CHARACTERISATION OF TOMATO JUICE AND DIFFERENT TOMATO-BASED JUICE BLENDS FORTIFIED WITH ISOMERISED LYCOPENE EXTRACT

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Juices and beverages with bioactive compounds are consumed for their nutritive and health benefits. Beverages fortified with various functional ingredients are an important product category within the functional food segment. In this study tomato juice with various proportions of apple, carrot and sweet corn juice together with isomerised lycopene extract were used for formulation of functional beverages. Chemical composition and physicochemical properties of prepared juice blends was evaluated and a sensory analysis was conducted in order to identify the most acceptable blends. The addition of concentrated isomerised lycopene extract with β-carotene to tomato juice as well as juice blends significantly increased the levels of lycopene and especially the levels of *cis*-lycopene in the final products. The content of total lycopene in the juice blends varied from 16.21 mg/100 g to 25.65 mg/100 g, whereas the content of *cis*-lycopene – from 9.16 to 14.46 mg/100 g. The juice blends containing apple juice had the lowest pH and the highest titratable acidity. Higher percentage of apple, sweet corn or carrot juice in the blends resulted in higher contents of TSS in the functional beverage. The addition of apple, carrot, and sweet corn juice significantly changed the initial color of the tomato juice. The lowest colour difference (ΔE) values had tomato-carrot juice blends (6.8–7.3), whereas the highest ΔE had tomato juice with 25 and 35 % of sweet corn juice (10.6 and 14.3, respectively). Sensory evaluation revealed that the most acceptable taste had tomato-apple juice blend with 35 % apple juice and tomato-carrot juice blend with 40 % carrot juice.

Keywords: functional foods, juice blends, lycopene extract, sensory properties, tomato.

INTRODUCTION

There has been an explosion of consumer interest in the active role of food in the well-being and life prolongation, as well as in the prevention of initiation, promotion, and development of chronic, age-related diseases (Granato et al., 2010). Functional food is "any fresh or processed food that is claimed to have a health promoting and/or disease-preventing property beyond the basic nutritional function of supplying traditional nutrients". Functional foods may help prevent disease, reduce the risk of developing disease, or enhance health (de Pinho Ferreira and Lima Reis, 2012). Non-alcoholic beverages fortified with vitamins or other functional ingredients are an important product category within the functional food segment.

Vegetable juices are attracting more attention due to the phytochemical value of many vegetables. Tomato products are increasing in popularity due to the scientific findings that lycopene, the major carotenoid pigment responsible for the red colour in tomatoes, has anti-inflammatory and anti-carcinogenic properties. Unlike β-carotene and other closely related carotenoids, lycopene has no pro-vitamin A activity (Horvitz et al., 2004). Interestingly, the bioavailability of lycopene is increased in processed, thermally heated tomato products (Horvitz et al., 2004; Bohn et al., 2013).

The absorption of carotenoids, also lycopene, from fruits and vegetables in the human tract hamper their specific localisation in plants and their lipophilic nature. A key limitation of lycopene use is that its intestinal absorption is low. Carotenoids are attached to cellular components and surrounded by organelle membranes, cell membrane and cell wall

(Palmero et al., 2013). The composition and structure of the food have an impact on the bioavailability of lycopene and may affect the release of lycopene from the tomato tissue matrix. Lycopene absorption in human intestinal cells depends on administered matrix and on food preparation and processing conditions. It was reported that lycopene from tomato oleoresin capsules and tomato juice (processed tomatoes) was better absorbed from the intestine than lycopene from raw tomatoes (Böhm and Bitsch, 1999). Mechanical and thermal treatment is quite effective in improving lycopene bioaccessibility by breaking down food matrix. Moreover, during exposure to light, heat and/or oxygen lycopene can undergo isomerisation and degradation. In nature, lycopene is almost exclusively found in the *trans*-form. However, many studies have indicated that the bioavailability of the *cis*-isomer of lycopene is greater than the all-*trans*-isomers (Horvitz et al., 2004). In human plasma, lycopene is an isomeric mixture containing more than 60 % of the total lycopene as *cis*-isomers (Qiu et al., 2006). Therefore it may be assumed that lycopene extract comprising higher percentage of *cis*-isomers would be more readily absorbed by the human body and that such extract, if added to the product, would improve its functionality.

Functional foods generate one of the most promising and dynamically developing segments of food industry therefore, in this study, chemical and organoleptic assessment of tomato juice and different tomato-based juice blends fortified with isomerised lycopene extract was carried out in order to determine the most acceptable blend and the levels of lycopene isomers and other phytochemicals in such products.

MATERIALS AND METHODS

Preparation of isomerised lycopene extract. Isomerised lycopene extract was prepared with supercritical carbon dioxide SFT–150 (Supercritical Fluid Technologies, USA) from dried tomato by-products left after juice preparation. The flow rate of CO_2 in the system was controlled manually by the micrometering valve (back-pressure regulator). The volume of CO_2 consumed was measured by a ball float rotameter and a digital mass flow meter in standard liters per minute (SL/min) at standard state (P_{CO_2}=100 kPa, T_{CO_2}=20 °C, ρ_{CO_2}=0.0018 g/mL). The extracts were collected in glass bottles. The conditions for extraction were set as follows: extraction time 240 min, pressure 55 MPa, extraction temperature 40 °C. The process consisted of static (10 min) and dynamic extraction steps. Static extraction time was included in total extraction time.

Juice preparation and its enrichment with isomerised lycopene extract. Tomato, apple, carrot and sweet corn juice was prepared in Biochemistry and Technology laboratory of Institute of Horticulture (Research Centre for Agriculture and Forestry, Babtai, Lithuania). Prior to processing, tomatoes ('Admiro F1'), apples ('Auksis'), carrots ('Garduolės') and sweet corn ('Overland') were thoroughly washed and prepared for juicing. Juice was extracted using Hurom Juicer (Hurom Group Corporation). Then three different tomato-apple juice blends 85:15, 75:25 and 65:35, v/v (TApl15 %, TApl25 %, TApl35 %, respectively), three different tomato-carrot juice blends 60:40, 50:50 and 40:60, v/v (TCar40 %, TCar50 %, TCar60 %, respectively), and three different tomato-sweet corn juice blends 85:15, 75:25 and 65:35, v/v (TCor15 %, TCor25 %, TCor35 %, respectively) were prepared. Before thermal treatment 3 %, by weight, of concentrated isomerised lycopene extract with β-carotene was added to all the juice blends prepared. Juices with lycopene isomerised extract were homogenized and pasteurized (10 min at 95 ± 5 °C), then poured to 350 ml glass jars. The pasteurized juice were sealed with metal covers and kept in the dark room at ambient temperatures until analysis (approximately 2 weeks).

High-performance liquid chromatography (HPLC) analysis of lycopene isomers and β-carotene. The stability of all-*trans*-lycopene and β-carotene in the juice extracts (extracted with hexane with 1 % of BHT) was analysed by the slightly modified reversed phase HPLC method of Ishida, Ma and Chan (2001) connected to a detector Waters 2489 UV/Vis ("Water Corporation", USA). Detection of lycopene and β-carotene was at 473 nm. The mobile phase consisted of methanol and methyl-*tert*-butyl ether at a flow rate of 0.650 ml min^{-1}. The injection volume was 10 µl. The column temperature was 22°C. The samples were filtered through a 0.45 mm polyvinylidene fluoride (PVDF) syringe filter ("Millipore", USA) before injection. To quantify lycopene in the extract samples, a calibration curve was generated using an authentic all-*trans*-lycopene standard. Levels of *cis*-lycopene isomers are given in all-*trans*-lycopene equivalents.

Colour measurements. Colour change was measured by a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory Inc., USA) (Urbonaviciene et al., 2012). The apparatus (45/0 geometry, illuminant D65, 10 observer) was calibrated with a standard tile (X = 81.3, Y = 86.2 and Z = 92.7). A cylindrical glass cell filled with 3 ml of sample was placed on the top of the light source (2.5 cm opening) and covered with a white plate. Inclusion of air bubbles was prevented. The recorder X, Y and Z tristimulus values were converted to CIE L*, a* and b* colour values. Regarding light reflection, the L*, a* and b* parameters (lightness, redness and yellowness indices, respectively, according to CIE L*a*b* scale) were measured, and chroma (C) (Equation 1), hue angle (h°) (Equation 2) and the total colour difference (ΔE) (Equation 3) were calculated:

$$C = (a^{*2} + b^{*2})^{1/2} \qquad\qquad (1)$$

$$h^{\circ} = \arctan(\frac{b^*}{a^*}) \qquad\qquad (2)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad\qquad (3)$$

The L*, C, a* and b* volumes were measured in NBS units, and hue angle was measured in degrees from 0 to 360°. A NBS unit is a unit of the USA National Standard Bureau, and it corresponds to one threshold of colour distinction power, i.e. the least distinction in colour that the trained human eye can notice (McGuire, 1992). The colour parameters were processed with the software Universal V.4-10 program. Colour measurements were performed in triplicate.

Electronic tongue system and sampling procedure. An E-tongue (α-Astree, Alpha MOS Company, France) was employed to classify and characterize the juices. This instrument mainly consists of seven potentiometer chemical sensors (ZZ, BA, BB, CA, GA, HA, and JB), a reference electrode of Ag/AgCl, data acquisition system, and basic data analysis software. The sensitivity of the seven chemical-sensors is different from that of the five tastes (sourness, saltiness, sweetness, bitterness, and savory). Experiments were carried out with filtrated juice to avoid the influence caused by solid particles. The amount of each juice sample was 80 mL to ensure that the sensors could be fully immersed in the liquid. The measurement time was set to 120 s for each sample, which was long enough for the sensors to reach stable signal values, and the sensors were rinsed for 10 s using deionized water to minimize and correct the drift of sensors. Measurements were carried out at 20 ± 3 °C.

The ascorbic acid (vitamin C) content was determined by the titrimetric method using a 2,6-dichlorophenolindophenol sodium salt solution (AOAC, 1990); chloroform was used for intensely coloured extracts.

The total soluble solids (TSS) were determined using a digital refractometer (ATAGO PR-32, Atago Co., Ltd., Tokyo, Japan).

Titratable acidity (TA) was determined by titration with 0.1 N NaOH to a pH 8.2 end point and expressed in percents of citric acid equivalents.

The *pH* was measured using an inoLab Level 1 pH meter with SenTix 81 (WTW) electrode.

Sensory assessment. Regular consumers (32 people) of fruit and/or vegetable juices participated in consumer evaluation session. All consumers were aged between 20 and 65 years of age and it was an approximately equal split of males to females. Samples were labeled with 3-digit blinding codes and presented in a balanced order. Five-point hedonic scale was used for evaluation of the samples (5–the highest acceptability, 1–the lowest acceptability). In addition the panellists were asked to indicate the defect, which they had observed in the sample if 3 points or lower score was given.

Statistical analysis. The mean values and standard deviations of the experimental data were calculated using SPSS 20 Software (SPSS Inc., Chicago, USA). Mean values were further compared, using Turkey's test, and differences were considered to be statistically significant when $p \leq 0.05$.

RESULTS

Chemical analysis results revealed that juice blends containing apple juice (TApl15 %, TApl25 %, TApl35 %) had lower pH and higher titratable acidity compared with control and tomato juice blends with carrots or sweet corn (TCar and TCor). Since sweet corn and carrots are generally low in acids the tomato-sweet corn and tomato-carrot juice blends had higher pH values and lower titratable acidity. The pH value of juice with the highest percentage of sweet corn (TCor35 %) was above 4.5, therefore such juice would require more severe thermal treatment regime which is used for the processing of low acid foods.

Juice blends with apple, carrot and sweet corn had higher TSS contents compared to control (tomato juice). Compared to the control juice the TSS content in TApl was by 22–59 % higher, in TCar – by 9–19 % and in TCor – by 8–11 % higher. Higher percentage of apple, sweet corn or carrot juice in the blends resulted in higher contents of TSS in the product. The content of ascorbic acid in control juice was 8 mg/100 mL, whereas its content in juice blends in most cases was slightly lower. The lowest content of ascorbic acid (lower by 10–13 %) was found in tomato-carrot juice blends.

The initial lycopene content in tomato juice was 7.94 mg/100 g, where *cis*-isomers constituted only 11.2 % of the total lycopene concentration. The initial content of β-carotene in tomato juice without extract additive was 0.96 mg/100 g. The addition of isomerised lycopene extract to tomato juice as well as juice blends significantly increased the levels of lycopene and especially the levels of *cis*-lycopene, which is more readily absorbed than all-*trans*-lycopene, in the final products Table 1.

Table 1. The total lycopene and its isomers content in tomato and juice blends with lycopene isomerised extract additive

Samples	Concentration mg/100g in juice			
	β–CAR	*trans*-LYC	Total *cis*-LYC	Total LYC
Control (without additive)	0.96 ± 0.030b,c	7.57 ± 0.125b	0.37 ± 0.031a	7.94 ± 0.024a
TApl15 %	0.93 ± 0.106h	9.67 ± 0.123e	12.67 ± 0.040h	22.34 ± 0.026g
TApl25 %	0.74 ± 0.135g	8.61 ± 0.096d	11.27 ± 0.047f	19.88 ± 0.051e
TApl35 %	0.63 ± 0.127f	7.04 ± 0.077a	9.16 ± 0.057b	16.21 ± 0.036b
TCar40 %	4.67 ± 0.014e	9.68 ± 0.075e	12.37 ± 0.020g	22.05 ± 0.237f
TCar50 %	4.22 ± 0.015d	8.23 ± 0.074c	10.96 ± 0.015e	19.19 ± 0.025d
TCar60 %	6.95 ± 0.007a	7.1 ± 0.129a	9.54 ± 0.040c	16.64 ± 0.031c
TCor15 %	1.17 ± 0.047c	11.19 ± 0.076g	14.46 ± 0.030j	25.65 ± 0.151i
TCor25 %	0.98 ± 0.015b,c	10.1 ± 0.108f	12.95 ± 0.050i	23.05 ± 0.040h
TCor35 %	0.75 ± 0.026a,b	8.49 ± 0.101c,d	10.81 ± 0.035d	19.30 ± 0.100d

Different letters in the same column indicates significant differences between the samples ($p \leq 0.05$)

The content of total lycopene in the juice blends varied from 16.21 mg/100 g (TApl35 %) to 25.65 mg/100 g (TCor15 %), whereas the content of *cis*-lycopene from 9.16 to 14.46 mg/100 g (in TApl35 % and TCor15 %, respectively). As expected, lower contents of lycopene were in the blends with higher percentages of apple, carrot and sweet corn juices. Tomato-carrot juices had the highest contents of β-carotene among the tested juice blends. Its content in TCar was 4.4–7.2 times higher compared to control juice. The content of β-carotene in TCar gradually increased from 42.20 to 69.5 mg/100 mL as the percentage of carrot juice in the blend was increasing. It is interesting to note that previously it was shown that an ingestion of β-carotene and lycopene improved absorption of lycopene in human (Johnson et al., 1997).

Although the content of lycopene in the juice blends was reduced with addition of fruit and vegetable juices that initially contain no lycopene it can be assumed that tomato-apple juice blends where enriched with health beneficial compounds that are present in apple juice and that normally are not found or found in negligible amounts in tomatoes such as hydroxicinamic acids, dihidrochalcones, flavonols, catechins and oligomeric proanthocyanidins (Gerhauser, 2008). Oligomeric procyanidins have attracted increasing attention in the fields of nutrition and medicine due to their potential health benefits observed *in vitro* and *in vivo* (Aron and Kennedy, 2008). Similarly, tomato-sweet corn juice was enriched with biologically valuable constituents that are present in corns such as ferulic acid, a potent antioxidant and anticarcinogenic agent (Balakrishnan et al., 2008).

The principal components analysis (PCA) was used to classify the juice samples according to the similarity in taste. A PCA score plot for data obtained from E-tongue is shown in Fig. 1. PC1 vs. PC2 was shown, together explaining 85.927 % of the variance. As it is seen, not all the juice samples could be distinguished reliably with the help of PCA in two dimension principal components.
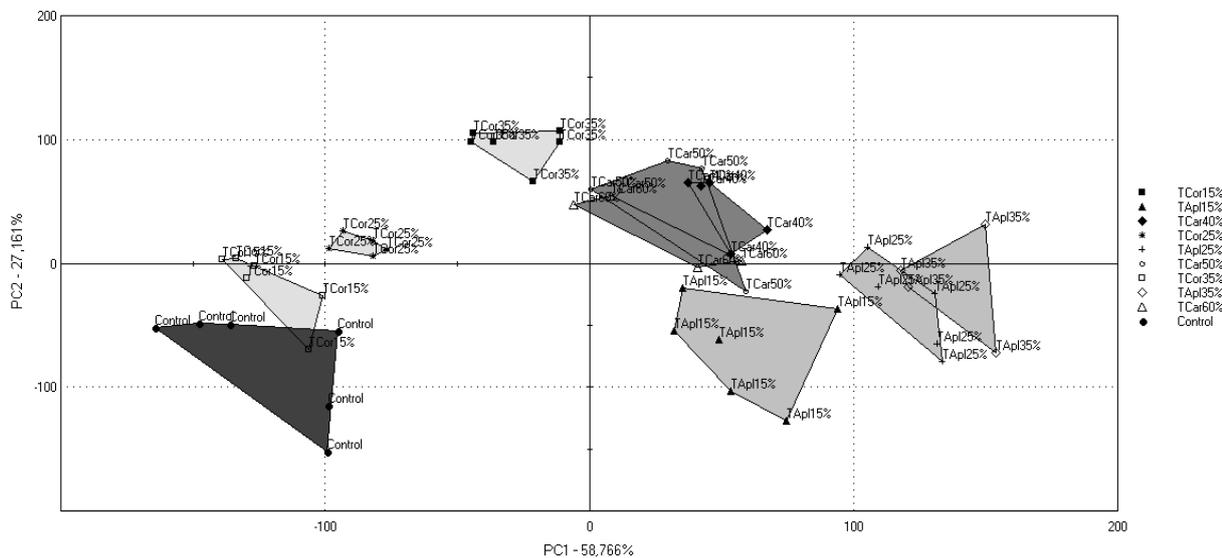

Figure 1. Discrimination of PCA plots for tomato (control) juice and different tomato-based juice blends

The PCA scores plot on the PC1 and PC2 shows overlap of tomato-carrot juice samples (TCar40 %, TCar50 %, TCar60 %) and two tomato-apple juice samples (TApl25 %, TApl35 %). This indicates that overlapping samples were quite similar in taste and that E-tongue could not differentiate between those samples. However, as it is seen from PCA graph there was distinct discrimination among the tomato-sweet corn (TCor15 %, TCor25, TCor35 %), tomato-carrot (TCar40 %, TCar50 %, TCar60 %) and tomato-apple clusters (TApl15 %, TApl25 %, TApl35 %) showing that different tomato-based juice blends taste very differently. The juice samples that are closer to one another in the PCA score plot share more similar taste characteristics, according to which the tomato-sweet corn juice blend with the lowest amount of sweet corn juice (TCor15 %) was most similar to the control sample (tomato juice), whereas the most distinct from control (furthest clusters from the control) were tomato-apple juice samples.

The estimated CIEL*a*b* parameters of tomato juice and juice blends are shown in Table 2. Tomato-apple juice blends had the lowest lightness (L*), redness (a*) and yellowness (b*) values compared with the control juice and juice blends with carrots and sweet corn. TCor25 % and TCor35 % juice blends were the lightest (L*=44.06 and 46.56, respectively) and had the highest yellowness values (b*=23.1 and 26.4, respectively).

Chroma (C*) is an expression of the purity or saturation of the colour. The highest C* had TCor blends, followed by TCar, control juice and TApl. The hue angle (h°) of juice blends with carrots, apples and sweet corn was lower compared with the tomato (control) juice. The h° value of control juice was 44.83 indicating that the redness component had larger influence on the overall colour of the product (0° indicates that the juice is red and 90° indicates that the juice is completely yellow). The addition of apple, carrot juice and in particular sweet corn juice increased the influence of the yellowness component on the overall colour of investigated juice blends.

Table 2. CIEL*a*b* colour parameters of control (tomato) juice and tomato based juice blends

	a*	b*	L*	C*	h*	ΔE
Control	13.2 ± 0.18d	13.2 ± 0.20b	42.4 ± 0.03a	18.7 ± 0.20c	44.8 ± 0.53a	−
TApl15 %	11.5 ± 0.09c	12.1 ± 0.07a	33.6 ± 0.09a	16.7 ± 0.11b	46.4 ± 0.11b	8.9 ± 0.69b
TApl25 %	10.9 ± 0.10b	12.1 ± 0.21a	33.5 ± 0.10a	16.2 ± 0.09a,b	48.0 ± 0.76c,d	9.3 ± 0.10b
TApl35 %	10.3 ± 0.02a	11.5 ± 0.05a	33.5 ± 0.03a	15.4 ± 0.02a	48.2 ± 0.18c,d	9.5 ± 0.03b
TCar40 %	15.3 ± 0.09e	16.6 ± 0.08c	36.9 ± 0.02b	22.6 ± 0.12d	47.3 ± 0.02b,c	6.8 ± 0.08a
TCar50 %	16.3 ± 0.01f	17.9 ± 0.10d	38.3 ± 0.04c	24.1 ± 0.07e	47.7 ± 0.15c,d	7.0 ± 0.04a
TCar60 %	16.6 ± 0.08f	18.8 ± 0.17e	39.2 ± 0.01d	25.1 ± 0.15f	48.5 ± 0.23d	7.3 ± 0.14a
TCor15 %	15.6 ± 0.17e	19.9 ± 0.27f	40.2 ± 0.02e	25.3 ± 0.12f	51.9 ± 0.67e	7.5 ± 0.19a
TCor25 %	16.3 ± 0.03f	23.1 ± 0.15g	44.1 ± 0.07g	28.3 ± 0.14g	54.8 ± 0.16f	10.6 ± 0.16c
TCor35 %	16.4 ± 0.52f	26.4 ± 0.82h	46.6 ± 0.42h	31.0 ± 0.96h	58.2 ± 0.27g	14.5 ± 0.15d

Different letters in the same column indicates significant differences between the samples ($p \leq 0.05$)

The total colour difference (ΔE) values higher than 5 indicate large colour differences between samples that are easily perceived by an inexperienced observer. The total colour difference values indicate that the addition of apple, carrot, and sweet corn juice significantly changed the initial colour of the control juice. The lowest ΔE values had TCar (6.8–7.3), whereas the highest ΔE had TCor25 % and TCor35 % (10.6 and 14.5, respectively).

Consumer acceptance of functional foods is far from being unconditional, with one of the main conditions for acceptance pertaining to taste. It is important to recognize that functional benefits may provide added value to consumers but cannot outweigh the sensory properties of foods (Siro et al., 2008). Therefore, sensory evaluation was performed in order to select the best tasting functional tomato-based juice blend. The results of the acceptability test are shown in Table 3.

Table 3. Acceptability scores of tomato based juice blends

	Taste	Odor	Appearance	Consistency
TApl15 %	3.0 ± 0.38a,b	3.3 ± 0.32 a,b,c	4.5 ± 0.19c,d	3.0 ± 0.38 a,b
TApl25 %	3.8 ± 0.42b	3.8 ± 0.40b,c	4.5 ± 0.19c,d	3.5 ± 0.43 b,c
TApl35 %	4.8 ± 0.17c	4.0 ± 0.27b,c	4.3 ± 0.17b,c,d	4.5 ± 0.19d
TCar40 %	3.8 ± 0.32b,c	4.0 ± 0.32b,c	4.0 ± 0.27b,c	4.0 ± 0.27c,d
TCar50 %	3.5 ± 0.33b	4.1 ± 0.22c	4.5 ± 0.18c,d	4.1 ± 0.09c,d
TCar60 %	3.3 ± 0.32a,b	3.3 ± 0.32a,b,c	4.8 ± 0.16d	3.5 ± 0.33b,c
TCor15 %	3.3 ± 0.42a,b	3.8 ± 0.42b,c	4.0 ± 0.25b,c	3.3 ± 0.32a,b,c
TCor25 %	3.0 ± 0.47a,b	3.0 ± 0.54a,b	3.8 ± 0.32b	3.0 ±0.47a,b
TCor35 %	2.3 ± 0.42a	2.3 ± 0.32a	2.8 ± 0.29a	2.5 ± 0.19a

Different letters in the same column indicates significant differences between the samples ($p \leq 0.05$)

According to the results, the most acceptable taste had tomato-apple juice blend that contained 35 % apple juice; followed by tomato-carrot juice blend that contained 40 % carrot juice. The tomato-sweet corn juice with the highest percentage of corn juice (35 %) was one of the least preferred. Some panelists commented on TCor35% blend as being pungent. The TCar50 % blend had the highest ranking for odor acceptability followed by TCar40 %, TApl35 %, TApl25 % and TCor15 % blends which also received quite high odor acceptance scores (between 4 and 3.75 points). The appearance of TCar60 % blend was most preferred to that of the other blends. The appearance of TApl15 %, TApl25 %, TApl35 %, TCar50 % was fairly liked, whereas the appearance of TCor35 % and TCor25 % was the least acceptable to panelists. The panelists commented on TCor35 % and TCor25 % blends as being too light-coloured. Result of the Tukey's test showed that the consistency of the TApl35 % blend was most preferred by the panellists. The consistency of TApl25 %, TCar40 %, TCar50 %, TCar40 % blends was fairly acceptable, whereas the consistency of TCor35 % blend was the least prefered. Most of the panellists named TCor30 % and TCor25 % blends as being too thick.

CONCLUSIONS

In this study lycopene-fortified tomato based juice blends were characterised. The study demonstrated that isomerised lycopene extract is a good vehicle for the fortification of fruit and vegetable juices with biologically active phytochemicals. The added isomerised lycopene extract increased the content of lycopene and especially the percentage of its *cis* isomers in the juice blends, thus improving functional properties of the products.

Based on the electronic tongue results the tomato-sweet corn juice blend with the lowest amount of sweet corn juice (TCor15 %) was most similar to the control sample (tomato juice), whereas the most distinct from control were tomato-apple juice samples. Furthermore, it was shown that the taste and appearance of TApl 25%, TApl 35%, TCar40 %, TCar60 % and TCor15 % were acceptable to consumers. The most acceptable taste had tomato-apple juice blend that contained 35 % apple juice and tomato-carrot juice blend that contained 40 % carrot juice.

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