Journal of Food Engineering 90 (2009) 171–178

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Effects of processing parameters on colour stability of strawberry nectar from puree

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ARTICLE INFO

Article history:
Received 5 December 2007
Received in revised form 16 June 2008
Accepted 21 June 2008
Available online 27 June 2008

Keywords:
Strawberry (*Fragaria* × *ananassa*)
Nectar
Freezing
Storage temperature
Colour stability
Anthocyanins
Polyphenoloxidase
Peroxidase

ABSTRACT

The effect of processing steps on the colour stability and the anthocyanin content of nectars made from strawberry puree was investigated, including (i) the use of frozen strawberries, (ii) processing temperature (10 °C vs. 20 °C), (iii) sieving, (iv) pH reduction and (v) storage temperature (20 °C vs. 4 °C). The use of frozen strawberries significantly improved the colour stability of the nectar even for long storage periods, and allowed the production of strawberry nectars with a shelf-life of up to 12 months without any additives. Moreover, half-life of anthocyanin monomers increased significantly. A negative effect of a higher processing temperature on the content of anthocyanins after pasteurisation could be observed as well as the reduction of the pH value during processing on the colour and the content of anthocyanin monomers. Sieving had no significant effect on colour stability and the content of anthocyanin monomers. Storage temperature had a strong impact on colour stability and degradation of anthocyanin monomers. At 4 °C, the nectar colour remained acceptable over even more than 12 months. Compared to frozen strawberries stored at −80 °C, frozen strawberries stored at −18 °C had a lower activity of polyphenoloxidase (PPO) and peroxidase (POD) of about 53% and 22%, respectively. No complete inactivation of PPO and POD could be achieved during the different processing steps. POD activity was more affected by pH treatment than PPO activity.

1. Introduction

Strawberries (*Fragaria* × *ananassa*) belong to the most popular fruits and their production, sales and processing is of considerable economic importance in Western Europe. Consumers seem to be primarily attracted by the bright red colour and typical strawberry aroma of the fresh fruits. Moreover, there is also an increasing number of processing companies offering a broad variety of strawberry products (frozen fruits, concentrates, jam, juice, nectar, syrup, dairy products, etc.) in the market.

As a rule, high quality fruits are allocated for direct marketing as fresh fruits, while second quality strawberries are subject to further processing. Therefore, processing companies often depend on varieties, which are primarily optimized for the fresh market. These are particularly scarlet red fruits with a long shelf-life. For processing companies, however, other traits such as colour intensity and colour stability are of more relevance. Elsanta is the most important strawberry variety in central and north Europe, and therefore of high commercial interest. It is characterized by excellent shelf-life, good fruit consistency and high yield.

Strawberries owe their attractive colour to the presence of two types of anthocyanidin pigments; derivatives of bright red pelargonidin (predominant) and dark red cyanidin (minor). The content of anthocyanins varies between 10 and 80 mg/100 g in fresh fruits (Heinonen et al., 1998; Kalt et al., 1999; Zabetakis et al., 2000) and 21–333 mg/l in juice (Bakker et al., 1994; Garzon and Wrolstad, 2002; Gimenez et al., 2001). Pelargonidin 3-*O*-glucoside is the main component in strawberries (82–100%) (Bakker et al., 1994). However, at least 13 other anthocyanins (e.g. pelargonidin 3-*O*-rutinoside, pelargonidin 3-*O*-glucoside-succinate, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside) have been found in lower concentrations in different varieties (Bakker et al., 1994).

Apart from anthocyanins, other polyphenolic compounds (e.g. derivates of hydroxycinnamic acid, hydroxybenzoic acid, monomere flavan-3-oles, procyanidins, flavonols) have been found in strawberries (Herrmann, 1996). Both genetic background and growing conditions strongly influence pre-harvest and post-harvest fruit quality. Colour stability is influenced by self-association (condensation of anthocyanins) (Brouillard and Dangles, 1994) and copigmentation (interactions of anthocyanins with polyphenols) (Bishop and Nagel, 1984), since this leads to compounds,

Abbreviations: AF, acceptance factor; AM, anthocyanin monomers; HL, half life; PPO, polyphenoloxidase; POD, peroxidase; TA, titrable acidity.

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doi:10.1016/j.jfoodeng.2008.06.018

which have a higher stability than the anthocyanin monomers (Markakis, 1982). Copigmentation has a hyperchromic effect (increased chroma C^*) and causes a bathochromic shift (higher absorption maximum) which results in a product appearing violet (Chen and Hrazdina, 1981). Interestingly, copigmentation is more intense in berry juices than in purified anthocyanin molecules of these juices (Wilska-Jeszka and Korzuchowska, 1996). Phenolic acid enrichment also improves and stabilizes the colour of berry juices during storage (Rein and Heinonen, 2004). In addition, there are many parameters influencing colour stability: pH value, temperature, metalions, light, oxygen, non-enzymatic browning reactions and L-ascorbic acid (Bakowska et al., 2003; Garcia-Viguera et al., 1999; Markakis, 1982; Wrolstad et al., 1970).

Unfortunately, products processed from strawberries have only a short shelf-life. The attractive red colour often does not even endure the processing steps. After a few weeks or even days of storage, the red colour is replaced by dull, brownish pigments. According to the literature, a key role in colour degradation is attributed to the presence of oxidoreductases such as polyphenoloxidase (EC 1.14.18.1) (PPO) and peroxidase (EC 1.11.1.7) (POD) (Grommeck and Markakis, 1963; Serradell et al., 2000; Wesche-Ebeling and Montgomery, 1990). Inactivation of these enzymes may obviously be achieved by different processing steps (Civello et al., 1995; Grommeck and Markakis, 1963; Serradell et al., 2000; Wesche-Ebeling and Montgomery, 1990) although there are often large variations in the results obtained (Chisari et al., 2007; Grommeck and Markakis, 1963).

In a pilot scale study, the effects of processing on the colour stability and the anthocyanin content of nectars made from strawberry puree were investigated. The following processing steps were evaluated: (i) the use of frozen strawberries, (ii) processing temperature, (iii) sieving, (iv) pH reduction and (v) storage temperature. Additionally the residual PPO and POD activities at selected processing steps – as well as the influence of freezing temperature on the enzyme activities were determined.

2. Materials and methods

2.1. Strawberries

Field-run strawberries (*Fragaria* × *ananassa*, cultivar Elsanta) were grown in Rust (Austria) in June 2005 and June 2006. Fruits were harvested at commercial ripeness each day in the morning. They were either processed immediately or packed for freezing within 2 h. Strawberries were packed in 5 kg plastic bags and stored for 4 months (design 1 and 2) or 5 weeks (design 3) at −18 °C. Frozen strawberries were kept for 24 h at 4 °C before they were used for nectar production.

2.2. Nectar production

Strawberries were milled with a roller crusher (Wottle, Austria), sieved (Wiesböck, Austria) and milled again with a colloid mill (Fryma, Switzerland). The puree was mixed with water, citric acid and sugar in order to achieve nectar (20 or 40 l for each variant, 40% puree, 14 °Brix, 7.0 g/l titrable acidity). The nectar was degassed in a vacuum tank at −0.6 bar for 15 min. Subsequently, the nectar was filled in 0.2 l white glass bottles using a vacuum filler (Rapf, Austria), pasteurized (85 °C, 10 min) in a tunnel pasteurizer (Balik, Austria) and stored at 4 °C or 20 °C in the dark.

2.3. Reduction of the pH value during processing

In order to lower enzyme activities the pH value of the puree was lowered to pH 2 by addition of 84% orthophosphoric acid (VWR, Austria) after the sieving step. After the degassing step, the pH value was adjusted to the original pH value by adding 3 N NaOH (Merck, Germany).

2.4. Chemical and physical analysis

Titrable acidity (TA) was measured at the titration-endpoint of pH 8.1 (0.1 N NaOH) and expressed as g/l tartaric acid (factor 0.75). The pH value was determined with a pH-meter (Multiline P4, WTW) in combination with the pH-electrode SenTix 41-3 (WTW, Germany). Brix-value (°Brix) was measured by a hand-held refractometer (Atago, Japan). The firmness of the fresh strawberries (10 strawberries per variant) was measured using a penetrometer (Setop, Durofel, stamp 10, France). Values were expressed as percentages (Durofel-%). Colour components were measured using the CIELAB-system. L^*, a^* and b^* values of strawberries, purees and nectars were measured with a Minolta Chroma Meter CR-200 (tristimulus method), which was calibrated using the standard white reflector plate No. 11033069 (design 1) and a Minolta CM 3500d (spectrophotometric method, D65, 30 mm, 10°, gloss excluded, colour data software CM-S100w, ver. 1.4, Japan), which was calibrated with a white reflection plate No. 16471004 (design 2 and 3).

The calculations of C^* (chroma) and h (hue angle) were carried out as described in Kammerer et al. (2007).

To quantify the colour changes during processing and storage, an "acceptance-factor" (AF), based on sensorial tests with consumers, was calculated as described previously (Gössinger et al., 2007).

The anthocyanin monomers (AM) were measured by HPLC following the method of Eder et al. (1990). All samples were stored at −18 °C before analysing. The results are given in mg/l pelargonidin 3-*O*-glucoside equivalents. The half-life (HL) of anthocyanin monomers was calculated as previously described (Arabashahi and Lund, 1985; Cohen and Saguy, 1985).

2.5. Determination of enzyme activities

PPO activity was performed by a spectrometric method as described in Serradell et al. (2000) and POD activity was carried out according to the Worthington manual (Worthington Biochemical Corporation, 1972). The activities were calculated as delta A per min and 1 mg protein. Protein content was determined by a modified Lowry procedure (Sandermann and Strominger, 1972) using BSA as a standard.

2.6. Statistical analysis

All experiments were performed twice. For all samples, analyses of pH value, soluble solid content, titrable acidity, colour and anthocyanin monomers were run twice whilst measurement of firmness (10 strawberries), PPO and POD activity and protein content in triplicate.

Statistical analysis was carried out using SPSS 12.0 (Statistical Package for the Social Science). Effects of the various factors and test of significance were calculated following the method of Klepp-

Table 1
Description of the factor steps of designs 2 and 3

Design	Factor	Parameter	–	+
2	A	pH value reduction	No	Yes
	B	Sieving	No	Yes
	C	Storage temperature	20 °C	4 °C
3	A	Freezing	No	Yes
	B	Processing temperature	10 °C	20 °C
	C	pH value reduction	No	Yes
	D	Storage temperature	20 °C	4 °C