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PAPER

PROPERTIES OF STRAWBERRIES PUREE STORED IN THE FREEZER

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ABSTRACT

Four different strawberry varieties were used for puree production and stored at -18 ºC for 12 months. Every three months samples were tested for rheological parameters, polyphenols content and antioxidant activity. Mathematical models for rheological behaviour of the samples were determined together with consistency coefficient (k) and flow behaviour index (n). Fluidity of the samples increased over time, but pseudoplastic behaviour remained through tested period. The biggest decrease of polyphenol content was observed between 9 and 12 months of storage, while antioxidant activity decreased the most during first three months by DPPH and ABTS method.

Keywords: antioxidant activity, polyphenols, rheology, strawberries

1. INTRODUCTION

Current guidelines for fruit and vegetable consumption recommend five portions per day (HARTMANN *et al*., 2008). Due to seasonal character of many fruits, as well as hectic lifestyle, these recommendations are not easily achievable especially when targeting fresh fruit and vegetables. Thus, replacement of one or several of portions by fruit juices, concentrates or purees is suggested (HARTMANN *et al*., 2008). Consumers have increased interest to high-value food products, especially fruit, vegetables and other functional foods (BISHARAT *et al*., 2013). Therefore, food manufacturers are facing the challenge to create new products which, beyond basic nutrients, also provide certain health-promoting properties (OBRADOVIĆ *et al*., 2015). Strawberries are one of the most consumed berries worldwide. In European market average strawberries intake is 2.16 kg/year per person including raw and processed fruit (GASPEROTTI *et al*. 2015). These popular fruits are favoured for their attractive taste, and are considered as rich source of micronutrients and phytochemical compounds such as water soluble vitamin C and polyphenols (phenolic acids, anthocyanins, flavonols, tannins and other) (KLOPOTEK *et al*., 2005; OSZMIAŃSKI *et al*., 2009; BODELÓN *et al*., 2013; ŻEBROWSKA *et al*., 2019). Diet rich in fruit and vegetables is beneficial for human body. It lowers the risk of many diseases: diabetes, atherosclerosis, cardiovascular disease, inflammatory-related illnesses, and cancer. This is attributed to vitamins, dietary fiber and polyphenols (NOWICKA *et al*., 2019). Strawberries have very strong antioxidant activity. They have 1.3 times activity of oranges, 2 times that of red grapes, 5 times that of apples and bananas and 13 times that of honeydew melon (OSZMIAŃSKI *et al*., 2009). For all mentioned reasons, strawberries are considered as a functional food although exact mechanism involved is still generally unclear (GASPEROTTI *et al*., 2015). Unfortunately, strawberries are very perishable due to high water content and soft structure, and consequently have extremely short postharvest shelf-life (HOLZWARTH *et al*., 2012; PEINADO *et al*., 2012). Therefore, there is a huge demand for strawberries puree for use as a base product for preparation of juices and soft drinks, for addition to ice-creams and yoghurts (BODELÓN *et al*., 2013), or it can be sold directly to consumers in canned or frozen forms (DIAMANTE *et al*., 2016). Purees are usually preserved by freezing or by heat. Freezing results in cell destruction allowing reactions between genuine enzyme activities and their corresponding substrates. Thawing is especially critical since polyphenoloxidases (PPO) are responsible for polyphenols destruction (HOLZWARTH *et al*., 2012). In order to create puree of satisfying quality and nutritional value it is necessary to determine optimal storage time. Therefore, the objective of this study was to explore influence of freezing on strawberry purees. Rheological properties, polyphenol content and antioxidant activity were evaluated. Changes in the rheological properties of fruit purees that have undergone freezing or freeze–thaw treatments are of practical significance for their acceptance and consumption (DIAMANTE *et al*., 2016). Reduction in antioxidant activity during processing and storage may reduce the health beneficial effects of food products (OSZMIAŃSKI *et al*., 2009) and that was the reason for test puree samples for above mentioned parameters. Some works have reported the rheological characterization of different fruits like mango and papaya (EL-MANSY *et al*., 2005), blueberry (ANTONIO *et al*., 2007, NINDO *et al*., 2007), raspberry, strawberry, prune, peach (MACEIRAS *et al*., 2007, ERGOVIĆ RAVANČIĆ *et al*., 2012), nectarine and blackberry (ERGOVIĆ *et al*., 2009; ERGOVIĆ *et al*., 2010), but to the best of our knowledge they haven't followed rheological parameters together with nutritional characteristics over a period of time in order to determine how storage time in the freezer affects mentioned characteristics.

2. MATERIALS AND METHODS

2.1. Sample preparation

Sample S-1 was prepared from wild strawberries harvested in woods near Požega town, Slavonia region, Croatia. Strawberries for sample S-2 (Albion variety) and S-3 (Clery variety) were purchased from the local farmers and for sample S-4 (Joly variety) in the local supermarket. Fruits were cleaned and blended in kitchen blender at room temperature for 3 minutes, divided in small portions (100 mL), sealed in polyvinyl chloride freezer bags (at atmospheric pressure and temperature, vacuum or modified atmosphere haven't been used) and kept in chamber freezer at -18 ºC, until the analysis. Every three months three bags were thawed at room temperature as parallels for the analysis.

2.2. Sugar and acidity determination

Total and reducing sugars were determined according to the Luff-Schoorl method (GAFTA, 2018). Total acidity was determined by potentiometric titration.

2.3. Extract preparation

1 g of strawberry puree was extracted with 20 mL of acidified methanol (methanol/2% HCl, 95:5) at room temperature for 60 min with constant shaking in temperature-controlled shaker (Kottermann labortechnik) at 200 rpm and centrifuged (Tehtnica, Centric 322A). Glasses were covered with aluminium folium to prevent evaporation of **solvent.**

2.4. Total phenol content

Polyphenols were determined according the Folin-Ciocalteu method (OBRADOVIĆ *et al*. 2015, with modifications). An aliquot of the extract (200 μL) was mixed with 2 mL water and 100 μL Folin-Ciocalteu reagent (Kemika, Croatia). The mixture was allowed to equilibrate for 5 min, and then 300 μL of sodium carbonate solution (20%) was added. After incubation at room temperature in dark for 30 min, the absorbance of the mixture was read at 725 nm (Camspec M501, UK). Acidified methanol was used as a blank. Total polyphenols were determined with 3 replications. Gallic acid (Carlo Erba reagents, Italy) was used as a standard (calibration curve $y = 1.1979x - 0.0188$, $R^2 = 0.9984$), and results were expressed in mg of gallic acid equivalents per 100 g of sample.

2.5. Antioxidant activity determination (ABTS)

$ABTS^{\cdot+}$ radical was obtained by mixing 7.4 mM ABTS (Fluka, Switzerland) solution and 2.6 mM solution of ammonium persulfate in 1:1 ratio. Solution was left in dark through the night in order to develop stable radical, and then radical solution was diluted with ethanol in 2:70 ratio to obtain absorbance approximately 1.100 (A_{ABTS}). An aliquot of extract (0.2 mL), was mixed with 3.2 mL of diluted $ABTS^{\cdot+}$ radical. After incubation at room temperature in dark for 95 min, the absorbance of the mixture was read at 734 nm (A_{EXTR}), and ΔA was calculated as $A_{ABTS} - A_{EXTR}$. Trolox (Sigma Aldrich, USA) was used as a standard. Decrease in absorbance caused by trolox was done in the same way as for the samples, and standard curve ΔA/trolox concentration was created ($y = 489.13x - 17.903$, $R^2 = 0.9952$).

Determination of antioxidant activity was done in 3 replications. Results were expressed in μmol of the trolox equivalents per gram (OBRADOVIĆ *et al*., 2015).

2.6. Antioxidant activity determination (DPPH)

An aliquot of extract (50 μL) was mixed with 2 mL DPPH radical solution (0,1mM in ethanol). The absorbance of the mixture was read at 517 nm during period of 30 min, results were expressed as the mean of 3 replications. Pure ethanol was used as a blank.

$$\% \text{ inhibition} = [(A_0 - A_t)/A_0] \times 100 \quad (1)$$

A_0 - absorbance of DPPH radical solution,
A_t – absorbance after 30 minutes.

2.7. Rheological properties determination

The rheological properties were measured before storage in the freezer and after 3, 6, 9 and 12 months of storage by rotation rheometer, model VT 550 362-0001 HAAKE with concentric cylinders (RheoWin Pro 2.91 software). Diameter of inner rotating cylinder was 36 mm, inner diameter of outer stationary cap was 40 mm, gap between cylinders was 4 mm. The measurements were carried out in triplicate at 40°C, at shear rates 0 – 60 1/s. Temperature of 40°C has been selected as the closest to the temperature of "ready to eat" food containing puree. Puree is not expected to be eaten alone, it is usually used as a filling for some cakes like strudel, or as a dressing for pancakes and this is temperature of "ready to eat" product, close to the body temperature. For each shear rate computer recorded shear stress which was provided by the strawberry puree during rotation of the measuring cylinder of the rheometer. Flow curves are presented as the mean value of recorded results. Rheological parameters determined with experimental flow curves were fitted to Ostwald-de Waele (power law) model using a software (Excel 2016, USA).

$$\tau = k \cdot D^n \quad (2)$$

τ - shear stress (Pa),
k - consistency coefficient (Pas^n),
D - shear rate (1/s),
n - flow behavior indeks.
Samples were taken from the freezer and after thawing and reaching room temperature, rheological parameters were determined. Relation between shear rate and shear stress were presented graphically and determination coefficient (R^2) was calculated for each curve.

2.8. Data analysis

Chemical composition data were analysed by Statistica 12 software, using *post hoc LSD* at 95% level.

3. RESULTS AND DISCUSSION

Initial composition of strawberries puree samples is presented in Table 1. Parameters were tested as an indicator of ripeness to initially assess the starting material. S-1 sample, wild strawberries, had the highest sugar content and the lowest acidity. Sample S-2 is following with slightly lower content of sugars, but with much higher acidity, and the samples S-3 and S-4 were similar in sugar content, but sample S-3 had the highest acidity among all samples. These parameters were not expected to be significantly changed during storage, so they were not measured every three months.

Table 1. Composition of purees before storage.

	S-1	S-2	S-3	S-4
Total sugars (%)	15.40±0.22	13.42±0.16	8.22±0.26	8.64±0.04
Reducing sugars (%)	10.56±0,06	8.96±0.12	7.64±0.08	8.16±0.06
Total acidity (mmol/100g)	7.96±0.04	17.52±0.02	24.40±0.06	13.60±0.10

There are diverse phenolic compounds in strawberries, not only coloured anthocyanins, but also colourless phenols like ellagic acid, ellagitannins, *p*-coumaric acid and quercetins (HARTMANN *et al*., 2008). GASPEROTTI *et al*. (2015) identified and quantified 56 individual compounds in strawberries, with concentrations ranging from 1 μg/100 g to 40 mg/100 g. They also highlighted that this is not a complete list of polyphenols present in strawberries. Total phenol content in puree samples before and after 3, 6, 9 and 12 months of storage is presented in Table 2.

Table 2. Total phenol content in samples during 12 months of storage [A, B].

Sample	Total phenols (mg_{GAE}/100 g)				
	Before storage	After 3 months	After 6 months	After 9 months	After 12 months
S-1	422.59^{e}±4.72	415.10^{d}±2.82	404.60^{c}±5.06	400.89^{b}±1.55	368.98^{a}±0.44
S-2	196.92^{d}±6.96	192.66^{c}±0.16	189.96^{b}±0.98	188.12^{b}±0.46	170.49^{a}±1.47
S-3	164.68^{d}±0.99	152.15^{c}±0.83	151.62^{c}±3.39	147.49^{b}±1.41	135.21^{a}±0.47
S-4	185.70^{d}±3.12	165.69^{c}±0.89	163.35^{b}±3.94	163.91^{b}±4.53	151.47^{a}±1.19

[A]Results are expressed as mean of three repetitions ± standard deviation.
[B]Means followed by the same letter in the lines are not statistically different at 5% probability.

As presented by YILDIZ *et al* (2014) and DIAMANTI *et al* (2014), initial polyphenols content in wild strawberries puree (sample S-1) was more than double compared to cultivated strawberries purees (samples S-2 till S-4). Polyphenols content is in direct relation to the ripeness stage (sugar content and acidity presented in Table 1). Obtained results are similar to the values presented by GALOBURDA *et al*. (2014) and KLOPOTEK *et al*. (2005). It is already documented that strawberry phenolics such as pelargonidin, ellagic acid, *p*-coumaric acid, quercetin and kampferol derivatives are very unstable and undergo destruction during fruits transformation in frozen products especially in the thawing process by native and microbiological enzymes and by nonenzymatic oxidation

(AABY *et al*., 2007; OSZMIAŃSKI *et al*., 2009), but we couldn't find recommendations for storage time in freezer in order to preserve reasonable high level of polyphenols, and if initial value influences degree of degradation.
As it can be seen in Table 3, samples S-1 and S-2 had relatively low level of polyphenols destruction during first three months in the freezer (1.77 and 2.17%, respectively). Level of degradation continued over next months of storage, so both samples had percentage of degradation approximately 5% after 9 months of storage, which can be considered as good result. Between 9 and 12 months of storage a large decrease in polyphenol content can be seen and percentage of degradation during that period was higher than in previous 9 months. On the other hand, samples S-3 and S-4 had relatively high degradation during period of first three months (7.61 and 10.78%, respectively), which haven't changed significantly until the 9 months, so at the end of that period degradation was 10.44 and 11.74%. Still, the highest degradation was between 9 and 12 months. Therefore, it can be concluded that storage time of strawberry puree shouldn't be longer than 9 months in order to preserve phenolic compounds. In the end, percentage of degradation after 12 months of storage was higher in samples with lower initial values of polyphenols. It can be explained by the protective role of polyphenols (higher level of polyphenols-higher level of protection). Similar effect can be found in wines where red wines are less susceptible to degradation and require less chemical protection than white wines. HARTMANN *et al*. (2008) concluded that every processing step during production of juices and purees reduces the content of polyphenols, but they are better retained in purees than in juices. They also recommended a short enzymatic treatment of the mash with maceration enzymes in order to achieve maximal yield of polyphenols and antioxidant capacity. It is very important to obtain short enzymatic treatment because longer mash standing actually increases the loss. At the same time enzymatically treated puree was less viscous and smoother. While the nonenzymatically treated puree registered a water phase separation in less than 3 weeks of storage, which wasn't the case in this research. HOLZWARTH *et al*. (2012) reported that freezing technique did not have significant influence on polyphenols as well as on colour and ascorbic acid. Thawing method was, on the other hand, very important factor affecting mentioned parameters. Thawing at 20ºC and microwave thawing were favourable methods (compared to 4 ºC and 37ºC thawing). As previously explained, in this research samples were thawed at room temperature.

Table 3. Percentage of polyphenols degradation during storage compared to the initial value.

Sample	Degradation (%)			
	After 3 months	After 6 months	After 9 months	After 12 months
S-1	1.77	4.26	5.13	12.68
S-2	2.17	3.54	4.47	13.42
S-3	7.61	7.93	10.44	17.90
S-4	10.78	12.04	11.74	18.43

ABTS and DPPH methods have gained popularity for the study of antioxidant activity due to their speed and simplicity, and both of them are based on free-radical scavenging activity. Antioxidant activity by ABTS method is presented in Table 4 and by DPPH method in Table 5. Values are in accordance with the results presented by KLOPOTEK *et*

al. (2005) and NOWICKA *et al*. (2019). As expected, wild strawberries puree (S-1) had much higher antioxidant activity than other samples. Samples with higher content of polyphenols also had higher level of antioxidant activity by both methods. Contrary to polyphenols degradation, antioxidant activity by ABTS method in sample S-1 had the biggest decrease in first six months of storage. Sample S-3 (which had the lowest antioxidant activity at the beginning) lost almost 50% of the initial value by the end of a storage. Results obtained by DPPH method also show the biggest decrease in antioxidant activity during first three months. During the rest of the storage period further decrease was slower compared to first three months. Although polyphenols are the most important and the most popular antioxidants, there are also other molecules with antioxidant properties like products of Maillard reactions (OBRADOVIĆ *et al*., 2015), so direct correlation between polyphenols content and antioxidant activity is not always the case and it depends on method used. BAIANO *et al.* (2009) showed low correlation between amount of polyphenols in wines and antioxidant activity. They also concluded that beside previously mentioned antioxidants, antioxidant activity depends not only on the phenolic concentration, but also on the specific chemical structure of each phenolic compound.

Table 4. Antioxidant activity of samples during 12 months of storage (ABTS method) A, B.

Sample	Antioxidant activity (ABTS) (μmol TE/g)				
	Before storage	After 3 months	After 6 months	After 9 months	After 12 months
S-1	30.20^{d}±0.59	27.65^{c}±0.53	24.31^{a}±0.21	25.70^{b}±0.45	25.71^{b}±0.32
S-2	10.79^{e}±0.37	9.96^{d}±0.56	9.16^{c}±0.33	7.98^{b}±0.20	7.16^{a}±0.18
S-3	7.62^{e}±0.08	6.22^{d}±0.04	5.46^{c}±0.15	4.52^{b}±0.39	3.94^{a}±0.29
S-4	8.63^{d}±0.04	5.52^{c}±0.14	5.59^{c}±0.13	5.10^{b}±0.48	4.70^{a}±0.41

[A]Results are expressed as mean of three repetitions ± standard deviation

[B]Means followed by the same letter in the lines are not statistically different at 5% probability.

Table 5. Inhibition of DPPH radical after 30 minutes[A, B].

Sample	Inhibition (%)				
	Before storage	After 3 months	After 6 months	After 9 months	After 12 months
S-1	80.02^{d}±1.82	71.80^{c}±2.04	70.54^{b}±1.94	68.38^{a}±2.20	68.13^{a}±1.56
S-2	49.39^{c}±0.96	39.63^{b}±1.14	39.93^{b}±1.55	39.49^{b}±1.24	38.95^{a}±0.93
S-3	43.94^{d}±1.06	36.14^{c}±0.58	36.60^{c}±1.86	32.75^{b}±0,64	31.44^{a}±0.88
S-4	49.74^{d}±1.58	40.35^{c}±0.98	38.20^{b}±0.60	38.27^{b}±1,46	37.21^{a}±1.38

[A]Results are expressed as mean of three repetitions ± standard deviation.

[B]Means followed by the same letter in the lines are not statistically different at 5% probability.

Beside chemical and nutritional properties, knowledge of the rheological properties of food products is important for process design, control of the process, and consumer acceptability of a product. Rheological properties provide information on how to control flow properties of the product so that the desired product can be prepared. Rheological

properties are explained by rheological parameters: flow behavior index (n) and consistency coefficient (k) (LOVRIĆ, 2003; OSORIO *et al*., 2008). Fruit purees are suspensions of solid matter in fluid media and have been categorized as time-independent non-Newtonian fluids showing a pseudoplastic behavior (RUDRA *et al*., 2007; SOROUR *et al*., 2016).

Rheological properties of strawberry puree samples are presented in Fig. 1. Shape of the curve in Fig. 1. shows that strawberry puree is pseudoplastic system. Pseudoplastic non-Newtonian flow behavior occurs when shear stress is increasing at a diminishing rate, while increasing shear stress decrease when fluid is subjected to higher shear rates. It leads to a convex profile curve in which tangential slope is decreasing with increasing shear rate (KREITH, 1999; FIGURA and TEIXEIRA 2007). This behavior is caused by decreasing molecular interactions within the molecular structure of the fluid during flow. Pseudoplastic behavior of all samples remains regardless of storage time. It can be seen that freezing caused decrease of shear stress values in all samples compared to the starting sample. Deviations presented in stress-strain graphs are result of a samples' inhomogeneity, but still, measured values fit well to the Ostwald de Waele model for pseudoplastic systems (Table 6, R^2 ranging from 0,911 till 0,994). The same trends were obtained by several authors. ALVAREZ *et al*. (2006) studied the rheological behavior of strawberry jam, MACEIRAS *et al*. (2007), BUKUROV *et al*. (2012) and YALÇINÖZ and ERÇELEBI (2016) researched the rheological properties of strawberry puree. All mentioned authors fitted results of rheological measurements to the same model.

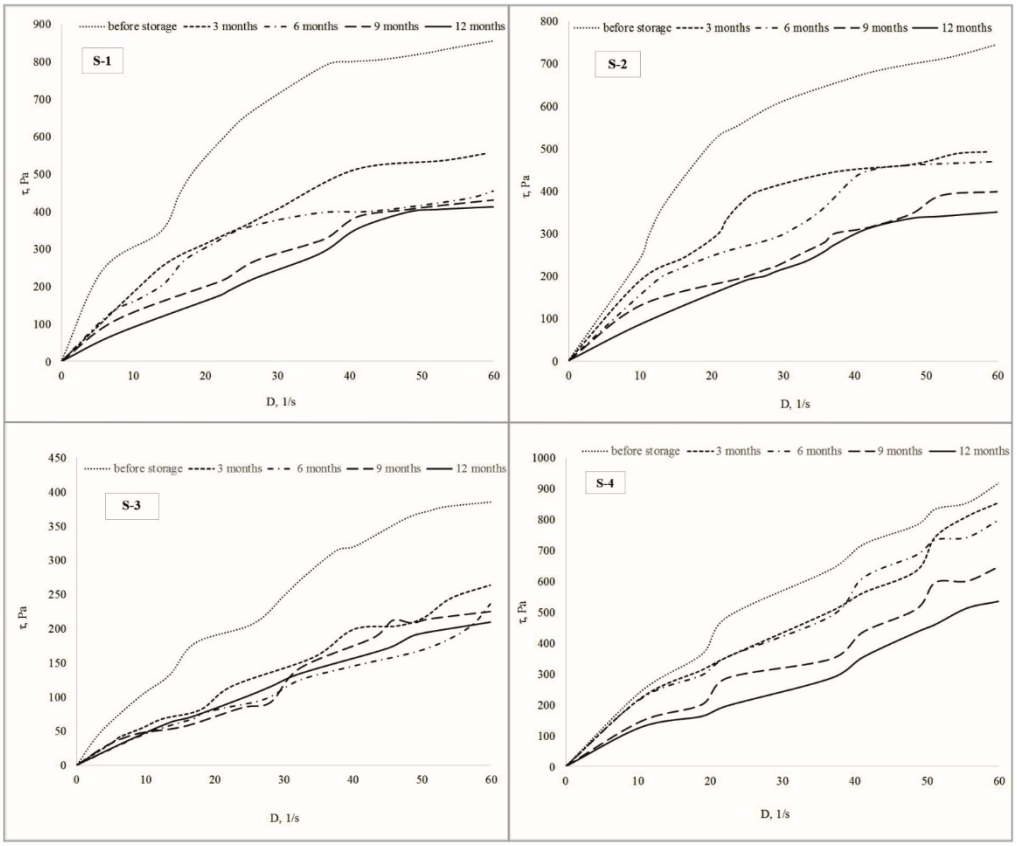

Figure 1. Rheological properties of strawberry purees during storage in the freezer.

Shear stress values were the highest in sample S-4 before and during storage with maximum shear stress 921 Pa and the lowest in sample S-3 with maximum shear stress 379 Pa at the shear rate 60 s^{-1}.

Table 6. Rheological parameters of strawberries puree samples during storage.

		Sample			
		S-1	**S-2**	**S-3**	**S-4**
Before storage	n	0.573	0.572	0.753	0.758
	k, $Pa \cdot s^n$	91.285	79.818	19.289	41.910
	R^2	0.953	0.911	0.994	0.991
After 3 months of storage	n	0.575	0.545	0.837	0.769
	k, $Pa \cdot s^n$	56.559	58.425	8.322	32.848
	R^2	0.985	0.930	0.994	0.979
After 6 months of storage	n	0.588	0.646	0.878	0.778
	k, $Pa \cdot s^n$	45.301	36.274	5.785	32.674
	R^2	0.941	0.953	0.988	0.983
After 9 months of storage	n	0.701	0.675	0.873	0.873
	k, $Pa \cdot s^n$	26.044	24.786	5.957	17.495
	R^2	0.987	0.962	0.961	0.971
After 12 months of storage	n	0.895	0.831	0.879	0.867
	k, $Pa \cdot s^n$	11.622	12.956	6.067	14.375
	R^2	0.990	0.9863	0.998	0.961

As shown in Table 6, flow behavior index values are within 0 and 1 (0,545-0,878), characteristic for pseudoplastic systems. Pseudoplastic fluid behavior is explained by cracking of the molecule structure when exposed to hydrodynamic forces and increasing the alignment of the constituent molecules. It can be seen that from the start samples S-1 and S-2 had lower values for flow behavior index than samples S-3 and S-4. This difference is obvious till 9 months of a storage, while after that flow behavior index is practically the same for all samples. Strawberry purees before storage in the freezer had the lowest values of flow behavior index and the highest values of consistency coefficient. Consistency coefficient is constantly decreasing during time in all samples. Before storage, samples S-1 and S-2 had higher k values than samples S-3 and S-4 (lower fluidity). Decrease in k value overtime indicates that fluidity of the sample increased during storage time. Moreover, weak physical bonds like electrostatic and hydrophobic forces might have been destroyed easily during shearing (ISANGA AND ZHANG, 2009). Destruction of cellular structure during freezing and thawing caused increase of flow behavior index value and decrease of consistency coefficient value. Although it is obvious that rheological behavior is changed during storage with increased fluidity, pseudoplastic behavior remained. Depending on the final purpose of puree, improvement of fluidity can be achieved by addition of hydrocolloids (ERGOVIĆ *et al*., 2010).

4. CONCLUSIONS

Based on the results of this paper it can be concluded that strawberry puree shouldn't be stored in the freezer longer than 9 months to avoid excessive polyphenol degradation because after 9 months of storage degradation of polyphenols accelerated in all samples. Regardless of the method used, antioxidant activity of all samples decreased significantly within the first three months of storage and continued to decrease further during the rest of the time. Fluidity of all samples increased during time, consistency coefficient decreased, flow behaviour index increased, but rheological parameters stayed within the limits for pseudoplastic systems. After all, depending on the final purpose of the puree optimal values for flow behaviour index and coefficient of consistency should be determined. Shelf life cannot be evaluated only based on polyphenols and additional tests are required.

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