Influence of Fruit Content and Storage Time on Gel Strength and Polyphenolic Content in Chokeberry Low Sugar Pectin-Gel Products

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ABSTRACT

The aim of this research was to investigate the influence of fruit level (20, 35, 45 and 75 %) and kind of sugar (sucrose or fructose) on polyphenolics' amount and gel strength in low sugar chokeberry pectin gel products during 6 months storage at +20°C. Pectin gel products were prepared form chokeberry purée obtained by cooking and pureeing of chokeberry fruits. Moreover, chokeberry fruits and purees were frozen and stored at -18°C during 6 months and also investigated in terms of polyphenolics. In all chokeberry products soluble solids were ranged 41.80 to 42.20 °Brix, pH from 3.06 to 3.48, and total acidity from 0.95 to 1.17 % (expressed as g citric acids per 100 g sample). Gel strength in all samples was measured by dr. D. Šulc pectinometer. Before storage gel strength of samples with fructose was slightly higher than those with sucrose, but during storage samples with sucrose showed more stable gel strength. Total phenols (TP), flavonoids (TF), and nonflavonoids (TN) were determined by Folin-Ciocalteu method. Higher amount of TP, TF and TN were observed in chokeberry purée compared to chokeberry fruit as well as in both frozen samples after 6 months storage compared to start samples. Further, TF were found in higher level than TN in all investigated samples. Further, higher amount of TP and TF was observed in all gel products with added fructose. Higher fruit level resulted with higher amount of TP, TF and TN in all samples. After 6 months storage TP, TF and TN decreased with retention of TP between 65 – 78 %. Finally, to produce more acceptable nutritive products it could be recommended to produce low sugar gel products with added fructose, and increased fruit level.

Keywords: chokeberry, polyphenolics, pectin gel products, low sugar jam, gel strength

INTRODUCTION

Chokeberry (*Aronia melanocarpa*) is a fruit native to eastern North America and East Canada (Hardin, 1973; Seidemann, 1993; Strigl et al., 1995; Jeppsson, 2000; Rugina 2012), from where its cultivation has been spread to Europe in 20th century firstly to Germany than Russia and more recently to East European countries (Seidemann, 1993; Strigl et al., 1995). Latterly it has become more popular in Croatia where has aroused great interest for both growing, processing and consuming due to high level of polyphenols and its remarkable positive influence on human health (Scalbert et al., 2005).. Chokeberry is extremely resilient and adaptable plant (Savjetodavna služba, 2014), and its berries are known as the richest polyphenols source (Sikora et. al., 2008) which possess health protective properties due to high antioxidant capacity. In chokeberry total polyphenols are determined from 40 to 70 mg g^{-1} dry matter (Sikora et al., 2008) what means 2-4 % of soluble solids belong to polyphenols (Oszmianski, Wojdylo, 2005; Slimestad et al., 2005). Among chokeberry polyphenols remarkable share make anthocyanins, according to some studies more than 50% (Jakobek et al., 2007, Sikora et al., 2008) and to some others study about 25 % (Oszmianski, Wojdylo, 2005). Kokotkiewicz et al. (2010) reported procyanidins as the most aboundant polyphenols in chokeberry. Further, in high concentration are presented hydroxycinnamic acids, represented mainly by chlorogenic, and neochlorogenic acids (Sikora et al., 2008), and caffeic acid and its derivatives (Zheng, Wang, 2003;

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Jakobek et al., 2007). Flavan 3-ols and flavonol glycosides are also identified in chokeberry (Määtta-Riihinen et al., 2003; Slimetsad et al., 2005). Phenolic acids belong to polyphenolic subcategory named nonflavonoids, and all others mentioned to subcategory named flavonoids (Belitz et. al., 2004. Flavonoids such as flavonols, quercetins, and anthocyanins, as well as phenolic acids, such as caffeic acid showed high antioxidant activity in chokeberries (Zheng, Wang, 2003). Further, anthocyanins are responsible for deep purple colour, and procyanidin (monomers include flavan 3-ol: (+)-catechin) and aforementioned acids are responsible for very tart taste of fresh chokeberries. Due to tart taste they are rarely consumed in fresh state (Sikora et al., 2008). Besides juice, pectin gel products as jams are usual products of chokeberry. Fruit level in jams and related products vary upon to recipe. According to legislation (COUNCIL DIRECTIVE 2001) gel products which contain minimally 350g fruit per kg of final product are called jam, and product which contain minimally 450g fruit per kg are called extra jam. During processing gel products fruit is exposed to high temperature and level of polyphenols as sensitive compounds could be decreased. Due to its biological activity it is desirable to preserve it in final products in as much as possible high concentration. Helstrom et al. (2007) studied the influence of several ways of processing chokeberry, and reported that stability of polyphenols depends on applied technology, and investigated subcategories of polyphenols showed different stability. Processing into jams caused losses from 25 % (boling time 5 minutes) to 40 % (boiling time 15 minutes) but affect the polyphenol profile, too. Phenolic acids showed the highest recovery after 15 min boiling followed by flavonols, then anthocyanins. Additionally, time of cooking did not effect on procyanidins (Helstrom et al., 2007).

Besides fruit level, legislation (COUNCIL DIRECTIVE 2001) defines minimally 60 % of soluble solids in jams and extra jams, with exceptions if low sugar jams or extra jams are produced. Mostly of soluble solids are sugars. In order to keep calorie count in order, many consumers prefer the consumption of low sugar jams (Levaj et al., 2010). Further, fructose is more preferred as sugar source than sucrose especially for diabetics (Uusitupa, 1994).

Therefore, the aim of this research was to investigate the influence of of fruit level (20, 35, 45 and 75 %) and sugar type (sucrose or fructose) on polyphenolics' concentration and gel strength in low sugar chokeberry pectin gel products during 6 months storage at ambient temperature.

MATERIALS & METHODS

Materials

Chokeberry (*Aronia Melanocarpa* cv.Viking) was used to produce low sugar jams (42 ºBrix) according to several recipes which differ in the fruit amount (200, 350, 450 and 750 g kg^{-1}). Food grade commercial crystal sucrose or fructose, citric acid and low ester amidated pectin were used, too. In plactic containers frozen chokeberry fruits were transported from producers to laboratory. In the same containers fruits were defrosted, moved in appropriate vessel, cooked and hot was pureed, through the sieve (0,8mm). Obtained purée was cooled, packed in polypropylene bags, frozen and stored at -18 °C till preparation gel products and during 6 months as well as frozen fruit to monitor stability of polyphenols.

Preparation of pectin gel products. Two kg of each gel products were prepared (4 with added sucrose and 4 with added fructose). Pureé was mixed with an amount of water and cooked under atmospheric pressure with addition mixture of one part of crystal sugar (sucrose or fructose) and pectin. Process of cooking/boiling was continued with stirring followed by addition rest of defined amount of sugar. After few minutes solution of citric acid was added and the mass was cooked until reaching 42 ºBrix. Amount of pectin and citric acid was adjusted to achieve the standard gel strength of products (50 g cm^{-2}).

Finally, products were filled into glass jars (240 mL). After filling and closing glass jars, gel products were pasteurized in water bath (20 min/85 ^{o}C) and cooled at ambient temperature. After 24 hours gel products were stored in the dark at +20°C during 6 months.

Methods

Physical and chemical parameters. Soluble solids were determined by measuring % Brix (Leica 7531L refractometer) and results were expressed in Brix percentages (^{o}Brix), pH values of the samples

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were determined by a pH meter (METTLER TOLEDO), and total acidity by titration with 0.1 M NaOH (expressed as g citric acids per 100 g sample).
Gel strength (g cm^{-2}) of gels products was measured by D.Šulc pectinometer (Šulc, 1983) which measures the force needed to rupture the gel. Gel products were kept at room temperature for at least 1 h before measuring
Polyphenolics. Total phenols (TP), flavonoids (TF) and nonflavonoids (TN) were determined using Folin-Ciocalteu colorimetric method (Singelton, Rossi, 1965).
All samples were analysed in triplicate and results were expressed as average values±SD. All results were statistically analysed using MANOVA (StatSoft Inc. Tulsa, SAD, Single User Version. University of Zagreb, 2014).

RESULTS & DISCUSSION

The values of the soluble solids, total acidity and pH of the fruits are in accordance with data in the literature (Kulling, Rawel, 2008). During the production of pureé, soluble solids and total acidity decreased, as a result of the production process of the pureé (Table 1).
Soluble solids of products were approximately 42% which was defined by the recipe. The total acidity and pH (Table 1).in pectin gel products were in accordance to earlier findings (Levaj et al. 2008).

Table 1. Physical and chemical properties of chokeberry fruit, pureé and low sugar pectin-gel products

Samples		Soluble solids (%)		Total acidity (%)		pH value	
Fruit		17,35 ± 0,07		1,54 ± 0,00		3,42 ± 0,01	
Pureé		10,90 ± 0,14		0,93 ± 0,01		3,48 ± 0,00	
Products / Kind of sugar		Sucrose	Fructose	Sucrose	Fructose	Sucrose	Fructose
Amountof pureé (g)	200	42,20 ± 0,00	42,05 ± 0,07	0,97 ± 0,01	0,96 ± 0,01	3,06 ± 0,01	3,11 ± 0,01
	350	41,95 ± 0,07	41,80 ± 0,00	1,06 ± 0,01	1,06 ± 0,01	3,12 ± 0,00	3,21 ± 0,01
	450	42,15 ± 0,21	42,10 ± 0,14	1,03 ± 0,00	1,02 ± 0,00	3,14 ± 0,01	3,21 ± 0,01
	750	42,10 ± 0,00	42,00 ± 0,00	1,12 ± 0,01	1,17 ± 0,01	3,44 ± 0,00	3,48 ± 0,01

During the preparation of gel products addition of pectin and citric acid was adjusted to achieve the standard gel strength of jam (50 g cm-2). So, all values of gel strength before storage were in range from 48 to 55 g cm-2 (Figure 1).
Though gel-product with 200 g of fruit is not defined by aforementioned legislation, except for citrus fruit, we wanted to establish this recipe, too. In addition product with 750 g kg^{-1} is also extra jam according to aforementioned legislation but with higher fruit level. During storage in all samples, a decrease in gel strength was noticed and it was greater in samples with fructose in spite of their slightly higher initial values in comparison to ones with sucrose. Also lower decrease in gel strength was observed in samples with 200 g of fruit with sucrose and fructose than in the other samples. Due to lower proportion of fruit content in those samples greater amount of pectin was added to achieve standard gel strength. From the obtained results it can be concluded that the added pectin is more efficient in preserving stability of the gel strength than native pectin present in the fruit purée. After 6 months of storage the sample of 200 g fruit with sucrose had the highest strength and sample with 750 g fruit with fructose the lowest gel strength. Finally, the mutual influence of gel product type, sugar type and storage time on the gel strength of investigated products have been statistically confirmed ($p \leq 0.05$).

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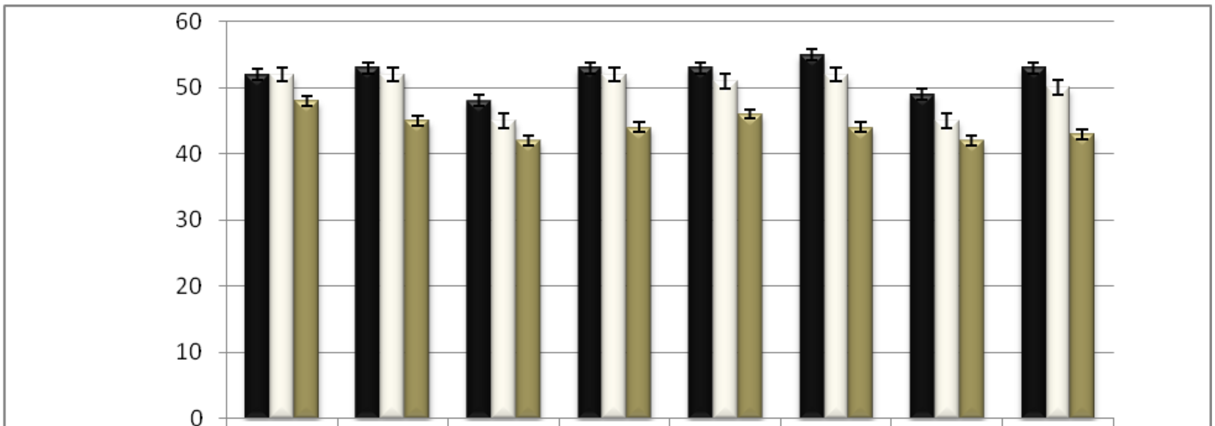

	200 S	350 S	450 S	750 S	200 F	350 F	450 F	750 F
before storage	52	53	48	53	53	55	49	53
after 3 months	52	52	45	52	51	52	45	50
after 6 months	48	45	42	44	46	44	42	43

Figure 1. Gel strength (g cm^{-2}) of chokeberry low sugar pectin-gel products during storage (200, 350, 450, 750 means g fruit per 1kg product, S – sucrose, F – fructose)

In the chokeberry fruit and purée high values of TP, TF and TN were determined (Table 2), and among them TF were determined in higher amounts than TN. These results correspond to earlier findings (Benvenuti et al., 2004; Oszmianski, Wojdylo, 2005; Rop et al., 2010). However, in purees were observed higher concentrations of TP, TF and TN than in fruit probably due to higher degree of cell membrane disintegration during fruit cooking and puréeing. It is known that mechanical and thermal processing of plant material, lead to the disruption of the natural matrix, and may enhance the bioaccessibility of bioactive food components (Parada, Aguilera, 2007). During storage of chokeberry fruit TP, TF and TN were increased. Interestingly, concentrations of TP, TF and TN in chokeberry purees slightly decreased after 3 months and after 6 months of storage were about the same concentrations as at the beginning.

Results of polyphenols in gel products are expressed per 100g of soluble solids. The highest proportion of soluble solids in pectin gel products belongs to added sugar, so soluble solids of fruit contribute to the total amount of soluble solids of products approximately only with 4 to 16 % in our cases. If actual level of fruit's soluble solids in total soluble solids of products are taking into consideration, it will be obvious that lower amount of polyphenols in products are more affected by recipe, less by processing. Theoretical or expected values of polyphenols in final products, calculated according the used amount of purée for certain product, and according to determined value of polyphenolics in purée, are even higher than experimentally found values. It could be concluded that polyphenols of chokeberry are quite stable during cooking. Helstrom et al. (2007) also reported that phenolic compounds in chokeberries were quite stable during the jam making. They reported individual phenols by HPLC so results are not completely comparable, particularly due to known interference of many substances, especially sugars (e.g. fructose) with the Folin –Ciocalteu reagent (Prior et al., 2005). So, non specifity of Folin-Ciocalteu method and interference with fructose (Prior et al., 2005) could be possible explanation for higher amount of TP and TF in all products with fructose. Results of TN in all products were very similar without uniform influence of kind of sugar.

Obtained results indicate lower level of TP before and after 6 months storage in extra jam and especially jam, commercially the most available pectin-gel products, in comparison with amount found in gel products with 750 g kg^{-1}. During storage decrease of all polyphenols was found. It was found that during storage TF were more stable in comparison to NF (losses were in range 68 – 98 % and 28 – 63 %, respectively).Further, after 6 months of storage TP in all samples decreased for approximately 37 % while in jam (350g fruit kg-1) the loss was about 23 %. Also, TF and TN in jam

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showed the highest stability. There is lack in the literature considering chokeberry polyphenols stability in jams during storage, but Wilkes et al. (2013) studied stability of polyphenols in chokeberry juices, and reported that anthocyanins declined linearly, whereas flavonols, total proanthocyanidins, and hydroxycinnamic acids were quite stable in juices stored for 6 months at 25 °C. The mutual influence of gel product type, kind of sugar and storage time on the TP, TF and TN of investigated products has been statistically confirmed ($p \leq 0.05$).

Table 2. Polyphenols content of chokeberry fruit, purée and low sugar pectin-gel products with various amount of purée during storage (mg 100^{-1} g^{-1} soluble solids)

Samples	Months of storage	Total phenols		Flavonoids		Nonflavonoids	
Fruit	0	5016.21±12.74		3699.64±15.39		1316.64±2.55	
	3	5106.27±12.74		3753.6±10.19		1352.67±2.55	
	6	5160.3±38.21		3775.22±56.04		1385.09±17.83	
Pureé	0	5920.3±60.82		4100.11±81.25		1820.53±20.27	
	3	5547.59±182.45		3758.72±166.07		1788.99±16.22	
	6	5934.63±40.55		4094.04±48.65		1840.6±8.11	
Products / Kind of sugar		Sucrose	Fructose	Sucrose	Fructose	Sucrose	Fructose
Amountof pureé (g) 200	0	270.09±3.16	324.4±2.10	172.62±4.21	228.42±2.10	97.47±1.05	95.98±1.05
	3	203.87±2.10	254.46±0.00	125.00±6.31	178.57±4.21	78.87±4.21	75.89±3.16
	6	180.06±2.10	232.14±3.16	118.3±3.16	180.06±5.26	61.76±105	52.08±1.05
350	0	505.21±3.16	554.32±1.05	325.15±1.05	375.74±5.26	180.06±2.10	178.57±2.10
	3	452.38±2.10	484.38±4.21	321.43±0.00	343.01±7.37	130.95±1.05	141.37±2.10
	6	397.32±1.05	427.08±2.10	320.68±1.05	348.96±1.05	76.64±1.05	78.13±2.10
450	0	1075.89±4.21	1107.89±1.10	827.38±6.31	860.86±3.16	248.51±3.16	247.02±6.31
	3	921.88±3.16	943.45±5.26	760.42±2.10	780.51±6.31	161.46±1.05	162.95±3.16
	6	674.85±1.58	723.21±3.16	580.36±0.00	638.39±6.94	94.49±2.10	84.82±2.10
750	0	1389.14±3.16	1478.87±3.58	1022.32±3.16	1116.52±6.31	366.82±1.02	362.35±3.16
	3	1214.29±4.21	1326.64±3.16	872.77±4.21	982.14±7.37	341.52±2.10	344.49±1.05
	6	882.44±1.05	989.58±2.10	776.79±3.16	883.18±4.21	105.65±1.05	106.40±1.05

CONCLUSION

Regardless of fruit level and sugar type, investigated pectin-gel products showed good stability of gel strength during 6 months storage in spite of reduced sugar amount. It was showed that cooking fruit as well as gel products had positive effect on concentration of polyphenols. But 6 month's storage of gel products resulted with decrease from 23–37 %. Further extra jam with 750 g fruit kg^{-1}still contains high concentration of polyphenols, so it is recommended to reformulate recipe and increase fruit level. Moreover, studies are needed for better understanding the behavior and stability of polyphenolics affected by kind of added sugar, during gel products producing and storage.

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