIS spectroscopy, respectively. This study is a part of a complex proceeding research focused on the influence of modification of production practices on selected physico-chemical parameters of fresh juices.

MATERIALS AND METHODS

The samples of pineapple juice with small pieces of pineapple fruit were provided by McCarter Company (Dunajská Streda, Slovakia). The company imports both pineapple juice and pineapple pieces (diameter of approx. 3 mm) in frozen state from suppliers operating in Costa Rica. After defrosting, juice and pieces are mixed together and immediately thermally treated by pasteurization up to 95 °C during 20 s. Then, the product is filled aseptically into the polyethylene terephthalate (PET) bottles with the volume of 200 ml. After the production, the samples of the same batch were split into 3 sub-groups, reference samples were delivered immediately into the laboratory and the remaining 2 groups, in a number of bottles corresponding to the chosen experimental setup multiplied by 2 (as due to replications), were stored at 7 °C under the different light conditions. One group was stored in the darkness in a closed box with temperature regulation, while the other was stored under the conditions that simulated the day light exposure. Samples from both these groups were delivered for analyses in regular time intervals during subsequent 26 weeks. Altogether, approx. 200 bottles of pineapple juices underwent the analysis described bellow. The temperature of 7 °C was chosen in order to simulate the storage conditions recommended by juice producer.

Chemicals

All chemicals used in HPLC experiments were of analytical or HPLC purity. For ascorbic acid determination, ascorbic acid (99% purity, Fluka Chemie, Sigma-Aldrich, Steinheim, Germany) was used as external standard to generate a calibration curve. Ortho-phosphoric acid (85% purity, Lachema, Brno, Czech Republic) was used for preparation of mobile phase. For the determination of 5-hydroxymethyl-2-furaldehyde (HMF), HMF standard (99% < purity), phosphoric acid, methanol and acetonitrile – all of gradient purity grade (Sigma-Aldrich), were used. In EPR and UV-VIS experiments, 2,2'-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) salt (ABTS, Polysciences, Warrington, Pennsylvania, USA); 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (Tempol), Folin-Ciocalteu's phenol reagent, gallic acid of analytical grade purity (Sigma Aldrich); $K_2S_2O_8$ (Merck, Darmstadt, Germany), and sodium carbonate (Lachema) were used. In all experiments, water of HPLC grade purity freshly prepared in the laboratory (Rodem 6; Ecotest, Zemné, Slovakia) with an average resistance of 18.5 MΩ was utilized.

Ascorbic acid determination

Sample preparation

The samples were prepared immediately prior to analysis in a way avoiding losses of ascorbic acid due to the light and air oxygen exposure. Just before the analysis, juices were diluted tenfold and twenty-fold for two concentration levels, and subsequently filtered through a 0.45 μm syringe filter.

Experimental setup

Ascorbic acid concentration was determined by a HPLC-DAD chromatograph Agilent 1100 (Agilent Technologies, Waldbron, Germany) equipped with quaternary pump, degasser, column thermostat and autosampler. The analytical column Zorbax SB-C18, 250 mm × 4.6 mm with the sorbent particle size 5 μm, and the precolumn Zorbax SB-C18, 12.5 mm × 4.6 mm with the same particle size (Agilent Technologies) were used. The mobile phase of 0.01 mol·l^{-1} phosphoric acid in deionized water was pumped at a flow rate of 0.7 ml·min^{-1}. The sample was injected in 50 μl volume. The analysis was performed at ambient temperature. For the detection, diode array detector set at 240 nm was used. The identification was based on the retention time evaluation and on the comparison of UV-spectra (spectral range, 210–360 nm) of the respective sample and pure standard. For the purposes of calibration, the linear regression diagnostics was carried out by the Excel software (Microsoft Office XP, Microsoft, Redmond, Washington, USA).

Method validation

For the validation of the described method, detection and quantification limit (1.4 mg·l^{-1} and 1.8 mg·l^{-1}, respectively), linearity range (from 1.80 mg·l^{-1} to 60 mg·l^{-1}), precision, and accuracy were assessed. The precision was evaluated as intra-day repeatability (n = 8) using control standard solution of ascorbic acid (concentration 60 mg·l^{-1} and 3 mg·l^{-1}). The obtained relative standard deviation (*RSD*) was 2.7% and 36.0%, respectively. Accuracy of the method was confirmed by the addition of a known amount of ascorbic acid to a juice sample that resulted in 84–90% recovery. In general, the accuracy tended to be worsening when the lower concentrations of ascorbic acid in pineapple juice were analysed. The calibration measurements were carried out with standard solutions of ascorbic acid, which covered six points linear range (from 0.725 mg·l^{-1} to 60.0 mg·l^{-1}), providing the correlation coefficient of R^2 = 0.99978.

Tab. 1. HPLC gradient separation conditions (mobile phase composition) used for HMF determination.

Time [min]	Volume of component in mobile phase [%]		
	Methanol	Water (0.01 mol·l^{-1} H_3PO_4)	Acetonitrile
0	0	100	0
1.5	2	95	3
2.1	2	95	3
3	8	86	6
11	8	86	6
11.5	94	0	6
20	94	0	6
20.1	2	95	3
30	2	95	3

HMF determination

Sample preparation

Juice sample was filtered through the folded filtration paper (diameter, 185 mm; Whatman Grade 604; Sigma Aldrich) and subsequently utilized.

Experimental setup

The instrument HPLC-PDA Agilent 1200 (Agilent Technologies) equipped with quaternary gradient pump at a flow rate of 0.8 ml·min^{-1}, autosampler and photo-diode detector set to 280 nm was used for separation and quantification of HMF in pineapple juice. Separation was performed on C18 SB column (Agilent Technologies), particle size 5 µm, 250 × 4.6 mm. Triple gradient elution was used, as indicated in Tab. 1.

HMF standard for external calibration was diluted in water and stored at 4 °C. Limit of detection of the applied procedure was 0.4 mg·l^{-1} and limit of quantification was 1.2 mg·l^{-1}. Uncertainty at a concentration level of 1.61 mg·l^{-1} reached ± 0.3 mg·l^{-1} [21].

EPR and UV-VIS measurements

Sample preparation

Immediately prior to the experiments, solid matters of pineapple juices were separated using a laboratory centrifuge (SciQuip, London, United Kingdom) at 10000 ×*g* at 5 °C (EPR measurements) or 20 °C (UV-VIS measurements) during 10 min. Supernatants were stored at 7 °C in darkness between the experiments.

The entire EPR experiments were performed in duplicates, using a portable X-band EPR spectrometer e-scan (Bruker Biospin, Karlsruhe, Germany) with accessory. The ability of pineapple juice samples to terminate $ABTS^{\bullet+}$ and Tempol free radicals was examined. The spectra were evaluated and $ABTS^{\bullet+}$ radical-scavenging activities were expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents ($TEAC_{ABTS^{\bullet+}}$) as previously described by Polovka et al. [22]. In case of Tempol assay, the results were recalculated to ascorbic acid equivalents (AAE), using the calibration curve of the standard solutions of ascorbic acid, and expressed in millimoles of ascorbic acid per litre.

All the UV-VIS experiments were carried out using UV-VIS-NIR spectrophotometer Shimadzu 3600 (Shimadzu, Tokyo, Japan) with accessory. The measurements were performed in duplicates, using the following setup: spectral range, 380 nm to 780 nm; sampling interval, 2 nm; slit width, 0.1 nm; in the quartz cell 100-QS-Suprasil (Hellma, Sigma Aldrich; optical path, 1 cm) against distilled water as a blank. Total polyphenolic compounds (TPC) concentration was estimated by Folin-Ciocalteu modified method, using a standard solution of gallic acid for calibration curve construction. Results were expressed as gallic acid equivalent (GAE) per litre [22].

Colour changes evaluation

Colour model CIE $L^*a^*b^*$ determined by CIE (Commission Internationale de l'Eclairage) classifies colour of any object in three dimensions; L^*, indicating lightness from 0 (black) to 100 (white); coordinates a^* and b^* represent greenness ($-a^*$), redness ($+a^*$), blueness ($-b^*$) and yellowness ($+b^*$), respectively. Their calculations were performed directly from the measured UV-VIS spectra by means of ColourLite Panorama Shimadzu software (LabCognition Analytical Software, Köln, Germany) using the D65 day light illuminant and 10° standard observer angle.

CIE $L^*a^*b^*$ values were used to calculate total colour difference according to the formula:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

expressing the magnitude of difference between the initial (reference) and stored pineapple juice samples [23]. The obtained colour differences were subsequently classified analytically as not noticeable (0 to 0.5), slightly noticeable (0.5 to 1.5), noticeable (1.5 to 3.0), well visible (3.0 to 6.0), and great (6.0 to 12.0) [24].

Statistical analysis

Measurements were performed in two replications with 2 repetitions each (thus $n = 2$) if not specified otherwise. The data obtained by individual methods/assays were statistically compared

on the basis of means, taking into account the values of standard deviations. Scientist software (MicroMath, St. Louis, Missouri, USA) was utilized for kinetic parameters calculation of ascorbic acid degradation.

RESULTS AND DISCUSSION

Ascorbic acid

In order to evaluate the changes in ascorbic acid concentration resulting from different light conditions of storage at 7 °C, pineapple juice was analysed in approx. two-week time intervals. As expected, the presence or absence of the light represented the principal factor significantly affecting the stability of ascorbic acid. Fig. 1 shows a gradual decrease of ascorbic acid concentration during the monitored time of storage. In accordance with expectations, concentration of ascorbic acid gradually decreased with the time of storage in dependence on light conditions. It is apparent that under the given experimental conditions, juice stored in darkness exhibited lower deviations in ascorbic acid concentration levels within the tested juice batch. Also, the dynamics of its decomposition was lower than that of samples exposed to the day light. Such behaviour was previously observed also for other types of fruit juices [4].

As also follows from the results (taking into account their trend lines), at the end of the monitored period, i.e., after 26 weeks, ascorbic acid concentration fell to 20% (day light) and 25% (darkness) of its original concentration.

It should be noted here, that when the decrease of ascorbic acid concentration at the end of juices shelf life declared by the producer (16 weeks) and at the end of the storage period (26 weeks) were contemplated, the respective differences following from different storage conditions reached approx. 50% and 59% for samples stored at day light and in darkness, respectively.

The observed decrease in ascorbic acid concentrations in juice stored at different light conditions were evaluated also by fitting the experimental data to the model of 1st order kinetic equation in the integral shape

$$c_t = c_{t=0} \exp(-kt) \qquad (2)$$

where $c_{t=0}$ and c_t represent an original concentration of ascorbic acid ($t = 0$) and the concentration at chosen time t of storage, respectively. k is the formal 1st-order rate constant of ascorbic acid degradation expressed as reciprocal day.

The calculated values of 1st-order rate constant of ascorbic acid degradation for juice samples stored in darkness reached (0.0077 ± 0.0006) d^{-1} and for those stored at day light (0.0091 ± 0.0009) d^{-1}. When compared on absolute basis, it is apparent that the difference between the respective rate constants achieved approx. 16%.

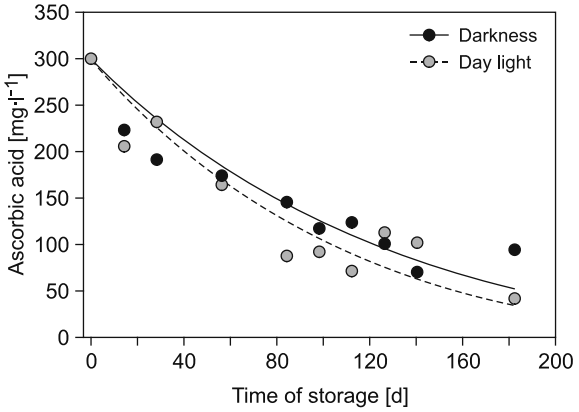

Fig. 1. Effect of storage time on ascorbic acid concentration in pineapple juice stored in darkness and at day light conditions.

Lines represent the fit of experimental values to the model of 1st-order kinetics.

The findings are in good agreement with some previously published data. Shaw et al. [25] assumed for citrus juice that, if the retention of their initial ascorbic acid concentration decreased to approx. 50%, it might indicate the end of their shelf life. In this context, Chia et al. [26] proved that, in pineapple juice pasteurized at 80 °C for 10 min and then stored at a temperature of 4 °C for 13 weeks, ascorbic acid concentration fell down to 50% after 8 weeks. As follows from the presented results, the initial concentration of ascorbic acid in pineapple juice before storage, i.e. (298 ± 14) mg·l^{-1} (100%), decreased to 50% after 10 and 13 weeks of storage at day light and in darkness, respectively.

The effect of juice thermal processing on ascorbic acid concentration is widely known. The rate of its decomposition is also influenced by the form, into which the respective fruit is processed. Uckiah et al. [27] pointed out that, after thermal processing of three pineapple products (juice, jam and sorbet) and their storage at 8 °C within nine days, the highest decomposition of ascorbic acid was noticed in pineapple juice, with a loss of 38.5% immediately after the processing and with continuous losses of approx. 0.6 mg per day during the storage period.

Tab. 2. Effects of storage and light conditions on the formation of HMF in pineapple juice with small pineapple pieces.

Time of storage [week]		0	2	4	8	12	14	16	18	20	26
HMF concentration [mg·l^{-1}]	Darkness	0.9	0.6	1.1	1.6*	1.2	1.5	2.1	1.1	2.3	2.0
	Day light	0.9	0.8	1.6*	1.6*	1.3	1.4	1.8	1.1	2.4	1.9

* – uncertainty at a concentration level of 1.61 mg·l^{-1} was ± 0.3 mg·l^{-1}.

HMF formation

Concentration of HMF was determined in pineapple juice stored under the described conditions, and its formation was found to be influenced mostly by the presence of hexoses, environmental conditions and the concentration of organic acids and/or final acidity. The determined concentrations of HMF in pineapple juice are presented in Tab. 2.

Very low concentration of HMF in the beginning of experiments confirmed just reasonable pasteurization process, which was also reflected by a very slight increase in the concentration of HMF in juice stored at 7 °C during the long time period of up to 26 weeks. The impact of light at this temperature of storage on HMF formation could be neglected as statistically non-significant (p = 0.8663). In view of the high saccharides content of pineapple juice, as well as the above mentioned crucial impact of organic acids on HMF formation, the results confirmed correct technological procedures including pasteurization. Storage temperature could not be underestimated as well, as the elevated temperature could influence transformation of hexoses to fructose or support Maillard reactions progress leading to the generation of acids with lower pK_a values. Taking into account all these factors, the chosen storage temperature of 7 °C and also the exposure of samples to day light/darkness were safe from the point of view of HMF formation in pineapple juice with small pineapple pieces.

Antioxidant characteristics monitored by EPR and UV-VIS

Results of all experimental characteristics determined by EPR and UV-VIS spectroscopy and their changes in pineapple juice stored at constant temperature and different light conditions are summarized in Tab. 3.

Regarding the changes in TPC concentration, it is obvious that without respect to storage conditions, their concentration in juice slightly decreased during storage until reaching a minimum after 8 weeks in both cases. Subsequently, their concentration increased and reached values almost as high as in the respective reference samples. The changes in TPC concentration were, in accordance with expectations, much more significant for samples stored under day light. Not enough information is available on pineapple juice to properly discuss all the observed phenomena. For orange juice, a gradual decay in TPC concentration of fresh juice resulting from storage, accelerated by the increased temperature, was proposed [28]. On the basis of our results, we can only suppose that, as a result of storage, decomposition occured, during which the low-molecular-weight

Tab. 3. Antioxidant characteristics of pineapple juice with small pineapple pieces stored at constant temperature (T = 7 °C) and different light conditions, determined by UV-VIS and EPR spectroscopy.

Time of storage [week]	TPC [mg·l^{-1}]		$TEAC_{ABTS}$$^{\bullet+}$ [mmol·l^{-1}]		AAE [mmol·l^{-1}]	
	Darkness	Day light	Darkness	Day light	Darkness	Day light
0	731.5 ± 5.2	731.5 ± 5.2	2.6 ± 0.0	2.6 ± 0.0	0.16 ± 0.01	0.16 ± 0.01
2	718.0 ± 6.6	731.4 ± 6.3	2.3 ± 0.0	2.1 ± 0.1	0.17 ± 0.02	0.15 ± 0.01
4	692.1 ± 3.3	655.9 ± 4.0	1.9 ± 0.1	2.2 ± 0.0	0.16 ± 0.01	0.15 ± 0.01
8	613.8 ± 5.0	604.1 ± 4.7	2.0 ± 0.2	1.8 ± 0.0	0.17 ± 0.01	0.16 ± 0.01
11	641.8 ± 8.1	612.6 ± 0.7	1.6 ± 0.1	1.9 ± 0.0	0.13 ± 0.01	0.13 ± 0.01
14	696.7 ± 19.8	679.7 ± 11.5	2.0 ± 0.0	2.3 ± 0.1	0.13 ± 0.01	0.15 ± 0.00
26	692.2 ± 5.7	714.9 ± 3.6	1.9 ± 0.0	1.5 ± 0.0	0.12 ± 0.01	0.08 ± 0.00

Values are presented as mean ± standard deviation (n = 2).
TPC – total polyphenolic compounds, $TEAC_{ABTS}$$^{\bullet+}$ – Trolox equivalents, AAE – ascorbic acid equivalents.

phenolic compounds were formed. Another aspect that should be taken into account is a known non-specificity of the Folin-Ciocalteu test, as the reactivity of Folin-Ciocalteu reagent components is negatively influenced by the presence of thiols, reducing saccharides and other compounds [29].

Results of $ABTS^{\bullet+}$ tests are depicted on Fig. 2. It is obvious that upon the time of the isothermal storage, a gradual worsening of antioxidant capacity of juice occured. In accordance with expectations, the decay of $TEAC_{ABTS^{\bullet+}}$ values was much more evident in juice stored under the day light, although some fluctuations in values are obvious, probably caused by juice inhomogeneity resulting from the presence of fruit pieces. The observed decrease of antioxidant capacity of the investigated juice as well as effect of light conditions on the decrease was most probably influenced also by the presence of some trace metals, e.g. Mn, Fe and Cu, determined by microwave plasma-atomic emission spectrometer (data not presented). These metals are either present naturally in fruit juices or their presence results from the processing (cutting, pressurization) and can actively influence the stability of juices via their role in some pro-oxidant processes, e.g. well known Haber-Weiss and Fenton (or Fenton-like) reactions [30, 31]. Even their active role in photo-oxidation processes should not be underestimated.

Tempol assay was selected because of the known sensitive reaction/response of this stable free radical to the presence of, in particular, ascorbic acid and also to other organic acids with a similar redox potential [32–34]. However, in spite of the expectations and losses of ascorbic acid concentration determined by HPLC, the effect of storage conditions on Tempol concentration was quite ambiguous, as in both cases, slight, statistically non-significant decrease upon the time of storage was noticed. Only at the end of the storage period, a significant, 50% and 23% decrease in AAE was confirmed for samples stored under day light conditions and in darkness, respectively. The observed effect can, on the other hand, indicate the existence of dynamic processes in juice, leading to the formation/elimination of compounds/intermediates with anti- and/or pro-oxidant properties. This assumption follows also from the above discussed trends in TPC concentration. As is also already indicated, the homogeneity and composition of the juice could play a key role in changes of the investigated parameters.

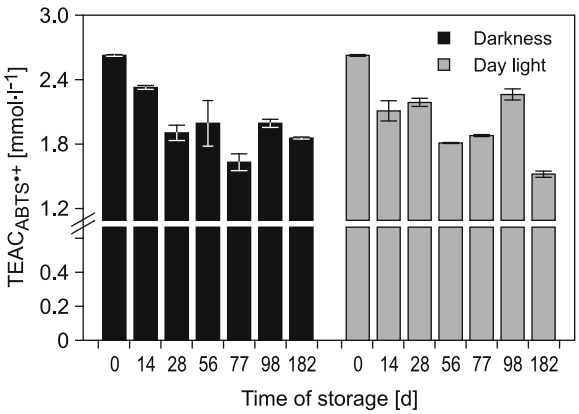

Fig. 2. Average $ABTS^{\bullet+}$ radical-scavenging ability of pineapple juice with pineapple pieces stored at constant temperature (T = 7 °C) and different light conditions characterized by EPR spectroscopy expressed as Trolox-equivalent antioxidant capacity.

Values are presented as mean ± standard deviation (n = 2).

Colour characteristics

Colour of fruit and vegetable juices has been traditionally used as an indicator of their physiological state, organoleptic and nutritional quality during the preservation/processing treatment and subsequent storage, as it is connected with the perception of some characteristics that seem to be representative of the quality of processed juices [9]. Some authors suggest that the change in pineapple juice colour is influenced by both non-enzymatic browning and pigment destruction, and the phenomena that occur during heating and storage of pineapple juice can be described by a combined model [35]. As indicated in the experimental part, pineapple juice under study was thermally processed, packaged and stored at different light conditions. All these operations could partially affect colour characteristics of the final product.

In order to quantify the colour changes in pineapple juices caused either by the preparation or their subsequent storage at a temperature of 7 °C under the different light conditions, total colour difference (ΔE) was evaluated from trichromatic CIE $L^*a^*b^*$ values. The obtained ΔE values as well as trichromatic parameters of pineapple juices are summarized in Tab. 4.

Relatively non-significant changes in L^* values during the storage of pasteurized pineapple juice containing the small pieces of pineapple are apparent from the presented data. In opposite to our findings, the L^* value of thermally pasteurized conventional pineapple or other types of fruit juices decreased significantly upon the storage period [26]. Slight increase of this value was observed in the pasteurized orange juice as a result of partial precipitation of the particles suspended in juice [36]. Juice matrix with addition of small

Tab. 4. Effects of storage of pineapple juice with small pineapple pieces at constant temperature (T = 7 °C) and different light conditions on changes in CIE colour parameters and of colour difference.

Time of storage [week]	Storage conditions								ΔE (darkness–day light)
	Darkness				Day light				
	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE	
0	97.7 ± 0.05	0.05 ± 0.02	4.78 ± 0.32	–	97.7 ± 0.05	0.05 ± 0.02	4.78 ± 0.34	–	–
2	97.8 ± 0.06	-0.99 ± 0.04	5.39 ± 0.44	1.08	97.8 ± 0.04	-0.49 ± 0.03	4.85 ± 0.37	1.01	-0.07
4	97.7 ± 0.05	-1.98 ± 0.03	4.62 ± 0.37	0.41	97.8 ± 0.06	2.49 ± 0.02	3.48 ± 0.24	1.37	+0.96
8	97.8 ± 0.06	-1.49 ± 0.05	5.57 ± 0.50	1.15	97.7 ± 0.04	-0.49 ± 0.04	6.37 ± 0.41	0.97	–0.18
11	97.7 ± 0.03	-1.14 ± 0.001	6.20 ± 0.28	0.89	97.6 ± 0.02	-0.49 ± 0.02	6.74 ± 0.18	1.56	+0.67
14	97.7 ± 0.04	-0.25 ± 0.02	8.97 ± 0.18	2.54	97.4 ± 0.03	-1.49 ± 0.03	11.6 ± 0.27	5.09	+2.55
16	97.5 ± 0.06	0.49 ± 0.04	10.1 ± 0.25	3.78	97.6 ± 0.05	-0.19 ± 0.02	7.66 ± 0.19	2.01	–1.77
17	97.7 ± 0.04	-0.04 ± 0.02	9.07 ± 0.47	2.60	97.5 ± 0.03	1.34 ± 0.04	10.8 ± 0.22	4.8	+1.58
19	97.8 ± 0.06	-1.39 ± 0.03	8.41 ± 0.33	2.43	97.4 ± 0.04	-1.44 ± 0.001	11.9 ± 0.17	5.27	+1.84
26	97.7 ± 0.05	-0.99 ± 0.04	8.74 ± 0.17	2.91	97.7 ± 0.04	-1.49 ± 0.002	9.57 ± 0.20	4.92	+2.01

Values are presented as mean ± standard deviation (n = 2).

pieces of fruit showed a positive effect in maintaining the lightness of pineapple juices during the storage.

The values of a^* and b^*, or their combined form (parameter chroma [26]), are often reported as criteria for the characterization of colour changes in fruit juices. As follows from the previously published papers, chroma parameter revealed the decreasing tendency for fruit juices treated by thermal treatment or stored at defined conditions [26]. In pineapple juice, a slight decrease of a^* value during the storage was observed compared to the reference sample. Similar shift of a^* value to slightly negative direction was observed by Lee and Coates [24] and Cserhalmi et al. [36] after thermal treatment of orange juice.

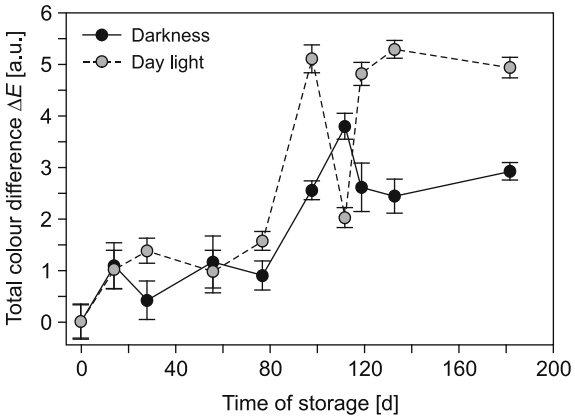

Fig. 3. Dependence of average total colour difference (ΔE) of pineapple juice with small pieces of pineapple on time of storage at constant temperature (T = 7 °C) and different light conditions.

Values are presented as mean ± standard deviation (n = 2).

Most important changes in colour characteristics of pineapple juice induced by their thermal processing and/or storage were found for the yellowness parameter b^*. In good agreement with observations of Chia et al. [26], a slight decrease of this value was observed during the 4th week of storage under both light conditions (Tab. 4), but the continuous long-term storage up to 26 weeks after the production led to a significant increase of b^* for both storage conditions. Significantly higher values of yellowness were found for pineapple juice samples stored in the day light conditions in comparison to these stored in darkness. The described changes of b^* significantly affected the values of total colour differences ΔE so that during storage, its gradual increase was found for both storage conditions, although some alterations appeared during the storage interval, as is also obvious from the data depicted on Fig. 3. As expected, the increase in ΔE is more intensive for juices stored under the conditions of day light. Although the approach is not fully appropriate in view of the variability of the evaluated ΔE, calculation of total colour differences using the linear regression data or taking into calculation only the colour characteristics determined for pineapple juice immediately after its preparation and at the end of the storage at chosen temperature, resulted in approx. 60% difference in this parameter between the samples stored in darkness and at day light.

According to classification of colour changes used by Cserhalmi et al. [24], both pasteurization of pineapple juice with small pineap-

ple pieces as well as the next short-term storage during 6 weeks caused only slightly noticeable changes ($\Delta E \sim 1.01$). On the other hand, long term storage (up to 26 weeks) induced noticeable colour changes in the juice stored in darkness (ΔE from 0.4 to 3.8), while for the juice stored at day light, well visible differences with ΔE above 6 were observed. These values are practically comparable to those reported previously for orange juice pasteurized and stored for 6 weeks at 2 °C ($\Delta E = 11.35$), canned strawberries after 24 weeks of storage at ambient conditions (ΔE from 13 to 20) or for orange juice gently treated by high-intensity pulsed electric fields technology and stored for 6 weeks at 2 °C ($\Delta E = 9.7$) [10].

CONCLUSION

Complex characterization of pineapple juices with addition of small pieces of pineapple fruit from the point of the losses of ascorbic acid, changes in antioxidant status and colour perception as well as the formation of HMF resulting from the storage at constant temperature and different light conditions upon 26 weeks was performed. This information is rather new as only limited information on the properties of fresh juice was available and information regarding the juice with addition of small fruit pieces was fully missing. Original results obtained clearly show that sample composition, processing but also storage conditions have significant effects on the juice shelf life as well as on its quality and other properties important for decision-making process of their potential consumers.

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