Nutritional Composition of Different Varieties of Apple Purees Sweetened with Green and White Stevia Powder

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Summary

The aim of this study was to analyze the chemical composition of apple purees of four different apple varieties: 'Golden Delicious', 'Idared', 'Jonagold' and 'Cripps Pink', in the fresh state (FP) and after thermal treatment by process of pasteurization with added green (PG) or white stevia powder (PW) derived from the leaves of the stevia plant. Also, to determine whether there is a significant influence of sweetening with stevia on the change of analyzed nutritional parameters of apple purees based on the results. Significant increase of dry matter content in PG and PW samples of apple purees was determined for approximately 10%, and also for total soluble solid content for 1%. High increase of natural invert content, for approximately five times higher, and some lower increase of total invert content of analyzed purees was determined in PG and PW samples in comparison with the FP samples. Total acid content and pH-value did not show any significant change during the process of pasteurization and sweetening with stevia. The pasteurization significantly influenced the slight vitamin C reduction as well as the high reduction of total phenols in PG and PW samples. Based on the obtained results it can be concluded that the thermal processing by pasteurization significantly affects the degradation of high antioxidant activity compounds. Adding a green and white stevia powder significantly influenced the alterations of some nutritional components analyzed in apple purees: the dry matter content, soluble solids, and natural invert content (sugar glucose). It is important to emphasize, that the main effect of green and white stevia powder was the increase of natural invert content i.e. content of glucose and fructose, thereby increasing the sweetness of product, without increasing the content of "harmful" sugars e.g. sucrose.

Key words

Stevia rebaudiana Bertoni, green stevia powder, white stevia powder, chemical composition, pasteurization

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Introduction

Nowadays, fruit production and processing are among the major industries in many countries. The trading and distribution of fruits have become an important international economic activity (Sinha et al., 2012). On the other hand, the development of modern processing technology of raw fruit materials as the main goal have the expansion of the fresh fruits processing, thereby increasing the supply of fruit products that have found widespread use in the branches of food and bakery industry (fruit fillings, jellies, jams, etc.). Production of fruit fillings among other technological operations necessary requires elimination of microorganisms i.e. the thermal processing of food and the necessity of taste correction with different types of sweeteners. In the food and baking industry, during the production of fruit fillings, sweetening with artificial sweeteners (saccharin, aspartame) is still very popular (Sinha et al., 2012) primarily because of their exceptional sweetness, even 50-100 times sweeter than sucrose (Pól et al., 2007). Modern medical researches prove that most artificial sweeteners are harmful for human health (Grenby, 1991; Weihrauch and Diehl, 2004; Valentine, 2005; Jae-Yong et al., 2011). From the mentioned reasons, consumption and implementation of sweeteners from natural sources, i.e. sweeteners extracted from natural materials, are increasingly used in food preparation. Sweetener from the group of natural compounds is stevioside isolated from the leaves of plant *Stevia rabaundiana* Bertoni, which is up to 300 times sweeter than sucrose (Alaam, 2007). The dry stevia leaves, which are used in the production of sweeteners, are about 10 to 15 times sweeter than sucrose (Raymond, 2010). Stevia products, besides that they possess exceptional sweetness, gained popularity by no caloric value (glycemic index is zero) (Puri et al., 2011), it is a sweetener with 0 calories (Kroyer, 2009; Seema, 2010). Stevia leaves that are used as a food additive i.e. sweetener, on the market are present in several forms: green powder obtained by grinding the dried green stevia leaves (Mishra et al., 2010), a white powder that is obtained by process of depigmentation of green powder and by process of extraction of stevioside from leaves (Brandle and Rosa, 1992), and solution that is obtained by extraction methods of sweet stevioside and rebaudioside A from green powder (Abou-Arab et al., 2010).

The aim of this study was: to analyze and to compare the chemical composition and nutritional quality of apple purees of different apple varieties, sweetened with green and white stevia powder, primarily intended for use as filler in the food industry; to compare whether there are significant differences of analyzed chemical parameters considering which form of stevia powder we sweetened apple purees with, specifically to determine differences in the results due to the use of green or white stevia powder.

Materials and methods

Analyzed apple purees were obtained by grinding process. The research included four different apple varieties: 'Golden Delicious', 'Idared', 'Jonagold' and 'Cripps Pink'. Prepared apple purees were stored at -18°C for a period of a one month before chemical analysis. After defrosting some of the samples, apple purees were sweetened with (a) 22.4 mg of green stevia powder and (b) 18.8 mg of white stevia powder and then thermally processed, by process of pasteurization at 85°C for 10 minutes. The main reason for mentioned added amounts of green and white stevia powder was the fact that white stevia powder is concentrated form of glycoside compounds i.e. stevioside and rebaudioside A, and it`s about 300 times sweeter than sucrose, while the green stevia powder is dry stevia leaves grinded in powder and it`s about 15 times sweeter than sucrose. In process of adding stevia in apple purees decisive were organoleptic properties. Several tasters concluded that adding of higher amount (mg) of green stevia powder contributes to the pleasant flavor. After pasteurization and sample preparation of apple purees of different apple varieties, following chemical parameters were determined: dry matter content (%) was conducted with etalonic method by drying at 105°C until constant mass (AOAC, 1995); total acidity (g/kg) by potentiometric titration (AOAC, 1995); total soluble solids (°Brix) by reading a results directly from the refractometer scale (AOAC, 1995); pH-value by pH-meter (AOAC, 1995); vitamin C content (mg/100 g fresh weight) by titrimetric method with 2,6-p-dichlorphenolindolphenol (AOAC, 2002); total phenols (mg GAE/100 g fresh weight) using Folin-Ciocalteu colorimetric method (Amerie and Ough, 1980; Singleton and Rossi, 1965) and sugar content (%) by Luff-Schoorl's method (AOAC, 1995). All spectrophotometric measurements were performed by UV-visible spectrophotometer UV-1650 PC.

Data were statistically analyzed in SAS, version 8.0 (SAS, 1990). Duncan's test of significance of differences (5%) was used.

Results and discussion

Analyzed chemical parameters of different varieties of apple purees are shown in Table 1. Dry matter content of analyzed apple puree samples before pasteurization and stevia addition was in range from 19.85% ('Golden Delicious') to 24.03% ('Jonagold'). Statistically, there were no significant differences between dry matter content of analyzed apple purees. The mean value of the dry matter content of all apple puree samples before processing (stevia addition and pasteurization) was 22.28%, which is slightly higher value than those obtained by other authors (Hui et al., 2006; Campeanu et al., 2009; Nawirska-Olszańska et al., 2011). Thus, other literature citations indicate data for dry matter content of fresh apple fruits while in this research were analyzed apple purees obtained by grinding process, and therefore a partial removal of water from the plant tissue, which had direct impact on the reduction of some water (juice) quantity in the puree samples. After thermal treatment by pasteurization process and adding a certain amount of green and white stevia powder, dry matter content of analyzed samples significantly increased. In apple puree samples with added green stevia powder (PG) dry matter content was in range from 31.27% ('Idared') to 35.95% ('Golden Delicious'), while in the samples with added white stevia powder (PW) in range from 32.55% ('Idared') to 34.54% ('Cripps Pink'). Displayed results indicate an average increase of dry matter content for 10%. Comparing the results of apple puree samples with added green and white stevia powder there were not determined any statistically significant differences. The increase of dry matter content in PG and PW samples has been expected, given that apple purees before pasteurization were sweetened with stevia powder (green and white) and thus directly influenced on the trend of increase of dry matter content (soluble and insoluble) in analyzed samples (Katalinić, 2006). Stevia as a natural sweetener is also specific for its rich nutritional content; it's a good source of a wide range of

Table 1. Basic chemical parameters of analyzed apple purees from different apple varieties, thermally treated by pasteurization and sweetened with green and white stevia powder

	D.M. (%)	TA (g/kg)	TSS (°Brix)	TSS/TA	pH
Variety (FP)	N.S.	**	**	**	**
Golden Delicious	19.85 d	0.25 g	12.60 d	49.74 cde	4.33 a
Idared	22.80 cd	0.35 c	14.90 a	41.77 fg	3.89 d
Jonagold	24.03 c	0.27 ef	13.67 bcd	50.62 cd	4.14 c
Cripps Pink	22.44 cd	0.37 b	13.13 d	35.15 h	3.94 d
Pasteurization + green powder (PG)	**	**	**	**	N.S.
Golden Delicious	35.95 a	0.20 h	13.47 cd	67.33 a	4.29 ab
Idared	31.27 b	0.33 d	15.40 a	47.14 de	3.86 d
Jonagold	32.81 ab	0.26 fg	14.67 ab	56.41 b	4.19 bc
Cripps Pink	33.32 ab	0.38 ab	14.43 bcd	37.67 gh	3.91 d
Pasteurization + white powder (PW)	**	**	**	**	N.S.
Golden Delicious	34.37 ab	0.21 h	14.62 ab	69.76 a	4.23 bc
Idared	32.55 ab	0.34 d	15.43 a	45.86 ef	3.87 d
Jonagold	33.95 ab	0.27 e	14.73 ab	53.93 bc	4.15 c
Cripps Pink	34.54 ab	0.39 a	15.03 a	38.89 gh	3.90 d
INT. V x P	N.S.	**	N.S.	**	N.S.

INT. V x P = interaction of variety and processing; N.D.= not determined; FP= fresh apple purees; PG= pasteurized with added green stevia powder; PW= pasteurized with added white stevia powder

minerals, essential amino acids, and phytochemicals (Abou-Arab et al., 2010) that contributed to the increase of dry matter content in apple puree samples. Total acids (TA) of apple purees were in range from 0.25 g/kg ('Golden Delicious') to 0.37 g/kg ('Cripps Pink') and significant differences between the analyzed varieties of apples were determined. The results of total acids obtained in this study are in agreement with the results obtained by other authors (Campeanu et al., 2009). It is important to emphasize that results of total acid content obtained in this research can be classified as relatively low, which is especially important for the consumption point of view. In general, the low acidity of the product means a good product quality for human consumption (Campeanu et al., 2009), primarily because the acidity is the basic component of the taste, and the important protection element against microorganisms for the final product (Katalinić, 2006). In apple purees of apple varieties 'Golden Delicious' (0.25 g/kg) and 'Jonagold' (0.27 g/kg) were determined the minimum values of total acid content, which was expected given that these varieties are generally characterized by a high sugar content and low content of total acids (variety characteristic) (Krpina, 2004; Miljković, 1991). Compare the values of total acidity for each apple variety before and after the pasteurization process and stevia addition (Table 1), we can conclude that There was no significant deviation of total acid content after heat treatment and adding stevia in samples of apple purees. For example, apple puree of apple variety 'Cripps Pink' had the highest total acid content before pasteurization (0.37 g/kg) and after pasteurization and addition of green (0.38 g/kg) and white stevia powder (0.39 g/kg). Based on mentioned results, it can be determined that the form of stevia i.e. whether it is green or white powder did not significantly affect the acidic content of apple puree samples. The obtained results were expected and they are in agreement with literature data of other authors considering the fact that the process of heat treatment by pasteurization does not affect the change in total acid content in samples of apple purees (Lewis and Heppell, 2000).

Total soluble solids (TSS; °Brix) in analyzed apple purees were in range from 12.60 °Brix ('Golden Delicious') to 14.90 °Brix ('Idared') and indicate statistically significant differences between the analyzed apple puree varieties. Obtained results are in agreement with other authors (Lopez et al., 1998; Kheiralipour et al., 2008; Campeanu et al., 2009). Statistically significant increase of total soluble solids in apple purees was determined after adding stevia green powder the values were in the range from 13.47 °Brix ('Golden Delicious') to 15.40 ° Brix ('Idared'), and after adding the white powder in the range from 14.62 °Brix ('Golden Delicious') to 15.43 ° Brix ('Idared'). Namely, mentioned increase of TSS in apple purees sweetened with stevia is expected due to the fact that stevioside belongs to a group of ditterpene glycosides, large three-dimensional molecules (Mishra i sur., 2010) composed of three glucose molecules linked to aglycone steviol (Puri et al., 2011; Lemus-Mondaca et al., 2012) that indicate good solubility and stability in a wide range of pH values of a solution (Abou-Arab et al., 2010). Also, comparing the TSS values in samples with added green and white stevia powder we determined that there was no significant difference.

The ratio of total soluble solids and total acidity (TSS/TA) is a major component in the definition of juiciness and harmony of flavors and are often used to provide technological characterization of fruits (Daughty, 1995). TSS/TA ratio of analyzed apple purees before processing (pasteurization and stevia addition) was in range from 35.15 ('Cripps Pink') to 50.62 ('Jonagold'). After addition of green and white stevia powder in apple puree samples TSS/TA ratio significantly increased, which was expected due to the fact that the addition of stevia in apple purees have a significant increase of the total soluble solids content (TSS). Purees of apple varieties 'Golden Delicious' and 'Jonagold' indicate the highest TSS/TA ratio and had the best harmony of the flavors; which is to expect because 'Golden Delicious' and 'Jonagold' are characterized by distinct aroma and flavor and for that reason are often used in the food processing industry (for example in the production of fruit juices) (Miljković, 1991).

Pasteurization and stevia addition did not significantly change pH-values of analyzed apple purees (Table 1). Before processing pH-values in purees of different apple varieties were in range from 3.89 ('Idared') to 4.33 ('Golden Delicious') and the obtained re-

Table 2. Antioxidant components of analyzed apple purees from different apple varieties before and after heat treatment by pasteurization and green and white stevia powder addition

	Vitamin C (mg/100g FW)	Total phenols (mg GAE/100g FW)	Non flavonoids (mg GAE/100g FW)	Flavonoids (mg GAE/100g FW)
Variety (FP)	*	**	**	**
Golden Delicious	4.90 abcd	50.56 d	27.05 c	23.50 d
Idared	4.33 cdef	106.37 a	65.89 a	40.47 a
Jonagold	5.24 ab	97.40 b	52.47 b	44.93 a
Cripps Pink	5.36 a	55.87 c	30.84 c	25.03 bcd
Pasteurization + green powder (PG)	**	**	**	**
Golden Delicious	4.42 bcde	15.13 i	N.D. g	N.D. f
Idared	3.87 ef	47.58 d	17.88 d	29.69 b
Jonagold	4.10 def	41.39 e	11.93 de	29.45 bc
Cripps Pink	5.00 abc	31.91 fg	16.04 de	15.87 e
Pasteurization + white powder (PW)	**	**	**	**
Golden Delicious	4.24 cdef	17.49 i	N.D. g	N.D. f
Idared	3.93 ef	33.14 f	17.97 d	15.17 e
Jonagold	3.56 f	24.55 h	10.39 ef	14.16 e
Cripps Pink	3.93 ef	28.53 g	14.90 fg	23.63 cd
INT. V x P	N.S.	**	**	**

INT. V x P = Interaction of variety and processing; N.D.- not determined; FP- fresh apple purees; PG- pasteurized with added green stevia powder; PW- pasteurized with added white stevia powder

sults are in agreement with other authors (Goodenough and Atkin, 1981; Picouet et al., 2009). As a basis for further scientific researches, it is important to emphasize that stevioside and rebaudioside A as a predominant sweet glycosides from stevia leaves show a significant thermal stability during pasteurization process in a wide pH range, and because of three-dimensional structure of their molecules, show considerable resistance to the low acid content and low pH (Abou-Arab et al., 2010; Mishra et al., 2010).

Antioxidant components analyzed in this study are shown in Table 2. Apple as a fruit species is specific in rich content of various phenolic compounds in the fruit, of which are predominant flavonoids and phenolic acids (Schieber et al., 2001; Manach et al., 2004; Scalbert et al., 2005; Markowski and Płocharski, 2006). Depending on the variety, total phenol content in the analyzed fresh apple purees was in range from 50.56 mg GAE/100 g fresh weight ('Golden Delicious') to 106.37 mg GAE/100 g fresh weight ('Idared'). Total phenol content of 'Cripps Pink' apple puree was 55.87 mg GAE/100 g fresh weight and of 'Jonagold' apple puree 97.40 mg GAE/100 g fresh weight, given that it can be determined that the total phenol content analyzed in the apple puree of varieties 'Jonagold' and 'Idared' was similar, as it was between varieties 'Golden Delicious' and 'Cripps Pink'. Flavonoid content of analyzed apple purees was in range from 23.51 mg GAE/100 g fresh weight ('Golden Delicious') to 44.93 mg GAE/100 g fresh weight ('Jonagold'). Also in the flavonoid content, the results are closer between apple purees of varieties 'Golden Delicious' (23.51 mg GAE/100 g fresh weight) and 'Cripps Pink' (25.03 mg GAE/100 g fresh weight) and between apple purees of varieties 'Idared' (40.47 mg GAE/100 g fresh weight) and 'Jonagold' (44.93 mg GAE/100 g fresh weight) i.e. the same trend was determined as for the total phenolic content. According to Table 2 and the above mentioned results, significant differences of total phenol and flavonoid content between apple varieties were determined. The above result was expected given that the composition of bioactive components in apple fruit including polyphenolic compounds significantly depends on the variety i.e. its genetic characteristic (Belitz and Grosch, 1999; Scalbert et al., 2005; Hui et al., 2006; Markowski and Płocharski, 2006). Obtained values of phenol and flavonoid content are lower than in the results of other authors (Hui et al., 2006; Hecke et al., 2006; Markowski et al., 2006). That is primarily the main consequence of freezing and storing of apple fruits through a long time period, but also a consequence of grinding process of apple fruits to the puree. A thin layer of apple fruit just below its peel contains the highest concentration of polyphenols (Hui et al., 2006; Ceymann et al., 2012). During the process of production of apple puree, peel of apples was not peeled, but together with the apple fruit endocarp milled into puree, and therefore the concentrations of phenols obtained in this study are slightly higher than in the results obtained by other authors who have analyzed the phenolic composition of apple puree without peel (Ceymann et al., 2012). Besides polyphenols, significant antioxidant component is also a vitamin C. The vitamin C content in analyzed fresh apple purees was in range from 4.33 mg/100 g fresh weight ('Idared') to 5.36 mg/100 g fresh weight ('Cripps Pink'). The vitamin C content of varieties 'Golden Delicious' and 'Jonagold' was 4.90 mg/100 g ('Golden Delicious') and 5.24 mg/100 g fresh weight ('Jonagold'). Mentioned results are in agreement with those obtained by other authors (Hui et al., 2006; Picouet et al., 2009). Possible differences of vitamin C content in apple purees considering reference data of other authors are primarily a result of different apple varieties that significantly affect the vitamin C content of each variety (Campeanu et al., 2009). After processing of apple purees by pasteurization process and stevia addition (green and white powder) a statistically significant degradation of total phenol and vitamin C content was determined (Table 2). The thermal treatment by pasteurization caused a total degradation of phenol content in the analyzed purees of different apple varieties by 38%, which was an expected result due to the fact that thermal treatment significantly contributes to the degradation of polyphenol compounds. The obtained results are in agreement with literature data of other authors (Markowski et al., 2005; Rembiałkowska et al., 2007; Mahdavi et al., 2010). Vitamin C belongs to the group of water-soluble vitamins and it is especially susceptible to significant degradation during post harvest

Table 3. The natural invert, total invert and sucrose content in apple purees of different apple varieties before and after pasteurization and green and white stevia powder addition

	Natural invert (%)	Total invert (%)	Sucrose (%)
Variety (FP)	**	**	**
Golden Delicious	8.46 i	6.72 k	N.D. c
Idared	7.76 j	6.24 l	N.D. c
Jonagold	4.80 l	9.12 g	4.10 a
Cripps Pink	5.76 k	7.20 j	1.37 b
Pasteurization + green powder (PG)	**	**	N.S.
Golden Delicious	36.39 b	8.55 i	N.D. c
Idared	37.50 a	9.16 f	N.D. c
Jonagold	32.34 f	9.18 e	N.D. c
Cripps Pink	30.85 g	9.67 c	N.D. c
Pasteurization + white powder (PW)	**	**	N.S.
Golden Delicious	34.94 d	11.16 b	N.D. c
Idared	35.10 c	8.99 h	N.D. c
Jonagold	34.10 e	9.34 d	N.D. c
Cripps Pink	28.46 h	11.90 a	N.D. c
INT. V x P	**	**	N.S.

INT. V x P = Interaction of variety and processing; N.D.- not determined; FP- fresh apple purees; PG- pasteurized with added green stevia powder; PW- pasteurized with added white stevia powder

handling of fruits and storage process (Gliszczynska-Swiglo and Tyrakowska, 2003; Nawirska-Olszańska et al., 2011). Also, the technological procedures of processing that involves heat treatment strongly affect the degradation rate of vitamin C (Markowski et al., 2005; Rembiałkowska et al., 2007). Adding a stevia as a sweetener in the processing of apple purees did not contribute to change of the content of polyphenol compounds and vitamin C, although stevia contains significant amounts of phenolic compounds (Singh, 2005; Abou-Arab, 2010).

Natural invert, total invert and sucrose content analyzed in samples of different apple purees are shown in Table 3. In fresh samples of apple purees (FP) content of natural invert was in range from 4.80% ('Jonagold') to 8.46% ('Golden Delicious'), content of total invert in range from 6.24% ('Idared') to 9.12% ('Jonagold') and sucrose content was only determined in apple puree samples of apple varieties 'Jonagold' (4.10%) and 'Cripps Pink' (1.37%). The obtained results of natural and total invert content in analyzed apple purees are in agreement with results obtained by other authors (Hui et al., 2006; Campeanu et al., 2009). Deviations in the results of sucrose content in apple purees of varieties 'Golden Delicious' and 'Idared' can be explained by the fact that the stated varieties of apples before grinding to a puree and analyzing, stood a long time period in inadequate storage conditions. Also in general, apple as a fruit species is not characterized by a high content of sucrose compared with glucose and fructose that are significantly more present in the composition of apple fruits independently of variety (Hui et al., 2006; Campeanu et al., 2009). After processing the purees by pasteurization and stevia addition (green and white powder) a statistically significant increase in the content of natural and total invert was determined (Table 3). Specifically, the content of natural invert in apple purees with added green (PG) and white stevia powder (PW) increased approximately five times. Lower trend of increase of total invert content in samples with added green and white stevia powder in comparison with fresh samples of apple purees was determined. There was no significant difference in the content of natural and total invert between PG and PW samples. The main reason for significant increase of the natural invert content after green and white stevia powder addition is the chemical composition of diterpene glycosides, which form the basis of the stevia leaves composition (Geuns, 2003; Giraldo et al., 2005), the basic raw material for obtaining stevia powder (Mishra et al., 2010). Glycosides of stevia plant (stevioside and rebaudioside A) are composed of aglycone steviol molecule and a certain number of glucose molecules (stevioside of three glucose molecules and rebaudioside from four glucose molecules) (Puri et al., 2011). Because of the polar groups containing in molecular structure (carbohydrate part of the molecule) listed diterpene glycosides of stevia plant indicate good solubility in aqueous solutions (Mitchell, 2006). In the hydrolysis process, molecule of diterpene glycoside decomposes on the sugar component (glucose molecules, natural invert) and aglycone steviol (non-sugar component, diterpenic carboxyl alcohol) (Bernal et al., 2011; Puri et al., 2011).

Conclusion

Thermal process by pasteurization, as well as other heat-invasive technological processing of raw materials significantly affects the degradation and final reduce of the concentrations of high antioxidant activity components, such as the different types of phenolic compounds and vitamin C. The use of new natural sweeteners such as extract of stevia plant can completely replace the use of refined sugar and artificial sweeteners in its primary task of enhancing the taste of sweetness of apple purees. Also, we can conclude that there is no significant difference in the analyzed chemical and nutritional parameters of apple purees sweetened with green or white stevia powder i.e. there were not established difference which of the applied stevia product (green or white powder) are better for use as a sweetener. The fundamental difference in the application of mentioned stevia products is in added quantity in apple puree samples. Specifically, to correct a sweet taste while preparing filling approximately 3.6 mg more of the green powder than the white powder (concentrate stevioside) should be added. However, from the health point of view the use of green powder is more acceptable due to the fact that it is obtained exclusively by process of milling without further extraction that often involves the need of chemical intervention.

References

Abou-Arab A.E., Abou-Arab A. A., Abu-Salem M. F. (2010). Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* Bertoni plant. African Journal of Food Science 4 (5): 269- 281

Alaam A.I. (2007). Sugar crops council: Future view. The Proceeding of Thirty-eight Annual Conference, Egyptian Sugar Expertese Society Hawamdia, Egypt

Amerie M.A., Ough C.S. (1980). Methods for Analysis of Musts and Wines, John Wiley and Sons, New York, USA 187-188; 192-194

AOAC (1995). Official methods of analysis (16th ed.). Washington, DC: Association of Official Analytical Chemists

AOAC. (2002). Official methods of analysis (17th ed.). Washington, DC: Association of Official Analytical Chemists

Belitz H.-D., Grosch W. (1999). Food Chemistry, Springer, Berlin, Germany, (992): 764-789

Bernal J., Mendiola J., Ibáñez E., Cifuentes A. (2011). Advanced analysis of nutraceuticals. Journal of Pharmaceutical and Biomedical Analysis 55 (4): 758–774

Brandle J.E., Rosa N. (1992). Heritability for yield, leaf: stem ratio and stevioside content estimated from a landrace cultivar of *Stevia* rebaudiana. Canadian Journal of Plant Science 72 (4): 1263-1266

Campeanu G., Neata G., Darjanschi G. (2009). Chemical Composition of the Fruits of Several Apple Cultivars Growth as Biological Crop. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37 (2): 161-164

Ceymann M., Arrigon E., Schärer H., Bozzi Nising A., Hurrell R. F. (2012). Identification of apples rich in health-promoting flavan-3-ols and phenolic acids by measuring the polyphenol profile. Journal of Food Composition and Analysis 26 (1-2): 128-135

Daughty, M.E. (1995). Fruit and vegetable processing. Fao Agricultural Services Bulletin. 119 Food and Agriculture Organization of the United Nations, Rome. Article from the Internet. Available from: http://www.fao.org/documents; cited on 20.10.2012.

Geuns J. M. (2003). Stevioside. Phytochemistry 64 (5): 913–921

Giraldo C., Marín L., Habeych D. (2005). Obtención de Edulcorantes de Stevia rebaudiana Bertoni. Revista CENIC Ciencias Biológicas 36: 3–10

Gliszczynska-Swiglo A., Tyrakowska B., (2003). Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. Journal of Food Science 68 (5): 1844–1849

Goodenough P.W., Atkin R.K. (eds.) (1981). Quality in Stored and Processed Vegetables and Fruit. Academic Press, New York, 398

Grenby TH. (1991). Intense sweeteners for the food industry: an overview. Trends in Food Science & Technology 2: 2-6

Hecke K., Herbinger K., Veberič R., Trobec M., Toplak H., Štampar F., Keppel H., Grill D. (2006). Sugar-, acid- and phenol contents in apple cultivars from organic and integrated fruit cultivation. European Journal of Clinical Nutrition 60 (9): 1136–1140

Hui Y.H., Barta J., Pilar Cano M., Gusek T., Sidhu S.T., Sinha N. (2006). Handbook of fruits and fruit products. Blackwell Publishing, Iowa, USA, (697): 265-297

Jae-Yong K., Juyi S., Kyung-Hyun C. (2011). Aspartame-fed zebrafish exhibit acute deaths with swimming defects and saccharin-fed zebrafish have elevation of cholesteryl ester transfer protein activity in hypercholesterolemia. Food and Chemical Toxicology 49 (11): 2899-2905

Katalinić V. (2006). Kemija mediteranskog voća i tehnologija prerade, skripta I. dio, Kemijsko tehnološki fakultet, Split

Kheiralipour K., Tabatabaeefar A., Mobli H., Rafiee S., Sahraroo A., Rajabipour A., Jafari A. (2008). Some Physical Properties of Apple. Pakistan Journal of Nutrition 7: 667-672

Kroyer G. (2009). Stevioside and Stevia-sweetener in food: application, stability and interaction with food ingredients. Journal of Consumer Protection and Food Safety, DOI 10.1007/s00003-010-0557-3

Krpina I., Vrbanek J., Asić A., Ljubičić M., Ivković F., Ćosić T., Štambuk S., Kovačević I., Perica S., Nikolac N., Zeman I., Zrinšćak V., Cvrlje M., Janković- Čoko D. (2004). Voćarstvo, Nakladni zavod Globus, Zagreb

Lemus-Mondaca R., Vega-Gálvez A., Zura-Bravo L., Ah-Hen K. (2012). Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chemistry 132 (3): 1121-1131

Lewis M., Heppell N. (2000). Continuous Thermal Processing of Foods: Pasteurization and Uht Sterilization, Aspen Publishers Inc, Maryland, USA

Lopez M.L., Lavilla M.T., Riba M., Vendrell M. (1998). Comparison of volatile compounds it two seasons in apples: Golden Delicious and Granny Smith. Journal of Food Quality 21 (2): 155-156

Mahdavi R., Nikniaz Z., Rafraf M., Jouyban A. (2010). Determination and Comparison of Total Polyphenol and Vitamin C Contents of Natural Fresh and Commercial Fruit Juices. Pakistan Journal of Nutrition, 9: 968-972

Manach C., Scalbert A., Morand C., Remesy C., Jimenez L. (2004). Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition, 79: 727-747

Markowski J., Płocharski W. (2006). Determination of Phenolic Compounds in Apples and Processed Apple Products, Journal of Fruit and Ornamental Plant Research, 14:133-142

Miljković I. (1991). Suvremeno voćarstvo, Znanje, Zagreb

Mishra P., Singh R., Kumar U., Prakash V. (2010). Stevia rebaudiana –A magical sweetener. Global Journal of Biotecnology & Biochemistry, 5: 62–74

Mitchell H.L. (2006). Sweeteners and Sugar Alternatives in Food Technology, Blackwell Publishing Ltd., Oxford, UK, (412): 341-347

Nawirska-Olszańska A., Biesiada A., Sokół-Łętowska A., Kucharska A.Z. (2011). Content of Bioactive Compounds and Antioxidant Capacity of Pumpkin Puree Enriched with Japanese Quince, Cornelian Cherry, Strawberry and Apples, *ACTA* Scientiarum Polonorum Technologia Alimentaria, 10: 51-60

Picouet P.A., Landl A., Abadias M., Castellari M., Viñas I. (2009). Minimal processing of a Granny Smith apple purée by microwave heating. Innovative Food Science and Emerging Technologies, 10: 545–550

Pól J., Hohnová B., Hyötyläinen T. (2007). Characterization of Stevia rebaudianaby comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. Journal of Chromatography A 1150 (1-2): 85–92

Puri M., Sharma D., Tiwari A.K. (2011). Downstream processing of stevioside and its potential applications, Biotechnology Advances, 29: 781-791

Raymond K.W. (2010). General Organic and Biological Chemistry, John Wiley & Sons, New York, USA vol. 516, 364-368

Rembiałkowska E., Hallmann E., Rusaczonek A. (2007). Influence of Processing on Bioactive Substances Content and Antioxidant Properties of Apple Purée from Organic and Conventional Production in Poland. 3rd QLIF Congress, Hohenheim, Germany

SAS Institute (1990). SAS/STAT User׳s Guide, version 8 edition. Vol. 2. SAS Institute, Cary, New York, USA

Scalbert, A., Manach, C., Morand, C., Rémésy, C., Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition, 4: 287-306

Scalbert A., Johnson I.T., Saltmarsh, M. (2005). Polyphenols: Antioxidants and beyond. The American Journal of Clinical Nutrition 81(suppl): 215S-7S

Schieber A., Keller P., Carle R. (2001). Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography, Journal of Chromatography A, 910: 265–273

Seema T. (2010). Stevia rebaudiana: A medicinal and nutraceutical plant and sweet gold for diabetic patients. International Journal of Pharmacy & Life Sciences, 8: 451-457

Singh S., Rao G. (2005). Stevia: The herbal sugar of 21st Century. Sugar Technology, 71: 17–24

Singleton V.L., Rossi J.A. (1965). Colorimetry of total phenolics with phoshomolybdic phosphotungstic acid reagents, American Journal of Enology and Viticulture, 16: 144-158

Sinha N., Sidhu J., Barta J., Wu J., Pilar Cano M. (2012). Handbook of Fruits and Fruit Processing, Wiley-Blackwell Publishing, Iowa,USA

Valentine L. (2005). The Stevia Alternative. The Epoch Times (Health)

Weihrauch MR, Diehl V. (2004). Artificial sweeteners- do they bear a carcinogenic risk? Annals of Oncology 15 (10): 1460–5

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