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EXPERIMENTAL RESEARCH ON ACTIVE COMPOUNDS AND NUTRIENTS IN NEW FRUIT SMOOTHIE PRODUCTS

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Abstract

The aim of this study is production and characterization of two smoothies based on blends of autochthonous fruits pressed or squeezed such as apple, grapes, plums, blueberries, apricots, peaches and vine shoot, without the addition of preservatives, stabilizers or chemical correctors for pH and acidity. Because vine shoot is a new ingredient, for first smoothie, two mixtures to observe the nutritional, sensory differences and the acceptability degree have been realised. Therefore, were realized three different mixtures as following: PMA from combining plums, apples, blueberries, grape and apple juices; PML was obtained from PMA with addition of vine shoot puree; PCS from combining apricots, peaches, apples purees, apple and grape juices. Physical-chemical (pH, DM%, TSS and TTA), nutritional (antioxidant activity, ascorbic acid and total polyphenols content) and sensory (color, acceptance and preference levels) characteristics were performed in order to characterize smoothies. It was observed that the differences between PMA mixture and PML were insignificant in all analyzes performed to characterize them. The vitamin C content of resulting smoothies may cover, 71% for PMA, 69% for PML and 66% for PSC from RDI.

Key words*: fruits, new products, nutrients, smoothie.*

INTRODUCTION

The smoothie concept was introduced for the first time in 1960 in the United States and re-emerged in 2000, consisting initially only of fresh fruit and vegetables (Titus, 2008). Smoothies can be defined as mixed beverages containing fruit, fruit juice, ice, yogurt or milk and are a very popular way to consume fruits. These products are usually purchased freshly prepared from "juice bars" or as a processed (slightly pasteurized) product from the hypermarket refrigerated section. Puree are characterized by a high nutrient concentration and low energy content. In most cases, fruit and vegetable mixtures are selected on the basis of color, flavor, texture, and in particular to guarantee high nutrient concentrations with low energy content. As a consequence, smoothies could contribute to the supply of fruit and vegetables, especially for people who can't consume fresh fruit and vegetables mainly because of market availability and / or convenience (Watzl, 2008).

Depending on the production and composition process, a smoothie may be enough to replace the nutritional value of at least one portion of fruit or vegetables (Di Cagno et al., 2011). If the percentage of fruit purée is higher, the product could also replace two portions of fruit (Muller et al., 2010) from daily requirements intake (RDI). Hagl et al. (2011) observed the effect of apple smoothie consumption on chronic colon diseases compared to apple juice or cider. Thus, they could indicate a higher prevention potential in the case of apple smoothies consumption in chronic colon diseases than in the case of the consumption of apple juice or cider.

So far smoothie market is poorly developed in Romania. These types of products are more present in the horeca segment, especially in coffee shops, where they are freshly prepared but it is quite different. The few smoothie products existing on the Romanian market belong to the international portfolio of producers.

The aim of this study is production and characterization of two smoothies based on blends of autochthonous fruits pressed or squeezed without the addition of preservatives, stabilizers or chemical correctors for pH and acidity. In order of this physical-chemical, nutritional and sensory characteristics were performed. The sensory characterization of smoothie was realized through consumers acceptance level for all smoothie products (PMA, PML and PCS), and consumers preferences between PMA and PML smoothies.

MATERIALS AND METHODS

Samples

Fruits and fruit juices were purchased from the regional market or harvested from the orchard depending on their maturity stage, namely: apricots - 10.07.2014, grapes juice - 12.09.2014, peaches - 12.09.2014, plums - 18.09.2014, apples - 21.10.2014, apple juice - 20.03.2015, vine shoot - 15.05.2015 and blueberries - 03.07.2015. All fresh fruits were blanched at 95°C for 5 minutes and homogenized for 1 min until it took puree form. Grapes and apples were squeezed in order to obtain juice. After processing operation, all juices and purees obtained were immediately packed and stored in freezer at -18 °C until new smoothie products were realized.

For the first product were made two mixtures (one with vine shoot juice and one without) in order to observe the nutritional, sensory differences and the acceptance level of vine shoot juice. Mixture 1, codified PMA consisted in combining plums, apples, blueberries, grapes and apple juices. Mixture 2, codified PML consisted in combining plums, apples, blueberries, grapes juice, apple juices and vine shoot puree.

The second product consisted in combining apricot, peach, apple, grape and apple juices.

Chemicals

Ascorbic acid, gallic acid and Folin Ciocalteu reagent were purchyased from Sigma-Aldrich, Germany, sodium acetate and sodium hydroxide were purchyased from Silal Trading SRL, Romania, oxalic acid, acetic acid CH_3COOH and blue bromothymol indicator were purchased from S.C. El-Chim S.R.L., Romania, xylene, from Chimopar S.A, Romania, Quercetin $C_{15}H_{10}O_7$ was purchased from Carl Roth GmbH+Co. KG,Germany, ethanol 99% from S.C. Connel 94 S.R.L, Romania and 2,6-Dichlorophenol-indophenol was purchased from Merk, Germany.

Physical – chemical characteristics

The pH values were measured with WTW INOLAB 720 series pH meter with domain between 0.00-14.00 and a precision of ± 0.01.

The dry matter content (DM %) was determined using the Precisa XM 60 thermobalance. Total soluble solids (Brix degree) were measured with Krüss Refractometer. The Schott automatic titrator was used for total titratable acidity (TTA). TTA was performed through titration (SR 6182-1:2008) of the homogenized sample with 0.1 N NaOH to an end point of pH 7.3. Results were expressed as citric acid/100 g product (factor 0.64).

Nutritional characteristics

Antioxidant activity

The antioxidant activity was performed through DPPH method after Villaño et al., (2007), with some modifications. The extraction of sample was realized through maceration in ethanol (75%) in dark at room temperature. Then 0.05 ml extract was added to 1.95 ml DPPH ethanol solution (60 μM), vortexed thoroughly, and incubated for 30 min in dark at room temperature (Gülçin, 2010). Calibration curve was realized with Quercitin. Absorbance was measured at 515 nm with an UV/Vis spectrophotometer Unicam Helios Gamma. Results were calculated using equations:

$$A_{AR}\ (QE) = (\%\Delta A_{515} - 3.4954) / 0.0811$$

where:

A_{AR} (QE) - antiradical activity expressed in quercetin equivalents

$$\%\Delta A_{515} = [(A_{515\ (t=0)} - A_{515\ (t=30)})/A_{515\ (t=0)}] \times 100)$$

Ascorbic acid content

The content of ascorbic acid was measured at 500 nm with the same UV/VIS spectrophotometer. 10 g of sample was extracted with 100 ml of 1% oxalic acid and filtered through a filter paper. Then 2 ml

extract, 1 ml oxalic acid 1%, 5 ml tampon solution, 2 ml indophenol (2, 6-Dichlorophenol Indophenol) and 20 ml xylene, were mixed in a centrifuge tube and centrifuged at 4 °C and 9000 rpm for 20 min. Results were expressed in milligrams at 100 g product and calculated using equation:

$Vit.C_{(mg/100g)} = [(V_0 - V_1) \times V_3 \times C / (V_4 \times V_2)] \times 100$

where:

V_0 - indophenol solution volume added for reduction,

V_1 - indophenol solution excess volume read on the standard curve,

V_2 - sample volume for analysis,

V_3 - sample volume brought for analysis,

V_4 – acid extract volume used for analysis,

C - ascorbic acid corresponding quantity for 1 ml indophenol solution.

Total phenolic content

The total phenol content was performed using the Folin-Ciocalteu method, according to the Arnous et al. (2002). The samples were extracted by macerating 5 g of sample in 25 ml of 75% ethanol in dark and room temperature. Then 1.58 ml of distilled water, 20 µl extract, 100 µl of Folin-Ciocalteu reactive, 300 µl of 20% sodium carbonate were mixed in a centrifuge tube and incubated for 2 hours in the dark. Absorbance was measured at 765 nm and the results were calculated through calibration curve with Gallic acid according to the equations:

Total polyphenols $(GAE)_{mg/100g} = (A_{765} - 0.0082) / 0.001$ $(R^2 = 0.9995)$

Where:

A_{765} - sample absorbance read at 765 nm,

GAE - concentration in gallon equivalents, mg/l,

$R^2 = 0.9995$ - correlation coefficient.

Sensory characteristics

Color indicators

Samples color was measured through HunterLab MiniScan ™ XE Plus Spectro-colorimeter with working conditions: Device geometry: 45º / 0º; Viewing area: LAV; Illuminator: D65; Observatory: 10º; Color system: CIELAB'76 and standardized according to its protocol. The CIELAB'76 color parameters, like: L * (luminance), a * (red-green coordinate), b * (yellow-blue coordinate), h * and chromaticity C * were calculated after four successive measurements.

Acceptance and preference tests

The evaluation of consumer acceptance was performed using a 9-point hedonic scale and nine possible ratings from "very good" to "I do not like it at all". The monadic test was used for determination of acceptance level of consumers and consist in exceeding a score of at least 70% of the rating scale.

The evaluation of consumer preference was performed using a panel's evaluators' in order to taste and answer at question like "What do you prefer?" (Lawless and Heymann, 2007). Therefore a pairwise comparison test (SR EN ISO 5495:2007) was performed. If tested product was preferred by at least 60% of evaluators this was labeled with a significantly higher preference level.

For both sensory tests, smoothie samples were coded randomly by numbers like: PMA-270, PML-184 and PCS -758.

Statistical analysis

All data were statistically evaluated using the variance analysis method (Anova software).

RESULTS AND DISCUSSIONS

In order to develop the two products, the required quantities of fruit puree and juice were determined according to their acidity, total soluble solids (Brix) and sensorial evaluation by 3 trained evaluators. More smoothies were made and evaluated (Table 1).

The accepted smoothies were: the third test for PMA witch consist in plums puree - 20%, apples puree - 30%, blueberries puree - 20%, grapes juice - 20% and apples juice – 10%; the second test for PML witch consist in plums puree - 20%, apples puree - 30%, blueberries puree - 15%, grapes juice - 20%, apples juice - 10% and vine shoot puree - 5%; the fourth test for PCS witch consist in apricots puree - 10%, peaches puree - 40%, apples juice - 10% and grapes juice - 40%. As evaluators remarks were noted: texture according to product specificity, pleasant taste and pleasant color.

Table 1. Fruit puree and juice mixtures in order to establish the final recipes

Mix code	No. of test	Smoothie compozition (g/100g product)	Physical-chemical characteristics		Evaluators remarks	Acceptance level
			Total titratable acidity (g citric acid/100g product)	Total soluble solids (°Brix)		
PMA	Test 1	Plums[a] - 30% Apples[a] - 30% Blueberries[a] - 20% Grapes[b] - 20%	0.55	12.5	- dense texture - astringent taste - pleasant color	REJECTED
	Test 2	Plums[a] - 10% Apples[a] - 30% Blueberries[a] - 20% Grapes[b] - 20% Apples[b] - 20%	0.56	12.5	- liquid texture (similar to the fruit nectar) - slightly astringent taste - pleasant color	REJECTED
	Test 3	Plums[a] - 20% Apples[a] - 30% Blueberries[a] - 20% Grapes[b] - 20% Apples[b] – 10%	0.51	13	- texture according to product specificity - pleasant taste - pleasant color	ACCEPTED
PML	Test 1	Plums[a] - 10% Apples[a] - 30% Blueberries[a] - 15% Grapes[b] - 20% Apples[b] – 20% Vine shoot[a] - 5%	0.55	12.1	- texture according to product specificity - slightly astringent taste - pleasant color	REJECTED
	Test 2	Plums[a] - 20% Apples[a] - 30% Blueberries[a] - 15% Grapes[b] - 20% Apples[b] – 10% Vine shoot[a] - 5%	0.49	12.5	- texture according to product specificity - slightly astringent taste - pleasant color	ACCEPTED
PCS	Test 1	Apricots[a] - 30% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 20%	0.9	12.1	- dense texture - sour taste - wrong color (Yellowish brown)	REJECTED
	Test 2	Apricots[a] - 25% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 25%	0.8	12.6	- dense texture - sour taste - wrong color (Yellowish brown)	REJECTED
	Test 3	Apricots[a] - 20% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 30%	0.7	12.6	- dense texture - acceptable color - slightly sour taste	REJECTED
	Test 4	Apricots[a] - 10% Peaches[a] - 40% Apples[b] - 10% Grapes[b] - 40%	0.58	13.5	- texture according to product specificity - pleasant taste - pleasant color	ACCEPTED

[a] – puree, [b] - juice

Physical – chemical characteristics

It was observed (Table 2) that the differences between the PMA mixture and PML were insignificant in all analyzes performed. Thus, the pH recorded a value of 3.61 (± 0.01) for PMA, while for PML a pH of 3.64 (± 0.01) was observed. For the PCS mixture pH value was 3.36 ((± 0.01)). The total titratable acidity values was similar for all tree smoothies as well as total soluble solids values. These values demonstrate that smoothie products have been performed correctly, thus achieving the acid-base balance necessary to obtain an equilibrate taste.

Table 2. Physico-chemical results for the smoothie samples (PMA - smoothie of plums, apples, blueberries; PML - smoothie of plums, apples, blueberries, vine shoots; PCS - smoothie from peaches, apricots, grape juice)

Sample	pH	Total titratable acidity (g citric acid/100g product)	Total soluble solids (°Brix)	Dry matter (DM%)
PMA	3.61 (±0.01)	0.51 (±0.02)	13 (±0.01)	23.42% (±1.5)
PML	3.64 (±0.01)	0.49 (±0.01)	12.9 (±0.01)	22.36% (±1.9)
PCS	3.63 (±0.01)	0.58 (±0.01)	13.5 (±0.01)	23.43% (±2.3)

Nutritional characteristics

Antioxidant capacity determination

Antioxidant capacity ranged between 604.94 µM quercitin equivalents for PCS smoothie and 1467.32 µM quercitin equivalents for PMA. Differences between PMA and PML smoothies are insignificant (Figure 1).

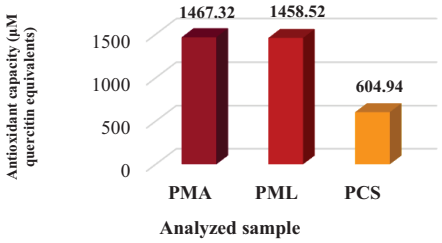

Figure 1. The antioxidant capacity values of the smoothie samples, where: PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice

Ascorbic acid content

According to the National Public Health Institute the RDI in ascorbic acid content is 80 mg per adult. Result obtained for ascorbic acid content content shown that the PMA smoothie can provide 71% of the RDI, the PML smoothie can provide 69% of the RDI and the PCS smoothie can provide 66% of the RDI. The content of ascorbic acid shown differences between PMA and PML smoothies with 2% in favor of PMA, but statistically are insignificant.

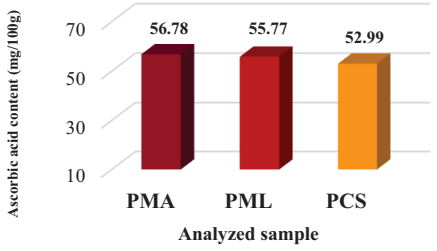

Figure 2. The ascorbic acid content of the smoothie samples, where: PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice

Total polyphenols content

Figure 3 shows that the total polyphenols content is lower for the PML smoothie compared to the PMA smoothie. The PCS smoothie recorded a total polyphenol content of 174.46 gallic acid equivalents / g of product.

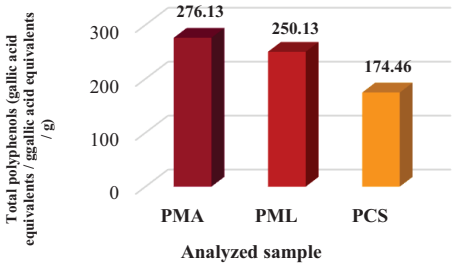

Figure 3. Graphic representation for total polyphenols content (PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice)

Sensory characteristics

In the agro-food sector, sensory evaluation can be defined as a systematic study of human responses to the physic-chemical and biological characteristics of food and their nutritional, properties (Croitoru, 2013). For smoothie sensorial characteristics was organized an evaluation panel with 31 evaluators trained before. The structure of the sample was represented by 67% of women and 33% of men in the age range 20-59 years. The number of valid questionnaires was 30 and one was canceled.

Color indicators

Following color analysis (Figure 4), differences between PMA smoothie samples and PML are also insignificant.

The PCS smoothie sample appears slightly shifted towards the red-green axis, due to the large (40%) amount of peach puree which has a pink color.

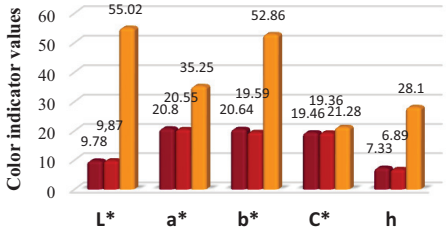

Figure 4. Graphic representation for color indicators of the smoothie samples (PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice)

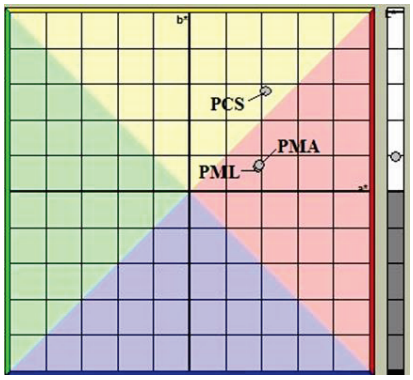

Figure 5. Graphic representation of CIELAB'76 system color for smoothie samples (PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots, PCS – smoothie from peaches, apricots and grape juice)

Acceptance tests

For "sweet" and "sour" evaluation was used a scale ranged between 1 to 5, where 1 means "too little", 3 means " exactly as it should", and 5 means "too much". For the product to be accepted, it must obtain at least 70% of the rating scale.

After sweet and sour were evaluated the PCS smoothie (Figure 6) was accepted by more than 70% of the panelists. In the case of PMA and PML smoothies, over 70% of evaluators noted the sweetness level by 3 ("exactly as it should"). Sour level was accepted only for the PMA smoothie, while the PML product receiving acceptance only from 60% of the evaluators, 26.7% believing that the sourness of the PML product is "too little", 13.33% saying it is "too much".

Also, the general impression of the products was evaluated and it was observed that PMA smoothie was accepted by 80% of the evaluators and the PML smoothie by the 70% of evaluators. Although the PML product was preferred by a smaller number of evaluators, the acceptability level set at the beginning of the test was reached.

In the case of PCS, the acceptance level was 86.65%, which means that 86.65% the evaluators offered points above 7.

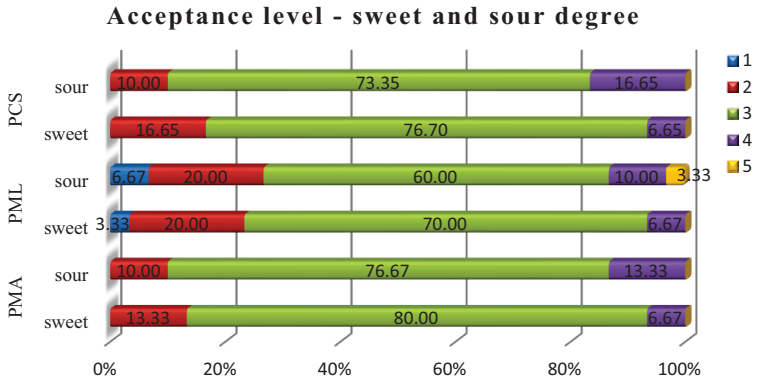

Figure 6. Graphic representation for the acceptance level of the smoothie products regarding the sweet and sour degree (1,2,3,4,5 - notes)

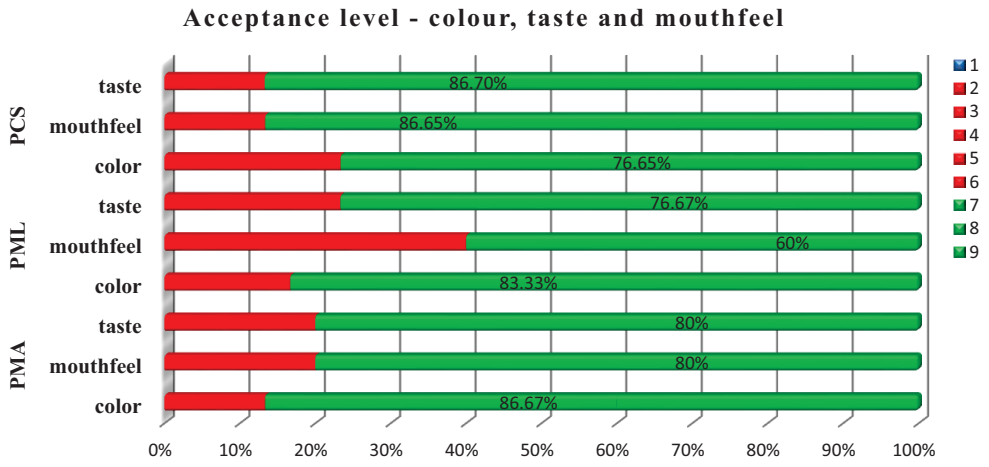

Figure 7. Graphic representation for the acceptance level of the smoothie products regarding the colour, taste and mouthfeel (1,2,3,4,5,6,7,8,9,- hedonic scale notes)

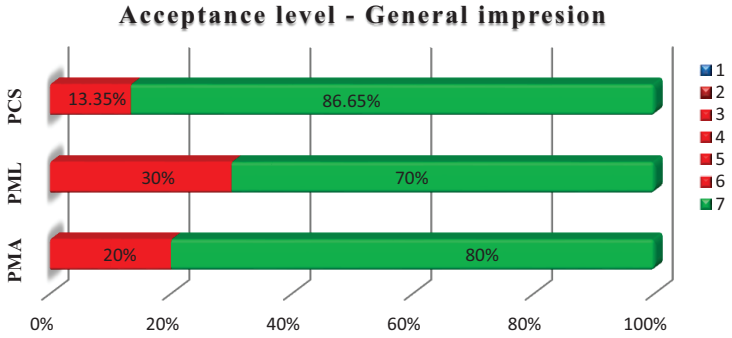

Figure 8. Graphic representation for the acceptance level of the smoothie products regarding the overall effect (1,2,3,4,5,6,7,8,9 - hedonic scale notes)

Preference tests

Following to the acceptance level measurements, the evaluators were asked to perform also the preference test for PMA and PML smoothies. Therefore, the PMA and PML smoothies obtained a score ranging from 40-60, with an approximately similar preference level (Figure 9).

The general impression of the products was also evaluated, and it was observed that the PMA product was accepted by 80% of the evaluators and the PML product of 83.33% of the evaluators. Although the PMA product was preferred by a smaller number of assessors, the preference level set at the beginning of the test was reached.

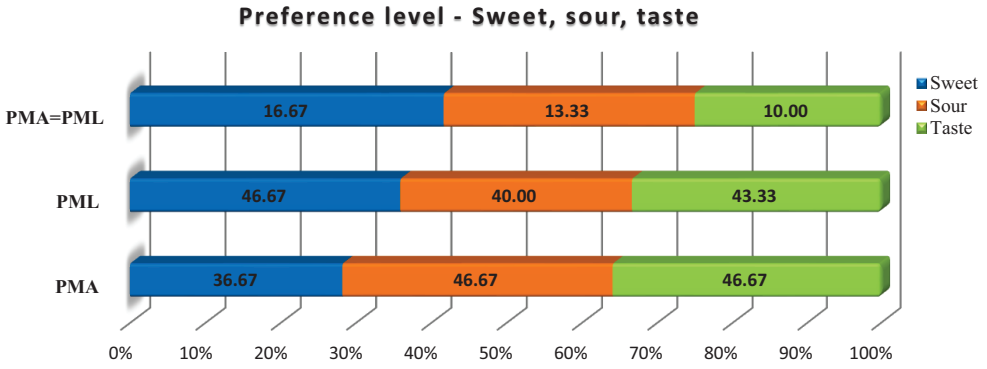

Figure 9. Graphic representation for the preference level of the PMA and PML, smoothie products, where: PMA – smoothie from plums, apples and blueberries; PML – smoothie from plums, apples, blueberries and vine shoots.

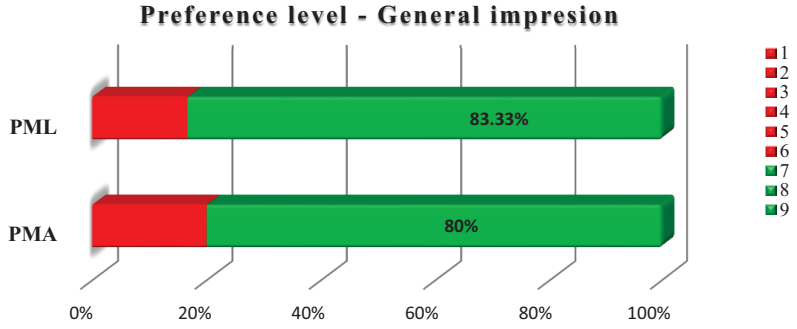

Figure 10. Graphic representation for the preference level of the PMA and PML, smoothie products regarding the overall effect (1,2,3,4,5,6,7,8,9 - hedonic scale notes)

CONCLUSIONS

The differences between the PMA mixture and PML were insignificant in all analyzes performed to characterize them.

The content of ascorbic acid shown differences between PMA and PML smoothies with 2% in favor of PMA, but statistically are insignificant. Insignificant differences between PMA and PML were also observed in antioxidant capacity and also in total polyphenol content.

The acceptance level of PCS smoothie was 86.65% which ranks him first.

In the preference level, the PMA and PML smoothies obtained a score ranging from 40-60, with an approximately similar preference level.

Discovering the similar physical-chemical, nutritional and sensorial characteristics of PMA and PML smoothies, the only major difference between them could be represented by the production price. Therefore, reference is made to the high price of blueberries as raw material, compared to vine shoots that are by-products of technological processes such as "pruning of shoots", a green operation in which shoots are shortened with 2-4 young leaves incomplete developed, in order to favor the fecundation process of the flowers.

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