HMF formation and colour change of bitter orange and sweet orange jams during storage

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Summary

In this work influence of preparation on 5-hydroxymethylfurfural (HMF) and colour of bitter orange jams and sweet orange jams was investigated. Samples were prepared without and with treatment of oranges with ascorbic acid in order to investigate the influence on prevention of browning in jams. Samples were stored for 365 days at 4 °C and at room temperature and formation of HMF and colour change during storage were measured. After jam preparation bitter orange jams had higher HMF content than sweet orange jams (231 mg/kg and 58.3 mg/kg, respectively). Treatment of oranges with ascorbic acid increased formation of HMF during preparation of jams (261 mg/kg and 95 mg/kg for bitter and sweet orange jams). During storage the same tendency was observed. Also, difference in colour between sweet and bitter orange jams was observed. The highest colour change was observed in bitter orange jams ($\Delta E^* = 5.66$) with addition of ascorbic acid. In this work the importance of pH value of jams and storage temperature for HMF formation and colour was emphasised.

Keywords: bitter orange jams, sweet orange jams, HMF, colour, storage

Introduction

Citrus fruits are structurally different from other fruit types. The most common citrus fruits used for consumption and production of fruit products are oranges, lemon, grapefruit, limes. Bitter oranges are grown in region with a special climate like in Adriatic coast region of Croatia, which have characteristic Mediterranean climate. The main purpose of bitter orange tree and its fruit was, and still is, decoration. Fruit of bitter orange is inedible since they taste bitter and acid due to bitter alkaloids (Sander et al., 2008). However, bitter oranges are used for their essential oil which is used in perfumes and as flavourings; also it can be used in small amounts in different food products and for production of dietary supplements (Sander et al., 2008). At the Adriatic coast of Croatia bitter oranges are used traditionally for preparation of jams and marmalades.

The common way of inhibition of the enzymatic browning of peeled and sliced fruits and vegetables is to dip, or immerse, them into anti-browning agents, such as ascorbic acid. Ascorbic acid has wide range of application, for enzymatic browning inhibition, but also as antioxidant, and for improvement of nutritive value of many food products. Ascorbic acid inhibits enzymatic browning very effectively, primarily because of its ability to reduce quinones to phenolic compounds before they undergo further reaction to form pigments (Iyengar and McEvil, 1992). However it is easily oxidised and decomposed and contributes to browning (Handwerk and Coleman, 1998; Clegg, 1996; Shinoda et al., 2004). The decomposition of ascorbic acid, together with nonenzymatic browning, is the main deteriorative reaction that occurs in orange juice. Other parameters that cause browning include sucrose loss due to hydrolysis and hydroxymethylfurfural (HMF) formation (Nagy, 1980; Villamiel et al., 1998). HMF can be an end product of ascorbic acid decomposition or carbohydrate breakdown (Nagy, 1980; Lee and Nagy, 1988; Villamiel et al., 1998).

Factors affecting degradation of ascorbic acid are pH, oxygen, concentration of ascorbic acid, temperature, light, metal, citric acid and so on (Shinoda et al., 2004).

There have been lots of studies conducted on HMF formation in orange juice, blood orange juice, lemon juice, grapefruit juice, model solutions of orange juice (Kacem et al., 1987; Villamiel et al., 1998; Gögüs et al., 2000; Arena et al., 2001; Koca et al., 2003; Shinoda et al., 2004; Shinoda et al., 2005; Cortés et al., 2008) but there is no data on orange jams, especially on bitter orange jams.

The aim of this work was to investigate difference in HMF formation and colour of bitter orange and sweet orange jams. In preliminary work, we found out that bitter orange jams had much higher HMF content than sweet orange jams. In this work oranges were treated with ascorbic acid prior cooking of jam for enzymatic browning inhibition in order to analyze if browning in bitter orange jams will decrease.

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Samples were stored for 365 days at 4 °C and at room temperature.

Materials and methods

Chemicals

Potassium hexacyanoferrate (II) ($K_4Fe(CN)_6x3H_2O$), zinc acetate $(ZnCH_3COO)_2x2H_2O$ and sodium matabisulphite ($Na_2S_2O_5$) were obtained from Kemika (Croatia).

Jam preparation

Jams were prepared according the traditional recipe. Bitter oranges were obtained from Dubrovnik, Croatia ("Deša" – Dubrovnik, unprofitable organisation) and sweet oranges were bought in local market. Oranges were peeled and cut into smaller pieces. Oranges, sugar, water with dissolved pectin (pectin was obtained by seed boiling) and lemon juice were mixed and cooked at 80 °C, until desired total solid content (62.5 % ± 0.5) of final product was achived (measured by refractometer). Jams were put into hot 200 mL jars, cooled down, and stored for 365 days at 4 °C and at room temperature (20 °C). In the samples with addition of ascorbic acid, oranges, after peeling and cutting into smaller pieces, were treated with ascorbic acid (1 %) in order to prevent enzymatic browning. Following steps were the same as in preparation of jams without ascorbic acid.

pH determination

pH of the samples were measured after jam preparation and during storage by pH Meter MP225, (Mettler Toledo).

Determination of HMF

HMF content was determined according to method after White (Bogdanov et al., 1997). The determination of HMF content is based on the determination of UV absorbance of HMF at 284 nm. In order to avoid interference of other compounds, at this wavelength, the difference between absorbance of a clear solution and the sample solution after addition of bisulphite is determined (Bogdanov et al., 1997). Measurements were conducted in duplicates.

Colour measurement

After preparation and during storage of orange jams colour was evaluated by colorimeter (Minolta CR-300). Samples were measured 10 times at different spots and mean values were taken for calculation of colour change. The chromatic values L^*, a^*, b^* were used to calculate the total colour change (ΔE^*) of the samples during the storage of samples, according the following formula: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$

Statistical analysis

Data of HMF content were analyzed by analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P < 0.05$. All statistical analyses were carried out using software program STATISTICA 7 (StatSoft, Inc, USA). Results were expressed as means ± standard deviation.

Results and discussion

pH value

Bitter orange pulp had much lower pH value (2.7) than sweet orange pulp (3.5). Difference in pH value remained after preparation of jams (Table 1). Jam prepared from bitter oranges had much lower pH value than samples prepared from sweet orange, 2.78 and 3.47 respectively. With addition of ascorbic acid during preparation of jams, in bitter orange jams there was slight decrease of pH value, 2.68, while in sweet orange samples this decrease was more pronounced, 3.17. During storage there was slight oscillation of pH values in comparison to samples after preparation. Gögüs et al. (2000) reported that in orange juice pH values ranged between 3.5 and 3.7 and there was no considerable change during the storage of the samples.

HMF content

Results of HMF content of bitter orange and sweet orange jams during 1 year storage at 4 °C and room temperature are presented in Table 2. After preparation of bitter orange and sweet orange jams, bitter orange jams had much higher HMF content due to much lower pH which is favourable for formation of HMF. HMF content in bitter jam was 231 mg/kg in contrast to sweet orange jams which had lower HMF content, 58.3 mg/kg. Addition of ascorbic acid during preparation of jams caused increase of HMF, 261 mg/kg and 95 mg/kg in bitter orange and orange jams, respectively.

Table 1. pH values of bitter orange and sweet orange jams during 1 year storage at 4 °C and room temperature.

pH					
Storage time (days)		**0**	**20**	**120**	**365**
BO jam	4 °C	2.78	2.73	2.63	2.64
	20 °C	2.78	2.77	2.63	2.63
BO jam + AA	4 °C	2.68	2.74	2.57	2.59
	20 °C	2.68	2.72	2.58	2.62
O jam	4 °C	3.47	3.36	3.19	3.24
	20 °C	3.47	3.39	3.22	3.23
O jam + AA	4 °C	3.17	3.16	3.07	3.05
	20 °C	3.17	3.23	3.09	3.06

BO – bitter orange; O – sweet orange; AA – ascorbic acid

Table 2. HMF content (mg/kg) of bitter orange and sweet orange jams during 1 year storage at 4 °C and room temperature and correlation coefficient of HMF formation during storage.

	HMF content (mg/kg)				
Storage time	**"0"**	**20 days**	**120 days**	**365 days**	**R**
Storage temperature 4 °C					
BO jam	231±6.4^{a}	235±4.5^{a}	307±5.5^{a}	444±6.1^{a}	0.9969
BO jam + AA	261±5.6^{b}	272±6.5^{b}	321±5.9^{b}	493±6.5^{b}	0.9950
O jam	58.3±2.1^{c}	63.2±4.3^{c}	124±4.2^{c}	193±5.1^{c}	0.9681
O jam + AA	95±6.4^{d}	105±5.4^{d}	136±5.6^{d}	216±3.5^{d}	0.9995
Storage temperature 20 °C					
BO jam	231±6.4^{a}	238±5.4^{a}	323±5.3^{a}	480±6.1^{a}	0.9966
BO jam + AA	261±5.6^{b}	305±5.3^{b}	348±4.5^{b}	511±5.3^{b}	0.9854
O jam	58.3±2.1^{c}	70.8±3.5^{c}	137±3.8^{c}	256±3.5^{c}	0.9946
O jam + AA	95±6.4^{d}	123±1.5^{d}	141±2.3^{c}	265±2.5^{d}	0.9765

BO – bitter orange; O – sweet orange; AA – ascorbic acid
Values in the same column with different superscripts (a–d) for each temperature of storage are significantly different ($P < 0.05$).

During storage increase of HMF content was observed in all samples, but higher increase was observed in samples stored at room temperature. After 1 year of storage at 4 °C increase of HMF content in bitter orange jams was 444 mg/kg and 493 mg/kg, without and with addition of ascorbic acid. Sweet orange jams had lower HMF content, after 1 year storage. HMF content in those samples was 193 mg/kg and 216 mg/kg, without and with addition of ascorbic acid. After 1 year of storage at room temperature HMF content was higher than in samples stored at 4 °C. HMF content in bitter orange jams was 480 mg/kg and 511 mg/kg, without and with addition of ascorbic acid. Sweet orange jams had lower HMF content, after 1 year storage. HMF content in those samples was 256 mg/kg and 265 mg/kg, without and with addition of ascorbic acid. The rate of the reaction was directly related with the temperature. Formation of HMF during storage at both investigated temperatures was linear (correlation coefficient in range from 0.9681 to 0.9995). Shinoda et al. (2004) reported that ascorbic acid mostly contributes to the browning within first two weeks of storage of orange juices model solutions and amino-carbonyl between amino acids and degradation products derived from ascorbic acid contributes the browning, while sugars do only after two weeks of storage. In their opinion that difference of origin of browning corresponds to two phases of browning, so in their results there is no linear increase of HMF accumulation during 60 days of storage. In our case HMF accumulation during storage was linear (step where can be noted that there are two phases of browning was not noticeable) probably due to very high concentration of sugar, so that step could be masked. The rate of the reaction depends on the amount of total soluble solids present in system (like juice, concentrate or jams). Therefore, once the orange juice is concentrated, the rate of nonenzymatic browning reactions is accelerated (Gögüs et al., 2000) and that could be applied to jams.

HMF is used as a marker for the heat treatment or long-term storage of foods rich in sugar since it is generally formed under acidic conditions (Sadilova et al., 2009). From the results presented in Table 1 and 2 it is obvious that with lowering pH values of jams higher HMF content was observed. Correlation between HMF content and pH value was also conducted, and it can be seen that there was very high correlation (from 0.9543 to 0.9866) between those two parameters (Table 4). Sadilova et al. (2009)

investigated HMF formation in sugar model solutions and reported that maximum HMF content were found when fructose was heated at pH 1, lower amount in sucrose and the lowest in glucose model solution. Generally, highest amounts of HMF were found at pH 1, i.e. under conditions favouring HMF formation. HMF was not detected even after heating of a glucose solution at pH 3.5 for 4 h. Shinoda et al. (2004) reported that with increase of ascorbic acid concentration in their model systems, browning of system increased. The same can be applied on our samples. Treatment of oranges before preparation of jams, ascorbic acid content increased and HMF formation was more pronounced after heat treatment of mixture for preparation of jams. Glucose, sucrose, and sorbitol protect L-ascorbic acid from destruction at low temperatures (23, 33, and 45 °C), while at higher temperatures (70, 80, and 90 °C) compounds with active carbonyls promoted ascorbic acid destruction (Rojas and Gerschenson, 1997).

Also, it is not excluded that amino acid composition of bitter and sweet oranges played important part in browning since it has been proven that amino acids (depending on type) stimulate browning (Kacem et al., 1987; Shinoda et al., 2004; Shinoda et al., 2005) and that degree of browning, when ascorbic acid and amino acids were present in system, was more than 3 times than that of ascorbic acid solution (Shinoda et al., 2004).

Colour change

Results of measurement of colour parameters L^*, a^* i b^*, and calculation of colour change (ΔE^*) of bitter orange and sweet orange jams are presented in Table 3. Sweet orange jams had higher L^* values, meaning they were lighter in colour than bitter orange jams after preparation. Values for a^* and b^* were also higher in the case of sweet orange jams in comparison to bitter orange jams. During storage, at 4 °C and at room temperature L^* values decreased, while a^* and b^* values increased. Decrease of L^* and increase of a^* and b^* were more pronounced in samples stored at room temperature. Through calculation of colour change (ΔE^*) it can be seen that during storage there was higher colour change which was more pronounced in samples stored at room temperature, which was expected. Bitter orange jams exhibited higher colour change at both investigated temperatures of storage than sweet orange jams. When samples were stored at 4 °C there were no so high difference between samples, without and with addition of ascorbic acid, in comparison when samples were stored at room temperature. Especially this difference between samples without and with ascorbic acid addition was noted in bitter orange samples. After one year of storage ΔE^* for bitter orange samples stored at 4 °C was 2.15 and 2.34 (without and with ascorbic acid, respectively), and for sweet orange jams colour change was lower 1.89 and 1.98 (without and with ascorbic acid, respectively). For samples stored at room temperature for bitter orange samples ΔE^* was 3.56 and 5.66 (without and with ascorbic acid, respectively), and for sweet orange jams colour change was lower, 2.74 and 2.98 (without and with ascorbic acid, respectively).

Table 3. Change of L^*, a^*, b^* and ΔE^* of bitter orange and sweet orange jams during 1 year storage at 4 °C and room temperature.

		Chromatic values							
Storage temperature		**4 °C**				**20 °C**			
Storage time (days)		**L^***	**a^***	**b^***	**ΔE^***	**L^***	**a^***	**b^***	**ΔE^***
BO jam	"0"	20.37	0.83	1.07		20.37	0.83	1.07	
	20	19.92	0.47	1.82	0.95	19.88	0.64	2.59	1.61
	120	19.35	0.99	1.98	1.38	19.06	1.06	2.88	2.24
	365	18.91	1.27	2.59	2.15	18.19	1.85	3.69	3.56
BO jam + AA	"0"	20.36	0.12	0.28		20.36	0.12	0.28	
	20	19.84	0.34	0.88	0.82	19.13	0.62	0.99	1.51
	120	19.31	0.71	0.99	1.4	18.55	0.98	1.53	2.36
	365	19.92	0.64	2.52	2.34	17.75	2.4	4.76	5.66
O jam	"0"	22.47	0.18	2.13		22.47	0.18	2.13	
	20	21.76	0.12	2.72	0.92	21.09	0.13	2.54	1.44
	120	21.48	0.21	2.89	1.25	20.95	0.52	3.13	1.85
	365	20.97	0.56	3.22	1.89	20.08	0.85	3.28	2.74
O jam + AA	"0"	22.11	0.1	2.92		22.11	0.1	2.92	
	20	21.81	0.15	3.75	0.88	21.55	0.41	3.96	1.22
	120	21.41	0.29	3.89	1.21	20.93	0.23	4.43	1.92
	365	20.96	0.72	4.41	1.98	20.15	0.98	4.99	2.98

BO – bitter orange; O – sweet orange; AA – ascorbic acid

Table 4. Correlation coefficient (R) between HMF content and pH values.

Storage time (days)	4 °C	20 °C
0	0.9638	0.9638
20	0.9647	0.9607
120	0.9866	0.9764
365	0.9616	0.9543

Conclusions

HMF formation and colour change of bitter orange jams and sweet orange jams, after preparation and during storage, was investigated. In this work importance of temperature and pH value of matrix on HMF formation was emphasised. Brown pigment formation and HMF accumulation, measured in this study, were the result of ascorbic acid oxidation, sugar amine reactions, and thermal degradation of sugars. Ascorbic acid is used for prevention of enzymatic browning, but it can be easily degraded especially at high temperatures, so its influence that it may have on prevention of enzymatic browning is negligible in this case. Addition of ascorbic acid caused decrease of pH which is favourable for HMF formation.

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