Influence of cultivar, storage time, and processing on the phenol content of cloudy apple juice

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original scientific paper

Summary

The aim of this work was to investigate the influence of cultivar and storage time on the total phenol content (TPC) of three actual apple cultivars (Topaz, Pinova, Pink Lady) and three autochthonous apple cultivars (Ruzmarinka, Ljepocvjetka, Paradija) as well to determine their physical and chemical characteristics. Total phenol content has been determined by Folin-Ciocalteu reagent during cold storage at 1 °C, for 60 days. Changes of apple juice samples phenolics in relation to oxidation time after pressing (0 hours, 2 h and 4 h) were examined. A significant variation in content of phenols in apple cultivars under investigation was noted. Paradija, autochthonous apple contained the highest content of phenols (1.003 g GAE/L), while Topaz, actual apple cultivar had the lowest content (0.596 g GAE/L), during the storage time. TPC of apple cultivars stayed at a relatively constant level during the storage, with the exception of Pink Lady and Paradija apples, where TPC changed significantly during cold storage. The level of TPC in cloudy apple juice samples of all apple cultivars decreased during the oxidation time after pressing.

Keywords: autochthonous and actual apple cultivars, physical and chemical characteristics, phenols content, storage time and cloudy juice

Introduction

The positive effects of secondary metabolites (which occur abundantly in plant foods) on human health are now widely referred (Hertog et al., 1993a; Dragsted and Strobe, 1993; Gordon, 1996; Aruoma, 1998; Joshipura et al., 2001; Wolfe and Liu, 2003; Boyer and Liu, 2004; Chinnici et al., 2004; Manach et al., 2004; Scalbert et al., 2005, Kevers et al., 2011).

The phenol compounds form one of the main classes of secondary metabolites with a large range of chemical structure and contribute to the organoleptic and nutritional quality of fruits and vegetables. Apples (Eberhardt et al., 2000) and processed apple products as well (Lea, 1999; Boyer and Liu, 2004) are rich in those compounds. Hertog et al. (1993b) determined that apples are the third largest resources of flavonoids in the Dutch diet, right after tea and onions, and in the United States 22 % of the fruit phenols consumed come from apples. Phenolic compounds in unpeeled and undamaged apple are separated from polyphenoloxidase (PPO). When cells are injured and exposed to oxygen, phenolic compounds rapidly oxidize to ortho-quinones, which in turn polymerize quickly to brown or black pigments, such as melanin.

Enzymatic browning is one of the main oxidative phenomena inducing the development of undesirable color, flavor and a loss of nutrients and it causes a great concern to food processors and scientists (Nicolas et al., 1993; Amiot et al., 1997, Piližota and Šubarić, 1998). The rate of the enzymatic browning in apples depends on the PPO activity and polyphenols concentration (Harel et al., 1964; Harel et al., 1966; Coseteng and Lee, 1987), both of which change during growth and maturation (Harel et al., 1966; Mosel and Herman, 1974; Macheix et al., 1990; Renard et al., 2007; Kevers et al., 2011), cultivar difference (Vamos-Vigyazo, 1995; Lata, 2008; Kevers et al., 2011), storage (Amiot et al., 1995; van der Sluis et al., 2001; Goulding et al., 2001; Lattanzio et al., 2001; Lata, 2008) and on processing (Lea, 1999; Lu and Foo, 2000; Boyer and Liu, 2004; Markowski and Plocharski, 2006).

Autochthonous apple fruit cultivars in Bosnia and Herzegovina are valuable sources of desirable genetic characteristics including important pomological, nutritional and technological characteristics of the fruits. Within several domestic and international projects, some efforts have been made in order to determine and preserve those cultivars (Begić-Akagić et al., 2006).

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The total phenol concentration in apples is reported to maintain a relatively at constant level during storage (van der Sluis et al., 2001; Goulding et al., 2001; Lattanzio et al., 2001). However, there is some disagreement concerning changes in phenols during maturation and storage. According to Coseteng and Lee (1987); Carbone et al. (2011), certain phenolic compounds, such as catechins and chlorogenic acid, reported the change in concentration during storage.
Processing of apples has been found to affect phenols content (Oszmianski et al., 2011; Ibrahim et al., 2011; Bourvellec et al., 2011). Apple juice phenolics change enormously during milling and pressing, mainly due to oxidation which is mediated by an active polyphenoloxidase (PPO) system (Lea, 1999; Boyer and Liu, 2004).
The purpose of the present study was to determine (i) the physical and chemical characteristics of autochthonous and actual apple cultivars (ii) total phenol content of different apple cultivars during storage (iii) the effect of storage time on stability of total phenol content of autochthonous and actual apple cultivars, and (iv) the stability of phenols in cloudy apple juice (freshly pressed juice at 0 h, 2 and 4 hours oxidation after pressing) which were prepared from apples immediately after harvesting and after 60 days of apple storage.

Materials and methods

The experiment was divided into two parts. The first part of experiment involved evaluation of total phenol content (TPC) of the actual and autochthonous apple cultivars immediately after harvesting (control t1), and after 7, 21, 45, and 60 days of cold storage at 1 ºC (t2, t3, t4 and t5). The second part of experiment involved measuring of the stability of phenols in cloudy juice samples obtained from the analyzed apple cultivars, immediately after harvesting and after 60 days of apples storage, as freshly pressed juice (0 h) and after 2 and 4 hours oxidation after pressing.

Chemicals

Folin-Ciocalteu reagent and 2,6-dichlorophenol indophenols were purchased from Sigma Chemical Co. Sodium carbonate, 0.1 M NaOH (standard titration solution), Luff-solution and Gallic acid were obtained from Semikem d.o.o. (Sarajevo, B&H).

Apple cultivars used for experiment

Actual apple cultivars, Topaz, Pinova and Pink Lady, were purchased from Srebrenik Orchards (Srebrenik Orchards, Tuzla, B&H) as well as autochthonous apple cultivars, Ruzmarinka, Paradija and Ljepocvjetka. For the storage study, apples were picked at technological stage of maturity. Harvesting time for Pinova, Topaz, Paradija and Ruzmarinka was the end of September while Pink Lady and Ljepocvjetka were picked in early November (2006). Apple maturity was determined by iodine-starch test and by penetrometer. All apples were stored under the normal atmosphere at 1 ºC and 87 % RH before they were purchased for analyses.

Physical and chemical analysis of apple cultivars

Experiment comprised 5 apples of each cultivar by trial. All experiment was carried out in three replications. Soluble solids content was measured with table Abbe refractometer (EUROMEX, Holland) and given in Brix (°Brix). Acids were measured by titration with 0,1 M NaOH and phenolphthalein as an indicator and given in mmol/100 g as malic acid. Reducing sugar content was determinated by Luff Schoorl's methods and vitamin C (L-ascorbic acid) by volumetric method - titration with dichlorophenol indophenols (DCPIP). Physic-chemical methods (soluble dry matter, acids, and L-ascorbic acid) for analyzing apple samples were made according to the current Regulation[1].

Apple juice preparation

The entire experiment was conducted under laboratory conditions of Faculty of Agricultural and Food Sciences, University Sarajevo. The preparation of cloudy juice samples is carried out in five periods: immediately after harvesting and after 7, 21, 45 and 60 days of storage. Preparation of 100 % cloudy apple juice samples included the following operations: inspection, washing, separating peel and stems, milling, pressing and filling. Juice samples were obtained from six randomly selected apples in each trial to minimize variation. Juice samples were squeezed over 8 layer cheese cloth. The juice presented edible portion of apple without the peel. Filling juice into bottles was performed immediately after squeezing and for the second part of experiment in three ways: freshly pressed juice, 2 and 4 hours oxidation after pressing. All experiment was carried out in three replications.

[1]*Pravilnik o metodama uzimanja uzoraka i vršenja hemijskih i fizičkih analiza radi kontrole kvaliteta proizvoda od voća i povrća (Sl. list SFRJ br. 29/83 – preuzeto Sl. list RBiH br. 2/92)*

Determination of Total Phenol Content

The total phenols contents in the apple samples were measured by using a modified colorimetric Folin-Ciocalteu method (Ough and Amerine, 1988). A volume of 0.2 mL apple juice and 1.8 mL of deionizer water were added to the test tube. Folin-Ciocalteu reagent 10 mL (1:10) was added to the solution and allowed to react for 6 min. Then, 8 mL of 7.5 % solution of sodium carbonate was aliquoted into the test tubes. The color was developed in 120 min, and the absorbance was read at 765 nm by spectrophotometer (SHIMADZU, model UV-1700, Japan). The measurement was compared to the standard curve of prepared gallic acid solution and expressed in g of gallic acid equivalents (GAE)/L of sample. Measurements were performed in triplicates.

Statistical Analysis

For determining the effect of cultivar on physical and chemical parameters included a descriptive analysis, followed by one-way analysis of variance (ANOVA) and determined differences were tested by Tukey test (significance level p=0.05).

Statistical test included a descriptive analysis, followed by two-factorial analysis of variance (ANOVA) for determining the effect of storage time on stability of phenols content of different apple cultivars and pair wise multiple comparisons were done by Dennett's significant difference test with the family error rate held at 0.05.

Results of phenols stability in juice samples, immediately after harvesting and after 60 days storage, as freshly pressed juice and 2 and 4 hours oxidation after pressing, were compared by three-factorial analysis of variance (using SPSS 16 program) and determined differences were tested by Tukey test (significance level p=0.05).

Results and discussion

Obtained results of physical-chemical analysis autochthonous and actual apple cultivars are shown below (Table 1).

Table 1. The physical and chemical characteristics of autochthonous and actual apple cultivars

Apple cultivar	Soluble solids (ºBrix)	Total acid (mmol/ 100 g)	Reducing sugars (%)	L-ascorbic acid (mg/100 g)
Pinova	12.00^{ab} ±1.080	4.39^{b}±0.164	6.94^{a}±0.223	0.82^{b}±0.161
Topaz	13.13^{a} ±0.818	8.84^{a}±1.238	5.48^{bc}±0.365	2.99^{a}±0.435
Pink Lady	13.57^{a}±0.419	5.67^{b}±0.330	4.55^{c}±0.223	4.12^{a}±0.651
Ruzmarinka	10.80^{b}±0.589	1.39^{c}±0.163	4.87^{c}±0.298	1.16^{b}±0.236
Ljepocvjetka	12.33^{ab}±0.624	4.80^{b}±0.589	5.00^{c}±0.234	1.14^{b}±0.217
Paradija	11.75^{ab}±0.312	1.73^{c}±0.095	6.28^{ab}±0.369	1.17^{b}±0.233
W0.05	2.33	1.96	0.926	1.20

*Values in the same column with different superscript(a-c) are significantly different by Tukey test

As it can be seen in Table 1, soluble solids in the apple cultivars ranged from 10.80 ºBrix (Ruzmarinka) to 13.57 ºBrix (Pink Lady). Generally, the soluble solids in actual apple cultivars were higher than the autochthonous and it ranged from 12.00 ºBrix (Pinova) to 13.57 ºBrix (Pink Lady). According to Begić-Akagić et al. (2006), soluble solids in the autochthonous apple cultivars are in the range from 8.9 ºBrix (Bukovija) to 12.81 ºBrix (Senabija). Soluble solids content of Topaz and Pinova apple cultivars are 13 ºBrix and 14 ºBrix (Fischer and Fischer, 2002), and to Babojelić Skendrović et al., (2007), for Pink Lady 16.4 ºBrix.

Acidity of the samples ranged from 1.39 mmol/100 g in Ruzmarinka to 8.84 mmol/100 g (Topaz). Tukey test has shown that cultivar Topaz has significantly higher total acid content than others and Ruzmarinka and Paradija have the lowest. Total acid of actual apple cultivar Topaz was consistent with the results obtained by Babojelić Skendrović et al. (2007), where the total acid of Topaz apple was 0.54 %. The content of reducing sugar in evaluated autochthonous apple cultivars ranged from 4.55 % (Pinova) to 6.94 % Pink Lady. According to Miličević (2005), content of reducing sugar in autochthonous apple cultivars ranged from 3.25 % to 5.38 % which was in line with obtained results. Results regarding the biochemical content of apples obtained by Campeanu et al. (2009), present a high content of total sugar, which varies between 9.53 and 12.34 %. The sugar content of apples differs depending on the weather conditions, cultivars, culture technology, position and exposition of the fruits in the crown (Mitre at al., 2009; Sestras et al., 2009). In our case, low contents of reducing sugar as well soluble solids are obtained may be due to weather conditions with low temperature and low nutritive element contents of soils, which permitted the assimilation of

sugars. L-ascorbic acid (vitamin C) varies between 0.82 mg/100 g and 4.12 mg/100 g (Pink Lady). According to Fischer and Fischer vitamin C ranged from 0.9 (Feedom) to 21.9 mg/100 g (Pilot). Vitamin C in autochthonous apple cultivars was between 2.13 mg/100 g and 4 (mg/100 g) Senabija (Begić-Akagić et al., 2006).

In Table 2 results of TPC of apple cultivars during storage are presented. The average phenol content during the storage of apple cultivars decreased. Generally, the decline of phenol content was in the range from 20.19 % (Pink Lady) to 24.49 % (Topaz), with the exceptions of Ljepocvjetka and Pinova, where the lowest decline of 9.99 and 14.69 % were noted.

Table 2. Changes of phenols content in apple cultivars during the storage time

Storage time	Phenols content of apple cultivars (g GAE/L)*					
	Autochthonous apple cultivars			Actual apple cultivars		
	Ruzmarinka	Ljepocvjetka	Paradija	Pinova	Topaz	Pink Lady
t1	$0.789^{a} \pm 0.074$	$0.791^{a} \pm 0.051$	$1.161^{a} \pm 0.126$	$0.735^{a} \pm 0.074$	$0.704^{a} \pm 0.079$	$0.738^{a} \pm 0.052$
t2	$0.797^{a} \pm 0.028$	$0.784^{a} \pm 0.014$	$0.989^{a} \pm 0.075$	$0.707^{a} \pm 0.064$	$0.59^{a} \pm 0.085$	$0.697^{a} \pm 0.042$
t3	$0.772^{a} \pm 0.013$	$0.802^{a} \pm 0.019$	$0.987^{a} \pm 0.139$	$0.702^{a} \pm 0.026$	$0.594^{a} \pm 0.136$	$0.649^{a} \pm 0.044$
t4	$0.66^{a} \pm 0.040$	$0.743^{a} \pm 0.021$	$0.967^{a} \pm 0.039$	$0.637^{a} \pm 0.012$	$0.562^{a} \pm 0.023$	$0.615^{b} \pm 0.026$
t5	$0.604^{a} \pm 0.033$	$0.712^{a} \pm 0.043$	$0.913^{b} \pm 0.048$	$0.627^{a} \pm 0.019$	$0.532^{a} \pm 0.025$	$0.589^{b} \pm 0.030$
Retentions of phenol content (%)	76.55	90.01	78.64	85.31	75.57	79.81
Mean cultivar**	$0.724^{bc} \pm 0.089$	$0.766^{b} \pm 0.047$	$1.003^{a} \pm 0.126$	$0.682^{c} \pm 0.063$	$0.596^{e} \pm 0.01$	$0.658^{cd} \pm 0.067$

*Different letters mark significant difference between means in the same column (P<0.05) by Dennett's significant difference test
**Values in the row with different superscript (a-e) are significantly different by Tukey test

During the cold storage, level of TPC of all apple cultivars slightly decreased. TPC of Paradija was significantly higher in the control sample (after harvesting t1) than during the 60 days storage ($p<0.05$). There are no significant differences in the TPC of Pinova, Topaz, Ruzmarinka and Ljepocvjetka between control samples and samples used during each storage period. After 21 days of storage, the TPC in Pink Lady was significantly different from the control sample. TPC measured in analyzed apple cultivars during storage was in line with Perez-Ilzarbe et al. (1997) where in the pulp, apple phenolic compounds decreased during the cold storage. Carbone et al. (2011) studied the influence of genotype, tissue type and cold storage on bioactive compounds of different apple cultivars, where total phenol content was dramatically reduced after cold storage (flesh 50 %; peels 20 %). On the other hand, results by Burda et al., (1990) showed that the concentration of the major phenolics, epicatechins, procyanidin B_2, and phloretin glycosides in apple flesh remained at a relatively constant level during the storage. Since individual phenolic compounds have shown to vary in their browning rates (Oleszek et al., 1989), it is important to know that the concentration of individual phenol in apples changes during the storage. The significant change of the TPC, during cold storage of the apples, such as Paradija and Pink Lady, may be due to higher concentration of the individual phenolic compounds, such as catechins and chlorogenic acid extracted during sample preparation.

Among the apple cultivars, the TPC in Paradija fruit was significantly higher 1.003 g GAE/L, when compared to the TPC in the other samples for Ljepocvjetka, Ruzmarinka, Pinova, Pink Lady, and Topaz (Table 2). The differences in phenol content between apple cultivars were significant ($p<0.05$), except for Ljepocvjetka and Ruzmarinka, and for Ruzmarinka, Pinova and Pink Lady ($p>0.05$).Total phenols of actual apple cultivar Topaz were consistent with the results obtained by Markowski and Plocharski (2006), where the total phenols in cloudy juice of Topaz apple was 539.4 mg/L.

There are no data available for any autochthonous apple cultivars from the region of Bosnia and Herzegovina. Certain lower phenol contents for some apple cultivars are probably due to sample preparation, since the peel was removed before preparation. This corresponds to data obtained by Awad et al. (2000), showing that the peel and seeds contain the highest levels of flavonols. The same results were obtained by Wolfe et al. (2003); Khanizadeh et al. (2008); Oszmianski et al. (2011), showing that TPC tended to be highest in the peel, followed by the flesh of analyzed apple cultivars and

apple pomace as opposed to phenols content in raw juice. Different type of phenols presented in different parts of the fruit affect the concentration of phenols in juice, puree and processed apple products. Only small amounts of quercetin glycosides and dihydrochalcones are extracted during juice production, because quercetin glycosides are presented mainly in the peel, and dihydrochalcones in the seeds (Markowski and Plocharski, 2006). According to Renard et al. (2007) flavonols were absent or at the trace level only in the flesh of analyzed apples, but in much higher concentrations in the peel, and their concentrations decreased in the apples for cider production but increased in the table apples.

According to numerous results published (Lea, 1999; Lu and Foo, 2000; van der Sluis et al., 2002; Guyot et al., 2003; Wolfe and Liu, 2003; Boyer and Liu, 2004) the processing of apples is found to significantly affect the phenol content. Apple juice obtained from Jonagold apples by pulping and straight pressing had 10 % of the antioxidant activity of fresh apples, while juice obtained after pulp treatment by enzymes had only 3 % of antioxidant activity. After pulp treatment by enzymes, the juice contained 31 % less phloridzin, 44 % less chlorogenic acid, and 58 % less catechin. Most of phenol compounds remained in the apple pomace (van der Sluis et al., 2002).

The analysis of variance of phenol content indicated a statistically significant effect of cultivar, storage time, oxidation time after pressing (Table 3), and interaction between cultivar and storage time, as well.

Table 3. Three-factorial analysis of variance (effect of cultivar, storage time, oxidation time after pressing)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Cultivar (Cv)	1.678	5	0.336	85.579	0.000
Storage term (st)	0.621	1	0.621	158.354	0.000
Oxidation (ox)	0.574	2	0.287	73.173	0.000
Cv* st	0.088	5	0.018	4.464	0.001
Cv* ox	0.052	10	0.005	1.323	0.235
st * ox	0.005	2	0.003	0.647	0.526
cv * st * ox	0.015	10	0.001	0.373	0.954
Error	0.282	72	0.004		
Total	48.310	108			
Corrected Total	3.315	107			

Table 4. Total phenol content (g GAE/L) in apple cultivars and in obtained juice samples immediately after harvesting and 60 days of storage

Cultivar	Phenol content (g GAE/L) in apple juice						Mean of storage term**		Mean of cultivar*
	Immediately after harvesting			60 days after storage			Immediately after harvesting	After storage	
	Oxidized apple juice			Oxidized apple juice					
	0h*	2h*	4h*	0h*	2h*	4h*			
Ljepocvjetka	0.791^{b} ±0.063	0.710^{b} ±0.041	0.683^{b} ±0.037	0.712^{b} ±0.053	0.595^{b} ±0.041	0.495^{b} ±0.026	0.728^{a}±0.064	0.601^{b}±0.100	0.664^{b} ±0.105
Paradija	1.161^{a} ±0.154	1.041^{a} 0.165	0.879^{a} ±0.034	0.913^{a} ±0.060	0.802^{a} ±0.050	0.646^{a} ±0.048	1.027^{a}±0.168	0.787^{b}±0.125	0.907^{a} ±0.189
Pinova	0.735^{b} ±0.091	0.597^{b} ±0.016	0.551^{b} ±0.075	0.627^{bc} ±0.023	0.574^{bd} ±0.054	0.496^{b} ±0.019	0.628^{a}±0.102	0.565^{a}±0.065	0.628^{c} ±0.102
Pink lady	0.738^{b} ±0.063	0.628^{b} ±0.055	0.596^{b} ±0.042	0.589^{c} ±0.037	0.527^{bd} ±0.027	0.462^{b} ±0.023	0.654^{a}±0.080	0.526^{b}±0.061	0.654^{c} ±0.080
Ruzmarinka	0.789^{b} ±0.091	0.674^{b} ±0.047	0.640^{b} ±0.062	0.604^{bc} ±0.041	0.477^{cd} ±0.020	0.420^{bc} ±0.026	0.701^{a}±0.090	0.500^{b}±0.086	0.601^{c} ±0.134
Topaz	0.704^{b} ±0.097	0.534^{b} ±0.074	0.534^{b} ±0.074	0.532^{c} ±0.030	0.412^{c} ±0.023	0.372^{c} ±0.023	0.591^{a}±0.111	0.439^{b}±0.075	0.515^{d} ±0.121
Mean of oxidation after pressing**	(0 h) 0.741^{a}±0.175		(2 h) 0.631^{b}±0.170		(4 h) 0.564^{c}±0.138		0.721^{a}±0.178	0.570^{b}±0.139	

*Different letters mark significant difference between means in the same column (P<0.05) by Tukey test
**Values in the row with different superscript (a-c) are significantly different by Tukey test

During the oxidation time after pressing, the level of phenols content in juice samples of all cultivars decreased.

The highest content of total phenols has Paradija juice in all cases, immediately after harvesting and 60 days storage (Table 4). In the same way this juice had statistically significant higher TPC than the other cultivars during observed time of oxidation after pressing with the exception of Ljepocvjetka, which is confirmed by Tukey test.

TPC of juice samples produced immediately after harvesting (0.721 g GAE/L) was statistically significantly higher than in samples produced 60 days after storage (0.570 g GAE/L), where the total phenols decreased for 20.94 %. Generally, the decline of phenol content was in the range from 17.44 % (Ljepocvjetka) to 28.67 % (Ruzmarinka), with the exception of Pinova, where the lowest decline of 10.03 % was noted.

TPC of apple juice samples decreased after the juice pressing, which is mediated by an active polyphenoloxidase (PPO) system. Each delay during the apple juice processing caused oxidation of phenol compounds. According to obtained results (Table 4.), the TPC of apple juice decreased significantly due to oxidation time after pressing.

Freshly pressed apple juice had 0.741 g GAE/L TPC while after 2 hours oxidation, after pressing, it decreased for 14.84 % and after 4 hours up to 23.88 %. Lea (1999) reported that phenols content in juice 6 hours after pressing decreased when compared to freshly pressed juice. This is consistent with the data obtained by Oszmianski et al. (2011) where during 6 months of puree-enriched cloudy apple juice storage, a significant change was observed in the content of polyphenols, especially in procyanidin fractions.

Conclusions

In this paper we investigated the stability of total phenols content of autochthonous and actual apple cultivars during storage time (up to 60 days) and during oxidation time after pressing of cloudy apple juice. Based on the achieved results, it can be seen that the analyzed autochthonous apple cultivars are a valuable raw-material base for food processing industry. Total phenols content of autochthonous apple cultivars was significantly higher than in actual apples. During the cold storage, the level of phenols content of all analyzed cultivars slightly decreased. The analysis of variance showed a statistically significant effect of cultivar, storage time, oxidation time after pressing juice on the phenol content in cloudy apple juice, and interaction between cultivar and storage time, as well. Since the individual phenolic compounds have tendency to vary during storage time, further studies are needed to determine the individual phenols in autochthonous apple cultivars, their changes during storage and for specifying the role of PPO.

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Received: November 9, 2011
Accepted: December 22, 2011