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POLYPHENOL-RICH SMOOTHIES: SENSORY AND CHEMICAL CHARACTERIZATION

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Abstract: The aim of this research is to achieve and characterize smoothies, rich in antioxidants (vitamin C, polyphenolic compounds), obtained by mixing carrots (*Daucus carota*) puree (CP), melon (*Cucumis melo l.*) puree (MP) and pomegranate (*Punica granatum*) juice (PJ). Smoothies were pasteurized at 85°C for 60 s and total extracted phenolic content (TPC), pH and ascorbic acid were analyzed. Also, colorimetric analyses (lightness-L*, redness-a*, yellowness-b*, chroma - C* and hue angle H*) were performed with a Chroma Meter CR-400/410 and sensorial analysis in fresh and thermal processed products stored over 30 days (4°C) as well. The vitamin C content ranged from 51.00±0.97 g/kg fw in pomegranate juice to 30.2±1.07 g/kg fw in melon-puree; total phenolic content was quantified between 1.82±0.81mg gallic acid equivalents (GAE)/g in the fresh carrot-melon-pomegranate (CMP) (with 60% carrot, 20% melon and 20% pomegranate juice) smoothie and 2.75±0.65 mg/g in the pomegranate juices. The study showed that the carrot smoothies with melon and pomegranate juice were attractive to consumers and may be important natural sources of polyphenolic antioxidants.

Keywords: color, juice, pomegranates, sensory attributes smoothies

INTRODUCTION

Since the first days of life and all the periods of childhood, nutrition has been the most important external factor influencing children's development. Fruit purees or fruit and vegetables are considered as main meals and delicious desserts rich in vitamins, minerals and fibers. Smoothies are easy to prepare and can be prepared before serving or later processed and preserved for a longer period, still maintaining their nutritional and sensory qualities. Fruit smoothies have become popular with consumers and are the major antioxidant sources and bioactive substances in the daily diet. Consumption of fruit and vegetables is important as it plays an essential role in preventing childhood obesity (Golley *et al*., 2011), and preventing many diseases, including certain cancers and osteoporosis, diabetes, coronary heart disease and stroke, neuronal degeneration and type II diabetes (Liu, 2003). In recent years there has been a growing interest in foods rich in antioxidants and fresher and better improved quality of food products. To increase the intake of fruits and vegetables, food industry focuses on the manufacture of smoothies due to an increased demand for ready-to-eat foods (Di Cagno, 2011). Mennella *et al*., reported that the Hispanic infants aged 4 to 5 months were more likely to eat pureed baby foods on a daily basis than non-Hispanic ones (Mennella *et al*., 2006). Keenan *et al*. (2010) developed a smoothie blend with apple (29.5), apple juice from concentrate (29.5), strawberry (21), banana (12) and orange (8%). Their results demonstrated that the high hydrostatic pressure (HHP) treatments had a significantly greater effect on smoothie color and antioxidant activity as compared to the thermal treatment, the beverage being stable and containing an important amount of anthocyanins. Similarly, antioxidant content of HHP processed smoothies decreased during storage and the level of reduction was greater than in thermally treated samples (Keenan et al., 2010).

Carrots (*Daucus carata L.)* of the *Apiaceae* (*Umbelliferae*) family is an important horticultural crop, distributed worldwide, contain large amounts of antioxidants, including carotenoids, vitamins and flavonoids (Leahu *et al*., 2013). Carrot roots are used as salad or assorted other vegetables, in food industry for juices, in various dishes especially when they are fresh. Orange carrots are highly considered as "good for the eyes" due to their high content in hydrocarbon carotenoids, a class of phytochemicals that are often precursors to vitamin A. α- and β-Carotene predominate in orange carrots (Arscott and Tanumihardjo, 2010).

As compared with watermelon, in Romania melon (*Cucumis melo*) is grown in small quantities and is only consumed freshly with evidence of its nutritional benefits. Organic melons have significantly higher ascorbic acid, whereas total phenolics content is higher (Salandanan *et al*., 2009).

In countries where traditional melons are grown, such as Central Asia, their pulp is prepared in different foods such as stewed fruits, juices and jams**.** Melon in the form of juice or paste without skin can be used as a fermentation substrate, to obtain alcohol (Briones *et al*., 2012). Melons can be consumed in dried form and the amount of sugar up to 50% is easily assimilable. The fruit is also a good source of functional components such as vitamin C (around 50 mg/ 100 g), calcium, potassium, carotene, magnesium, phosphorus, peptic substances, sodium and high water content. Melons (*Cucumis melo*) are excellent sources of β-carotene; having in view that β-carotene from melon is an important dietary antioxidant and precursor of vitamin A (Fleshman *et al*., 2011). A common feature of vitamin C deficiency leads to iron-deficiency anemia; therefore, the antioxidant properties of vitamin C may stabilize folate in food and plasma. The

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deficiency of vitamin C and zinc adversely affects the physical and mental growth of children and can impair their immune defenses (Maggini *et al*., 2010).

Pomegranate (*Punica granatum L.*) is a popular fruit due to its nutrition potential. It constitutes a good source of natural antioxidant substances and can be consumed fresh (including squeezed juice). Nowadays juices are used to produce alcoholic beverages, jellies, jams, topping, syrups and healthcare products. The composition of pomegranate juice depends on the cultivar type, environmental and postharvest factors, and storage and processing factors (Miguel *et al*., 2004).

Smoothies are non-alcoholic beverages made from fresh or frozen fruit and/or vegetables, which are blended and usually mixed with crushed ice to be immediately consumed or sterilized. Nowicka *et al*. (2016) found out that the use of sour cherry puree in the production of smoothies may be interesting from a nutritional perspective, as it is an excellent raw material for processing, with high bioactive potential, enriching and stabilizing the quality of the final products.

Therefore, the aim of this study was to determine the physico-chemical properties and sensory attributes of smoothie products after processing and also after one month storage period at 4 ^{0}C. The smoothies were obtained by mixing carrots, melon puree and pomegranate juices in appropriate proportions (60/30/10, 60/20/20 and 50/40/10) to obtain three varieties of smoothies.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu reagent and methanol were purchased from Merck (Germany). Gallic acid (99% purity), anhydrous sodium carbonate (99% purity), acetic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 90% purity) were delivered by Sigma-Aldrich (Germany).

Materials and production of smoothies

Mature fruit samples of carrots (*Daucus carota*), melon (*Cucumis melo L.*) and pomegranate (*Punica granatum*) were purchased from a local market in May 2016.

The carrots were washed with potable water; the parts that were affected by phytopathology were removed, sliced and hand peeled. After that, the carrots were homogenized into puree by using a home type blender (Braun 570 FP K HC) at room temperature. The melons were peeled and their remaining pulp was crushed and blended. To obtain pomegranate juice, the method consisted of manually peeling the fruits, separating the seeds and extracting the juice by a Phillips Electric juice centrifuge.

For the smoothie sample, fresh vegetables were prepared by mixing carrot and melon puree and pomegranate juices in appropriate proportions (60/30/10, 60/20/20 and 50/40/10), based on previous formulations which were well accepted by a trained sensory panel. Four samples were put into glass jars, pasteurized (10 min at 90 ^{0}C), and cooled to 20 ^{0}C.

After processing, the samples were cooled and stored at 25 ^{0}C in darkness. Finally, five different products were obtained: three semi-products (100% carrot purees (CP); 100% melon puree (MP); 100% pomegranate juice (PJ); and three smoothies. Each sample was prepared in two replicates.

Chemical analyses

The pH was measured directly in the fresh fruit smoothies which were placed in a plastic beaker and stirred continuously using a non-heating magnetic stirrer and a pH reading was taken after 5 min, using a digital pH meter, at room temperature.

Total phenolics were measured in duplicate samples of each extract, using the Folin-Ciocalteu method. The results are expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight (FW) of fruit material.

Extraction of ascorbic acid from samples

Preparation of samples: 4 grams of fruits or juice were extracted with 12 ml of acidified solutions (Perchloric acid and o- Phosphoric acid 1%) using a ceramic mortar and a pestle. The residue was re-extracted until the extraction solvents remained colorless (the total solvent volume was of 50 ml). The extract was filtered through a Whatman no. 5 filter paper.

Ascorbic acid separation, identification and dosage

The ascorbic acid in the samples was separated, identified and dosed in a HPLC SHMADZU system coupled with UV–VIS detector (DAD). A ZORBAX - C18 column (5μm, 250x4.6) was used. The column was eluted in isocratic system with a mobile phase that consisted of phosphate buffer pH = 3.5 (TFA): solution 0.02 m/l of monopotassium phosphate and ortho-phosphoric acid 10%, adjusted to pH = 3.5. The pump flow rate was set at 0.6 ml/min. The chromatograms were registered at 245 nm.

For ascorbic acid identification, a standard L-ascorbic acid (Sigma 99% standard L ascorbic acid) was used. For dosage of ascorbic acid in the samples, a calibration curve was constructed using dilutions of standard L-ascorbic acid in bi-distilled water (Leahu *et al*., 2016).

Color Measurement. The CIE L*, a*, and b* parameters were determined with a Chroma Meter CR-400/410. The CIE L*, a*, and b* parameters were defined as follows: CIE L* - black (0) to white (100) scale; CIE a* - red (+) to green (-) color scale; CIE b* - yellow (+) to blue (-) color scale [6]. The total change in the colour of samples (ΔE*) was calculated. Color of samples was measured after mixing the two fruit pulps and pomegranates juice (3 measures x 5 areas) in a Petri box.

Sensory analyses of the obtained smoothies, puree and juices was carried by fifty-five (55) panelists comprising of students of the Faculty of Food Engineering in the “Stefan cel Mare” University of Suceava immediately after processing using a 5-point scale with boundary indications: ‘I do not like it very much’ [1] e ‘I like it very much’ [5]. Panelists were randomly and blindly assigned the samples for assessment of sweetness, color, flavor and overall preference. The samples used for the sensory evaluation were provided to the panelists for the evaluation at 20 ^{0}C in uniform 50-mL plastic containers.

Statistical analysis

The data were analyzed using Minitab 17 Software and one-way ANOVA was applied. P-values<0.05 were considered statistically significant. Each measurement was carried out in two replicates. All the data were expressed as mean ± standard deviation. This statistical technique was also used to determine how well the model fitted the data by calculating S, R^2 and R^2 pred.

RESULTS AND DISCUSSION

The moisture, pH, ascorbic acid, total phenol content and color attributes of fresh carrots, melon and pomegranate are illustrated in Table 1. The data presented as a preliminary study on ascorbic acid content of fresh carrot, melon and pomegranate showed that these vegetables were a rich source of ascorbic acid displaying maximum values of 51.00 g/kg fw (*Punica granatum L.*) and 30.2 g/kg fw (*Cucumis melo L*) (Table 1).

Tab. 1.

Moisture, pH, ascorbic acid, total phenolic and colour parameters of vegetables used for analysis

Samples		Moisture%	pH	AA[1] mg/kg FW[3]	TP[2] mg/gFW	Colour			
						L*[4]	a*[5]	b*[6]	ΔE[7]
Carrot-puree (C)	*Daucus carota*	82.3±0.91	6.7±0.16	31.5±0.57	1.25±0.31	55.6±2.31	11.96±1.31	35.90 ± 2.58	8.12± 2.58
Melon-puree (M)	*Cucumis melo L.*	91.21±1.52	6.91±0.12	30.2±1.07	0.93±0.25	60.63± 0.24	14.72± 0.16	37.02 ± 0.098	7.75± 2.58
Pomegranate juice (PJ)	*Punica granatum L.*	52.32±1.78	3.94±0.15	51±0.97	2.75±0.65	33.25±0.20	6.75±0.12	(-2.05) ±0.16	6.48± 2.17

AA[1]-Ascorbic acid mg/kg/L; TP[2]- total phenolic mg GAE/g; FW[3] – fresh weight of fruits; L*[4] - lightness; a*[5] - indicates red for positive value and green for negative value; b[6] - indicates yellow for positive value and blue for negative value; ΔE[7] - total change in colour.

Al-Maiman and Ahmad (2002) found out that the ascorbic acid content in pomegranate (*Punica granatum L.*) fruit seeds decreased significantly with advance in maturity, being of 0.26 for green-unripe, 0.25 for half-ripe and 0.15 mg/100 g for full-ripe fruit. The initial counts of AA and TP in untreated carrots (control sample) were 31.5±0.57mg/kg and 1.25±0.31mg/g, respectively.

The experimental results demonstrate that lightness of the products processed depends on the addition of pomegranate juice: 20% > 10%.

The values of physico-chemical indicators obtained for control samples (C), smoothie samples with mixing carrot and melon puree and pomegranate juices with different proportions (60/30/10, 60/20/20 and 50/40/10) - (S), smoothie samples after one month storage - (Sm) were introduced in Minitab Software. The α*-level* used is the common one of 0.05, the null hypothesis = all means are equal, while the alternative hypothesis = at least one mean is different. In the first phase the obtained results were investigated in order to find out if the differences between group means are statistically significant. The obtained p-values are compared with α *- value*: when *p-value* ≤ α *- value* the alternative hypothesis are considered and null hypothesis is rejected. The null hypothesis is not rejected when *p-value* >α *- value*. From Table 2 it can be observed that p-values for pH and AA indicators are lower than α *- value* which means that the factors are statistically significant, the null hypothesis is rejected and not all group means are equal, while for TP, L*, a*, b* and ΔE the p-values are higher than α *- value.* In the last case the null hypothesis is not rejected, it seems that all group means are equal and it is recommended to increase the power of a hypothesis test using a larger sample for example [14].

In the second phase the group means are examined considering the boxplots (Fig. 1). From the Fig. 1a it can be observed that S samples have the highest pH response, while C samples have the last pH variable response. The medians are in the range 5.54 - 5.75 and there are no outliers. According to Fig. 1b the C samples have the highest AA response (median 33.9) and Sm samples have the lowest AA response (with a median value of 32.8). However, the lowest contents of ascorbic acid were determined in smoothies with 60%C:30%M:10% PJ. Vitamin C is the vitamin that usually degrades most rapidly and can be used as an index of freshness [17].

Slight vitamin C losses in stored frozen wild berries were also reported recently [15]. Total polyphenol content in the fresh CMP (60%C: 30%M:10% PJ) was of 1.40 mg/g, while in the CMP (50%C:40%M:10% PJ) it was of 1.35 mg/g. Therefore, smoothies with addition of 20% PJ were characterized by the highest content of polyphenol compounds: 1.82 mg/g. S samples registered the lowest value for TP, while C samples have the highest response for AA. In this case the median is in the range 1.36 to 1.4.

Tab. 2.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-value	p-value
pH					
Type of sample	2	0.0949	0.0474	11.43	0.009
Error	6	0.0249	0.0041		
Total	8	0.1198			
AA					
Type of sample	2	3.98	1.99	6.94	0.027
Error	6	1.72	0.2867		
Total	8	5.7			
TP					
Type of sample	2	0.0016	0.0008	1.06	0.405
Error	6	0.0047	0.0007		
Total	8	0.0064			
L*					
Type of sample	2	1.853	0.9267	0.17	0.847
Error	6	32.684	5.4474		
Total	8	34.538			
a*					
Type of sample	2	1.951	0.9756	0.85	0.474
Error	6	6.906	1.151		
Total	8	8.857			
b*					
Type of sample	2	2.022	1.011	0.74	0.514
Error	6	8.148	1.358		
Total	8	10.17			
ΔE					
Type of sample	2	0.7334	0.3667	2.15	0.198
Error	6	1.0229	0.1705		
Total	8	1.7564			

DF - total degrees of freedom; Adj SS - Adjusted sums; Adj MS - Adjusted mean; AA - Ascorbic acid mg/kg/L; TP - total phenolic mg GAE/g; L - lightness; a* - indicates red for positive value and green for negative value; b - indicates yellow for positive value and blue for negative value; ΔE - total change in colour.*

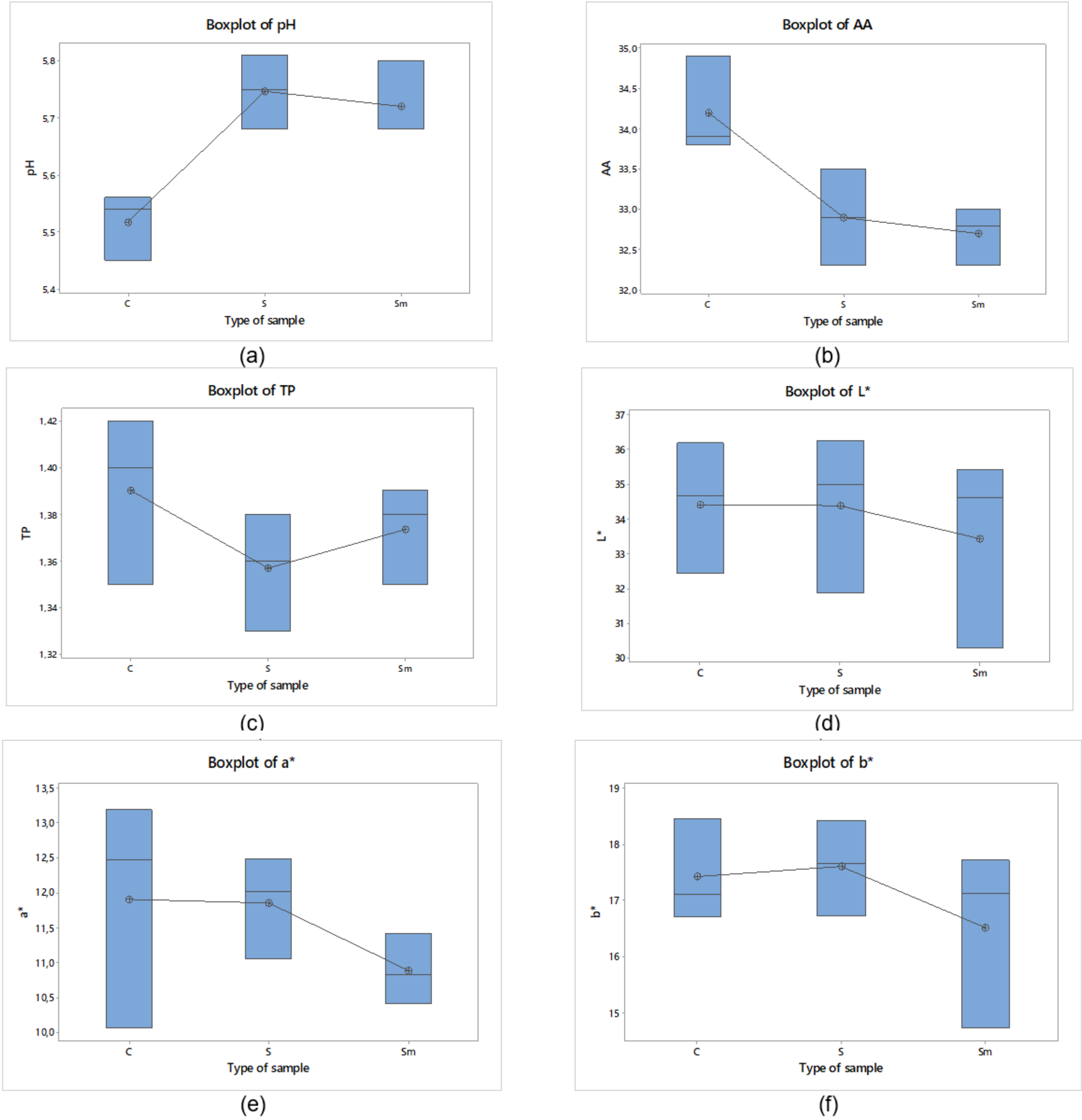
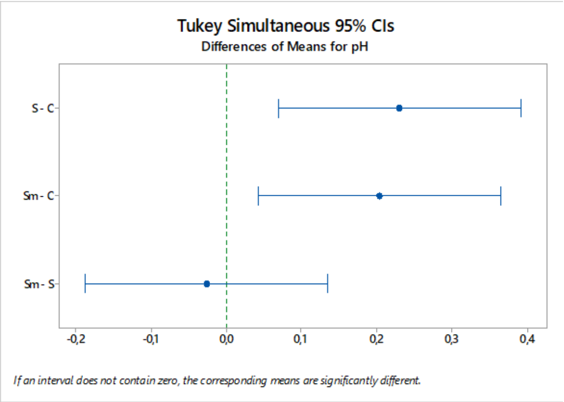


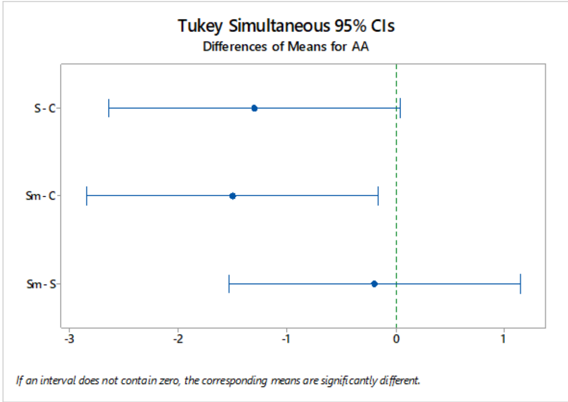
Fig. 1. Boxplot of (a) pH; (b) AA; (c) TP; (d) L^*; (e) a^*; (f) b^*

The value of the parameter L* in the analyzed smoothies ranged from 34.67 (50%C: 40%M: 10%PJ) to 36.18 (60%C:30%M:10% PJ). Only in the case of smoothies enriched by 20 % PJ there was a significant change in L*, lightness depending on the addition of the juice: 20% > 10%.

The group means can be compared using Tukey method and the results are illustrated in Fig. 2. In the case of pH versus type of sample and AA versus type of samples it was obtained that S and Sm samples are in the group A, while C samples are in the B group. In the case of TP versus type of sample we have observed that C and S samples are in the group A, while group B contains S and Sm samples. In this case C and Sm samples do not share a letter which indicates that C samples have a significantly higher mean than Sm. For the other indicators (L*; a*; b*; ΔE) all samples share the same group. The confidence level is 97.80% for each individual confidence interval. From Fig. 2 c, d, e, f and g it can be observed that the differences are not statistically significant since the confidence intervals include zero. The difference between the means of S-C and Sm-C for pH and of Sm-C for AA are statistically significant (Fig. 2a, 2b).



a)



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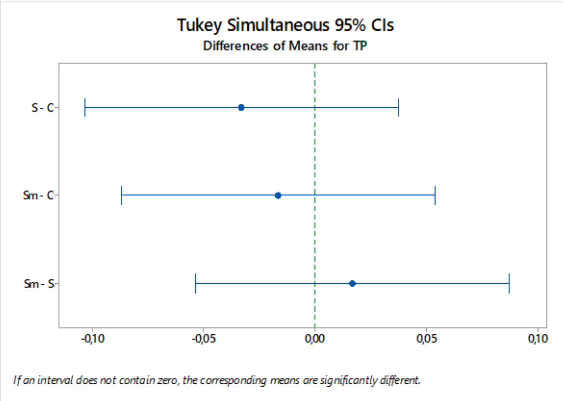
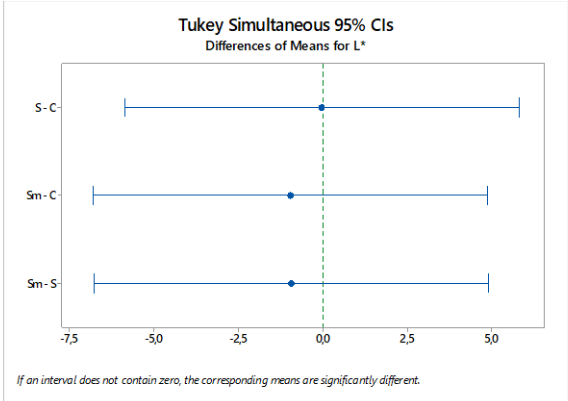
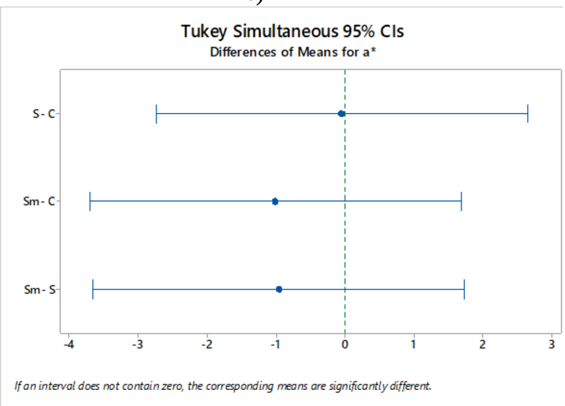


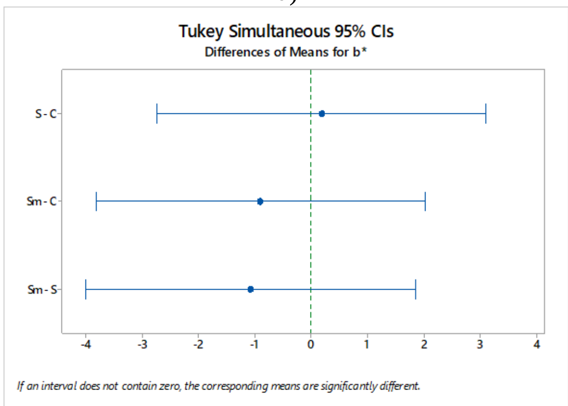
c)



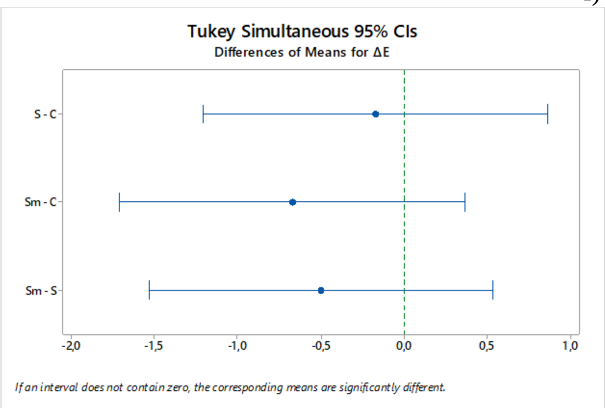
d)



e)



f)



g)

Fig. 2. Tukey Simultaneous

S an R-sq values were determined and used in order to determine how well the model fits the data. The standard deviation between the data points and the fitted values is provided by S values [14]. Model describes better the response when the value for S is lower (S=0.02 for TP; S = 0.06 for pH; S = 0.41for ΔE;

S = 0.53 for AA). It can be considered that the model fits the data when high values were obtained for R-sq. The values obtained for R-sq indicate that the predictors explain 79.20% of the variance in pH, 69.82% of the variance in AA and 41.76% of the variance in ΔE. The low predicted R-sq (5.37% for L*) suggests that the model will not predict new observations nearly as well as it fits the sample data. In order to verify the assumptions the residual plots must be checked (Minitab, 2014).

The statistical analyses confirmed that the pasteurization (10 min at 90 ^{0}C) process and any interaction related to the maintaining time at 90 ^{0}C did not affect the pH, content of TP of smoothie samples.

Fig. 3 illustrates the surface plots of physico-chemical indicators versus Carrot-puree (C) Melon-puree (M) and pomegranate juice (PJ). The surface plot of pH vs M; PJ shows that the highest values of pH are in the middle on the left side (M~30%; PJ~10%) and in the case of surface plot of pH and vs C; M it can be observed that the highest values of pH are in the middle on the right side (M~30%; C~60%). The highest values of pH are in the upper left corner which corresponds to high values of C and low values of PJ, while de lowest value of pH are in the lower right corner of the plot, which corresponds with low values of C and higher value of PJ. In the case of AA the highest values are in the upper left corner which corresponds to high values of M and low values of PJ, while de lowest value of AA are in the lower right corner of the plot corresponding to low values of M and higher values of PJ.

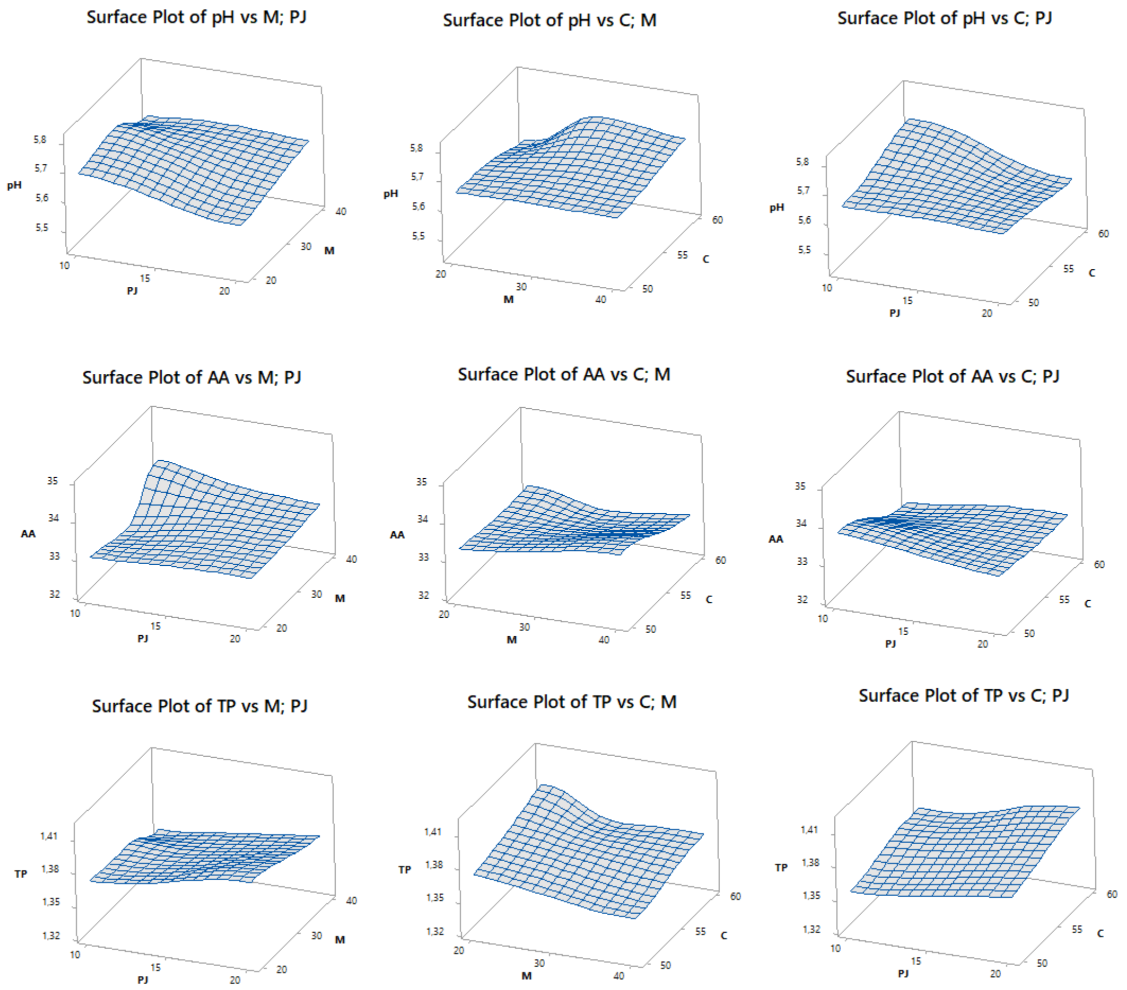


Fig. 3. Surface Plots

The highest value of TP were registered in the upper left corner which corresponds to high values of C and lowest values of M, while in the case of TP vs C and PJ the surface plot showed that the highest values of TP were obtained in the upper right corner of the plot corresponding to higher values of C and PJ (Fig. 3).

Sensory evaluation of smoothies

The sensory results provided by the trained panel were grouped according to complex sensory properties: aspect, colour, texture, flavor, aroma and taste (Fig. 4). However, as illustrated in figure 4, the addition of pomegranate juice into the vegetable puree causes sensory attribute differences. Color and aspect of smoothies attract the consumer and can help in impulse purchases. Flavor may be the most challenging quality attribute related to consumer’s acceptability. The score of color for S2 smoothie sample with 20% PJ (contained higher amount of TPC), was better than that of S1 smoothie with 60% C and 10%PJ.

Nowicka *et al.* (2016) studied the changes during storage of smoothies obtained by mixing sour cherry puree with apple, pear, quince and flowering quince juices. The quality evaluation in this study included visual ratings on a 9 point hedonic scale, L*a*b* color measurements of obtained products and viscosity measurements. These authors found that the products of the highest antioxidant activity were characterized by the worst taste according to consumers (Nowicka, *et al.*, 2016).

Sun-Waterhouse *et al.* (2010) reported that the 2.5 g apple fibers (total fiber content 67.4%, of which 16% is soluble) x 250 mg apple PP extract (APE, 78.5% phenolic) smoothie was given the highest overall ratings in terms of flavor, texture and overall acceptability.

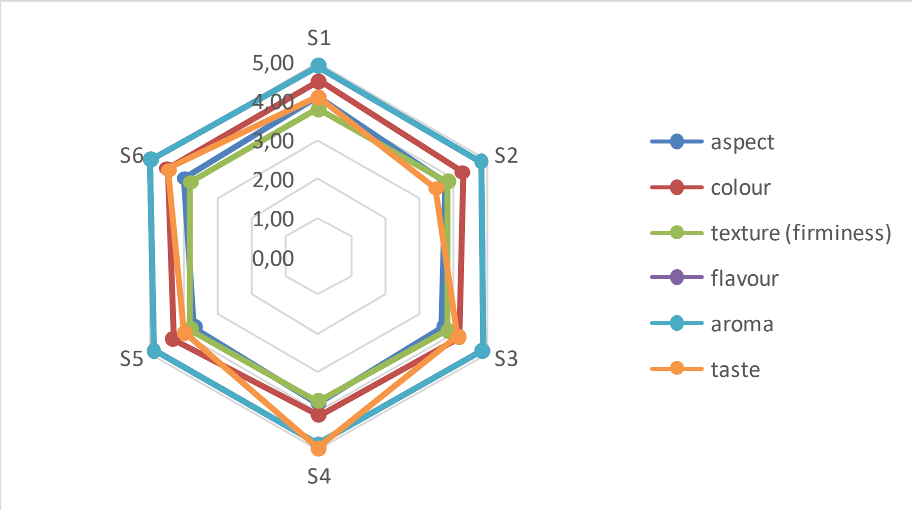


Fig. 4. Sensory quality of smoothie samples (S1 - Control 60%C: 30%M: 10% PJ; S2- Control 60%C: 20%M: 20%PJ; S3 - Control 50%C: 40%M:10%PJ; S4, S5, S6 - smoothie samples after one month storage)

In previous studies, Hurtado *et al.* (2015) reported that the sensory panel did not discriminate between smoothie flavours and that the „fresh-like" fruit flavour was maintained in smoothies pressurized at 350 and 450 MPa. The obtained results showed that HPP (high pressure processing) as compared with thermal processing provides noticeable benefits regarding enzyme inactivation, but has clear sensory disadvantages due to the development of cooked-fruit flavors (Hurtado *et al.*, 2015).

CONCLUSIONS

Thus, the aim of the present research was to achieve new all natural fruit smoothies rich in antioxidants that meet the quality requirements of a functional food. Fruit smoothies are good sources of fibers, vitamins and some beneficial phytochemicals such as carotenoids, phenolics and ascorbic acid. The sensory potential in treated smoothies using thermal treatment could be lost during storage as a result of thermal degradation of flavoring compounds during fruit heating.

The obtained results showed that the pH value of the smoothie samples registered insignificant changes, during the storage period. The physico-chemical analysis of samples showed that all smoothies can be used as functional food but according to the answers provided by the participants of the sensory analysis we can conclude that the sample S2 with 60% C, 20% M and 20% PJ is the most appreciated both fresh and also after one month storage.

Also, in this paper ANOVA was applied to investigate if the group means are different. The results showed that for pH and AA indicators the null hypothesis can be rejected and that not all group means are equal. Instead, for TP, L*, a*, b* and ΔE the null hypothesis is not rejected and it is recommended to use a larger sample. This fact is also suggested by R^2 and R^2 – predicted values. The results support the potential of these smoothies formula as food with health-promoting properties with remarkable content of ascorbic acid and polyphenols.

The novelty of this study is the investigation of a larger number of samples with different concentrations, in view of leading to the production of innovative functional beverages.

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