SUPPLEMENTARY MATERIAL

Low sugar jellies of berry fruits: The impact of low vs. high temperature regime on their chemical composition and antioxidativity

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ABSTRACT

This study focuses on the impact of low and high temperature regimes on the chemical composition and antioxidativity of low sugar berry fruits jellies. High quality fruits (strawberry, raspberry and blackberry) were collected from Western Serbia region, quite well recognised both nationally and internationally due to an extremely well developed practice in growing berry fruits. The obtained results have clearly indicated the importance of low temperature regime for enriched contents of both total phenolics and anthocyanins followed by an enhanced antioxidativity. *Rubus fruticosus* L. Čačak Thornless cultivar, the only autochthonous berry fruit variety screened herein, may be firmly recommended as a raw material for industrial production of low sugar blackberry jellies with exceptional characteristics. This innovative procedure of preparing berry fruit jellies have encompassed the application of low temperature regime (55°C), lower content of sugar (40%), seeds separation from jellies followed by no use of pectin throughout the whole process.

Keywords: strawberry, raspberry and blackberry jellies; TPC and TAC; DPPH and ARP; DC polarography; *Rubus fruticosus* L. Čačak Thornless cultivar, autochthonous variety

Experimental

Biological material

Berries from Western Serbia's Čačak and Arilje regions were available. Strawberry (*Fragaria* x *ananassa* L. Duch, Clery cultivar) was from the Čačak region. The raspberry (*Rubus idaeus* L., Willamette cultivar) and blackberry (*Rubus fruticosus* L., Čačak Thornless cultivar) were from the Arilje region. All berries were immediately frozen at -20°C upon arrival. The relevant berry fruits samples were provided from Fruit Research Institute, Čačak, Republic of Serbia (strawberry, raspberry and blackberry Nos. FA82250, RI82900 and RF82905, respectively), representing the oldest and most famous national institute in the field.

Determination of berry fruits moisture contents

The moisture of the fruits was determined by drying 1.0 g of fresh samples at 105°C for 2 hours, cooling in a desiccator and weighing.

Procedure for the preparation of berry fruits jellies

Raspberry and blackberry were spontaneously defrosted for 24 hours. The juice obtained in such a way was kept, while the pulp was additionally passed through a cloth, to separate it from the remained juice. Afterwards, the pulp was heated for 30 minutes at 55°C (due to obtaining a more fluid state) and passed through a filter, to separate it from seeds.

The fluid pulp was then mixed with juice and cooked for at least 2 hours at low temperature regime (55°C), to obtain a jelly product (Downing 1996). After, sugar was added until dry matter reached 60% (40 g sugar was added into 60 g jelly). After a short heating (5 min at 75°C), pH was checked and adjusted by addition of a few drops of 50% calcium citrate solution to a

preferred pH of 3.0 to 3.2 necessary for getting the product in the gelled state. Later on the jellies were poured in glass jars kept over night in UV-sterilisers.

The applied procedure for strawberries was practically the same one with a minor modification – actually, no separation of seeds was needed.

For comparison, the respective jellies were also made under high temperature regime, which meant that fruits contents were brought to a boiling point once before and after the addition of sugar and calcium citrate.

Determination of Total Phenolic Content

The jellies polyphenolic extracts were obtained by methanol extraction. The jelly (1 g) was mixed with 5 ml of the solvent, mechanically stirred for 15 minutes at 25°C and then separated by centrifugation. The Total Phenolic Content (TPC) was determined according to the Folin-Ciocalteau method by using a Thermo Fisher Evolution 600 spectrophotometer (Waltham, MA, USA) (Waterman & Mole 1994). Gallic acid was used as a calibration standard, while the results are expressed as milligrams of gallic acid equivalents (GAE) per kg of the jelly.

Determination of Total Anthocyanin Content

The Total Anthocyanin Content (TAC) of the berry fruits jellies was estimated in the same extract used for TPC determination. TAC was determined according to the procedure described in the European Pharmacopoeia 6.0, with minor modifications. The absorbance was measured at 528 nm by the aforementioned type of spectrophotometer using a 0.1% (v/v) solution of hydrochloric acid in methanol, as a reference. The percentage of anthocyanins, expressed as cyanidin-3-glucoside chloride, was calculated as previously reported (Matić et al. 2010).

Determination of Antioxidant Activity

The antioxidant activity of the berry fruits jellies was assessed by widely used DPPH and recently developed DC polarographic methods.

Spectrophotometric DPPH method

A common method with the stable radical DPPH was applied herein (Liyana-Pathirana & Shahidi 2005). The results are expressed as the concentrations of the samples per ml needed to decrease DPPH concentrations for 50%, highlighting their reciprocal values ARP (Anti Radical Power).

DC polarographic method

This polarographic method was modified as previously described (Sužnjević et al. 2011). In brief, it's based on the decrease of anodic current caused by Hydroxo-Perhydroxyl Mercury(II) Complex (HPMC) formation. The electrochemical measurements were performed using Polarographic Analyzer PAR (Princeton Applied Research) model 174A coupled with X-Y recorder (Houston Instruments, Omnigraphic 2000). A conventional three-electrode cell was used: the dropping mercury electrode (DME) as the working electrode, the saturated calomel electrode (SCE) as the reference electrode, and the platinum foil as the counter electrode. The results are expressed as the percentage of limiting current decrease with gradual addition of screened samples (%/ μL).

Statistical Analysis

All experiments were done in triplicate. The obtained data were subject to analysis of variance (ANOVA), due to comparison of the means. Significant differences between groups were calculated according to Tuckey's HSD ($p < 0.05$).

References

Downing DL. 1996. Preserves (jams), jellies, and related products. In: Downing DL, editor. A Complete Course in Canning and Related Processes. III. Maryland: CTI Publications, Inc; p. 385–462.

Liyana-Pathirana CM, Shahidi F. 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J Agric Food Chem. 53:2433–2440.

Matić I, Žižak Ž, Simonović M, Simonović B, Gođevac D, Šavikin K, Juranić Z. 2010. Cytotoxic effect of wine polyphenolic extracts and resveratrol against human carcinoma cells and normal peripheral blood mononuclear cells. J Med Food. 13:851–862.

Sužnjević DŽ, Pastor FT, Gorjanović SŽ. 2011. Polarographic study of hydrogen peroxide anodic current and its application to antioxidant activity determination. Talanta. 85:1398–403.

Waterman PG, Mole S. 1994. Analysis of phenolic plant metabolites. Oxford, UK: Blackwell Scientific.