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Anthocyanin content and colour development of pomegranate jam

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ABSTRACT

One of the most important parameters to which consumers are sensitive when selecting jams is the colour. Anthocyanin and colour development of pomegranate jams made from the 'Mollar' cultivar were analysed during five months. Different temperatures (5 °C and 25 °C) and light exposures (daylight and darkness) were tested during storage. Also the influence of pectin on jam preparation was evaluated. The results concluded that high methoxy pectins yielded better pomegranate jams because of their high a^* values (34% higher than low methoxy ones). Optimal storage conditions were achieved at 5 °C with no light exposure at all.

Keywords: Pomegranate; Jam; Anthocyanins; Colour and storage

1. Introduction

Pomegranate fruit is rich in anthocyanins which are pigments responsible for colour development of the skin and seeds. The following anthocyanins were identified in pomegranates: delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside, and pelargonidin 3-glucoside and 3,5-diglucoside (Du et al., 1975; Gil et al., 1995). Anthocyanins are crucial for the colour development and quality of many fresh and processed fruits. Though they are a good source of natural antioxidants, anthocyanins are quite unstable during processing and storage. Temperature, time of processing and storage have been found to exert a great influence on anthocyanin stability (Decareu et al., 1956; García-Viguera et al., 1999; Markakis, 1982; Martí et al., 2001; Meschter, 1953; Pilano et al., 1985). Loss of anthocyanins has been attributed to many factors such as pH and acidity, phenolic compounds, sugars and sugar degradation products, oxygen, ascorbic acid, fruit maturity and thawing time (Abers and Wrolstad, 1979; García-Viguera et al., 1998; Markakis et al., 1957; Rommel et al., 1990; Withy et al., 1993; Wrolstad et al., 1970).

The colour quality of many fresh and processed fruits may influence consumers' acceptance. It is therefore essential that jam be prepared and stored at a temperature at which colour stability will be maximized (García-Viguera et al., 1998).

The effect of pectin type on jam colour has not been extensively studied. It has been suggested the effect of pectins on pigment degradation of jam products (Lewis et al., 1995). Kopjar et al. (2009) investigated the influence of different pectin additions and their concentration on colour and textural properties of raspberry jams; they concluded that different pectins and their concentrations definitively affect colour and texture.

The goals of this study were to evaluate anthocyanin content of pomegranate jam and its colour development during storage at different temperature and light regimes, as well as the influence of commercial pectin on jam preparation.

2. Materials and Methods

The pomegranate cultivar *Mollar* was used on this study since it is the most commonly cultivated in Spain (Melgarejo and Martínez-Valero, 1991; Melgarejo and Salazar, 2003). Fully ripe pomegranate fruits were harvested at the gene bank located at the Higher Polytechnic Agricultural College (Orihuela Campus, Universitas Miguel Hernández, Alicante, Spain) during the first half of October 2002 and then stored for one day at 5 °C. Pomegranate jam was made during the next day.

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2.1. *Jam preparation*

Pomegranate jam was obtained according to a typical commercial protocol. Low and high methoxy pectins were used for jam production (Grindsted® Pectin LA 210 and Grindsted® Pectin RS 400, respectively). Different types of pomegranate jam were prepared as follows:

- High methoxy pectin jam (HM): 350 g kg^{-1}of edible seeds, plus 1.65 g kg^{-1} of pectin, 3 g kg^{-1} of citric acid, 0.5 g kg^{-1} of ascorbic acid and 1 g kg^{-1} of sorbic acid. The final sucrose concentration was 65° Brix.
- Low methoxy pectin jam (LM) recipe: 350 g kg^{-1} of edible seeds, plus 7 g kg^{-1} of pectin, 4.5 g kg^{-1} of citric acid, 0.5 g kg^{-1} of ascorbic acid and 1 g kg^{-1} of sorbic acid. The final sucrose concentration was 65° Brix.

Citric acid was used for adjusting pH values for proper gelatinization of pectins. The pH necessary for HM was 3.2–3.3 and for LM 2.8–3. Two pectins were dosed at different concentrations following Danisco Cultor España, S.A. recommendations.

2.2. *Anthocyanin extraction*

A 75 ml solution of methanol/acetic acid/water (25:1:24, v/v) was added to 5 g of pomegranate jam and stirred for 20 min at room temperature (22 °C) (García-Viguera et al., 1997). Afterwards the extract was filtered through glass wool, and concentrated under vacuum; the residue was then redissolved in 5 ml acidified water (3% formic acid, v/v). Finally, the aqueous solution containing anthocyanins was adsorbed onto a C_{18} *Sep-Pak* cartridge (García-Viguera et al., 1997).

2.3. *HPLC anthocyanin analysis*

An 1100 *Hewlett-Packard* High Performance Liquid Chromatograph (HPLC) with a C-18 column was used for anthocyanin identification and quantification using 5% formic acid (solvent A) and methanol (solvent B) as the mobile phase. Elution was performed at 1 ml min^{-1} using a gradient starting with 15% B, increasing to 30% B at 15 min, isocratic elution for 5 min, and finally increasing to 95% B at 25 min. Detection was achieved at 520 nm (García-Viguera et al., 1999; Hernández et al., 1999). All analyses were done in triplicate and results expressed as mean values.

The different anthocyanins were quantified by their peak areas in the chromatograms by comparison with an external standard of cyanidin 3-rutinoside (Apin Chemicals, UK). The total anthocyanins were calculated by summation of the amounts of the six different anthocyanins detected in each chromatogram as previously reported (Gil et al., 1995).

2.4. *Colour measurements*

A *Minolta* CR-300 colour spectrophotometer was used for this study. Analyses were performed by reflection on a 2.5 mm thick sample placed over a white surface. $L a^* b^*$ values were calculated using illuminant D65 (8 mm diameter measuring area) and a 10° observer according to the CIELAB 76 convention. All measurements were made by triplicate.

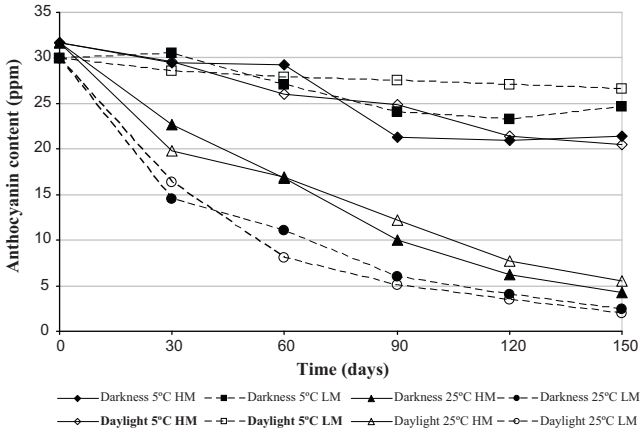

Fig. 1 – Total anthocyanin content evolution of pomegranate jams (low and high methoxyl pectins) during storage conditions (at 5 °C and 25 °C, daylight and darkness exposures).

2.5. *Storage of pomegranate jam*

Pomegranate jam samples were stored at different temperatures (5 °C and 25 °C) and light regimes (daylight and darkness). Both types of pomegranate jam (high methoxy pectin jam and low methoxy pectin jam) were analysed after preparation and then after 30, 60, 90, 120 and 150 days of storage.

2.6. *Statistical analysis*

All data were subjected to statistical analyses. Three replicates of each treatment were examined. Multifactorial ANOVA analyses were performed along with the least significant difference tests (LSD) to detect any statistically significant differences ($p \leq 0.05$). The statistical software package *SPSS* version 16.0 was used for data analyses.

3. Results and discussion

Anthocyanin content evolution of pomegranate jam over time is shown in Fig. 1.

When jam was stored at 25 °C, anthocyanin total content went down quickly in both low and high methoxy pectin jams. Regarding those jams stored at 5 °C, there were a 32% pigment degradation in HM ones and a 14% reduction in LM jams after 150 days. These results completely agreed with those obtained by García-Viguera et al. (1999) and Maier et al. (2009); strawberry jams showed lower anthocyanin content degradation at 5 °C than at 24 °C/18 °C after 180 days storage.

According to Markakis (1982) and Francis (1989), anthocyanin content could be negatively correlated to light exposure, but statistically significant differences could not be established among pomegranate jams stored either in dark or daylight conditions. Likewise, García-Viguera et al. (1999) found no significant effect of light exposure on strawberry jam pigment composition. These results could be definitively due to the protective effect of high amounts of sugar (Wrolstad et al., 1990).

All anthocyanin pigments showed a reduction over time. This statement completely agrees with pomegranate marmalade results obtained by Zafrilla et al. (1998).

Jam samples prepared with different types of pectin show different anthocyanin contents (Fig. 1). Samples with high methoxy pectin had lower values of anthocyanins than sam-