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Identification of Colored Components Produced in Sugar Beet Processing Using Gel-Permeation Chromatography (GPC) with UV and RI Detection

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ABSTRACT: Sugar juices in the production of beet sugar contain complex varieties of colored materials that have not been identified completely. Color of the sugar depends the color of juices from which sugar is produced. The mechanisms concerning color formation in beet sugar processing are complicated due to many parameters involved. The main mechanisms related to the color formation during purification stage are the Maillard reaction and alkaline degradation of invert sugars, but caramelization is more important in thick juices. In this study, gel-permeation chromatography (GPC) is used to separate and determine some color components in raw, thin and thick sugar beet juices. Subsequently, the separate components are detected by a UV coupled to a refractive index (RI) detector in series. GPC is a suitable technique for determining the molecular weight distribution of compounds in juices and can be used to demonstrate colored compounds through the sugar beet processing. GPC chromatography, using UV and RI as detector, is the analytical method proposed to study the nature of colored impurities and to characterize the stages of the process where colorants are generated. The results show that all of the juices in this study had low molecular weight colorant in the range of 0.2 up to 10 kDa.

Keywords: *Beet Juices, Colorant, Gel Permeation Chromatography*.

Introduction

Color is a generic term used to describe a wide range of components or colorants that contribute to the visual appearance of sugar. Colorants are materials made up of various molecular weights, pH sensitivity, ionic charge, chemical composition and affinity for the sugar crystal (Godshall, 1996). Many factors affect the quality and quantity of beet sugar and these factors are related to the formation of non-sugars (Coca *et al*., 2008). The major macromolecules in sugar processing include colorant and polysaccharides, along with minor amounts of protein, soluble lignin, colloidal silicates and possibly calcium complexes. (Godshall *et al*., 2002).

Color is one of the measures of the white sugar quality that depends upon the color of juices from which sugar is produced. Groups of colorant developed in sugar beet technology are given in Table 1. Juices and syrups formed during sugar beet processing contain compounds that impart yellow or brown color to the white sugar. The colored compounds are polymers with different molecular weights, structures and properties. These compounds are formed in the process as a result of sugar degradation reactions, pH changes, thermal effects and reactions between amino compounds and

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carbohydrates (Coca *et al*., 2004). The mechanisms concerning color formation in beet sugar processing is very complicated because of the many parameters involved (Godshall & Baunsgaard, 2000). The main mechanisms related to color formation during purification stage are Maillard reaction and alkaline degradation of invert sugars [hexose alkaline degradation products (HADPs)]. Maillard reaction products, melanoidins, are formed by the reaction of reducing sugars with amino acids. Melanoidins are recognized as being acidic and polymeric compounds, with a highly complicated structure (Cämmerer *et al*., 2002; DeMan, 1976; Yaylayan and Kaminsky, 1998). The complex process of polymerization during Maillard reaction produces varieties of polymeric materials with different molecular weights, structures and elemental compositions. Most of the reported polymers from Maillard model systems incorporate nitrogen (Wedzicha and Kaputo, 1992).

The HADPs together with the melonidins are responsible for up to 80% of color in sugar beet juices (Asadi, 2006). The production of colored HADP takes place at the common pH of a beet sugar factory (8–11). The formation of degradation products occurs mainly in the purification step where temperature increases up to 85℃ and pH rises up to strong basic values (11–12). The nature and structure of colored HADPs have not been elucidated but they are probably due to the extensive aldolization of intermediate di-carbonyl compounds in alkaline solutions (Coca *et al*., 2008; De Bruijn *et al*., 2002). Caramels form as thermal degradation products of sucrose, with high molecular weights that increase with time and temperature as a result of increasing polymerization. They have only a slight charge and are not pH sensitive (Davis, 2001).

The reason why certain colorants appear to be included in the sugar crystals much more easily than the other colorants with the same color intensity at 420 nm is less well understood. It is suggested that differences in molecular weight of colorants play a particularly decisive role in their transfer to sugar crystals; e.g. the higher the molecular weight, the more difficult it will be for the colorants to diffuse away from the crystallizing sugar and so the more likely it will be to become subject to inclusion in the crystals (De Bruijn *et al*., 2002).

Gel-permeation chromatography (GPC) is a suitable technique for determining the molecular weight distribution of compounds in juices and can be used to demonstrate colored compounds through the sugar beet processing. The separation is based on the distribution of different sized molecules between the mobile phase and the pore volume. GPC columns allow large molecules to pass through more rapidly than smaller molecules. Therefore, GPC columns separate the compounds present in juices according to their molecular weight. A UV detector allows the identification of UV absorbing compounds. In this way, compounds can be characterized by their UV–Vis spectra. A refractive index detector allows the detection of colored compounds that do not absorb UV radiation.

Table 1. Colorant formed in sugar beet technology (Erdogăn et al., 1996)

Name of the process	Melanins	Melanoidins	Caramels	HADPs
Diffusion	+	-	-	-
Purification	-	+	+	+
Evaporation	-	+	+	+
Crystallization	-	+	+	+

(+) sign shows the formation of the colorant

Coca *et al*. (2004) have summarized the main characteristics of the most troublesome colorants formed throughout sugar beet manufacture. They synthesized the main colorants formed in sugar beet processing then analyzed by gel permeation chromatography (GPC) and characterized by their molecular weights and UV–Vis spectra. The GPC analysis of a sugar beet thin juice showed the presence of colored compounds with molecular weights ranging from 0.4 to >100 kDa. Bento and Sa (1998) studied the potential usage of GPC for determination of high molecular weight compounds in raw sugars and other sugar process materials. When spectrophotometric Diode Array Detector (DAD) was used as detector and an Evaporative Light Scattering Detector, in series. By this arrangement both chromophoric and non chromophoric compounds are detected simultaneously (Bento and Sa, 1998).

In this study, GPC is used to separate and determine some colorant compounds in raw, thin and thick sugar beet juices. Subsequently, the separated components are detected by a UV coupled to a refractive index detector in series.

Materials and Methods

- *Samples Preparation*

Different sugar beet juices such as raw, thin and thick juices were taken from Hamedan sugar beet factory during 2015-2016 campaign. At first, the pH of syrups was adjusted to 7 ± 0.1 according to ICUMSA method (Wojtczak, 2003) and the samples were stored in the refrigerator in plastic containers. The samples were then filtered through 0.45 and 0.22 μm membranes, respectively, already injecting onto the column. Table 2 summarizes the average characteristics of raw, thin and thick juice solutions.

- *GPC analysis*

The molecular weights of the colored compounds were estimated by GPC. The separation was achieved with Shimadzu LC-20A instrument using Waters Ultrahydragel Linear column (exclusion limit 0.2 to 80 KDa) and 0.1 M $NaNO_3$ in water as mobile phase with the flow rate of 0.9 ml/min, temperature of 35℃. Polysaccharides with 12600, 4270 and 1400 molecular weights were employed as standards and identification were achieved using UV and refractive index (RI) detector. UV detector was set at 420 nm, since this wavelength was recommended by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Refractive index detector was also employed for the analysis and was coupled to UV detector.

Results and Discussion

- *GPC analysis of raw juice*

The results of GPC analyses corresponding to the raw juice are presented in Figure 1.

Raw juice (diffusion juice) is the product of the diffusion process that usually has about 15% dry substance and 0.5 to 1.5% insoluble substances. Its purity (sucrose content as % of dry substance) is 85 to 92%. Raw juice has a low purity (high non-sugar content) because in the diffusion process (unlike the extraction process), the components are not chosen selectively (Asadi, 2006; Van der Poel, 1998). The

Table 2. Characteristics of the samples

Samples	Dry Matter (%)	Purity (%)	Pol (%)	pH	Color (IU)
Raw juice	16	91.3	14.6	6.5	3100
Thin juice	14.4	92.6	13.33	8.7	2830
Thick juice	43.7	92	40.2	8.9	4120

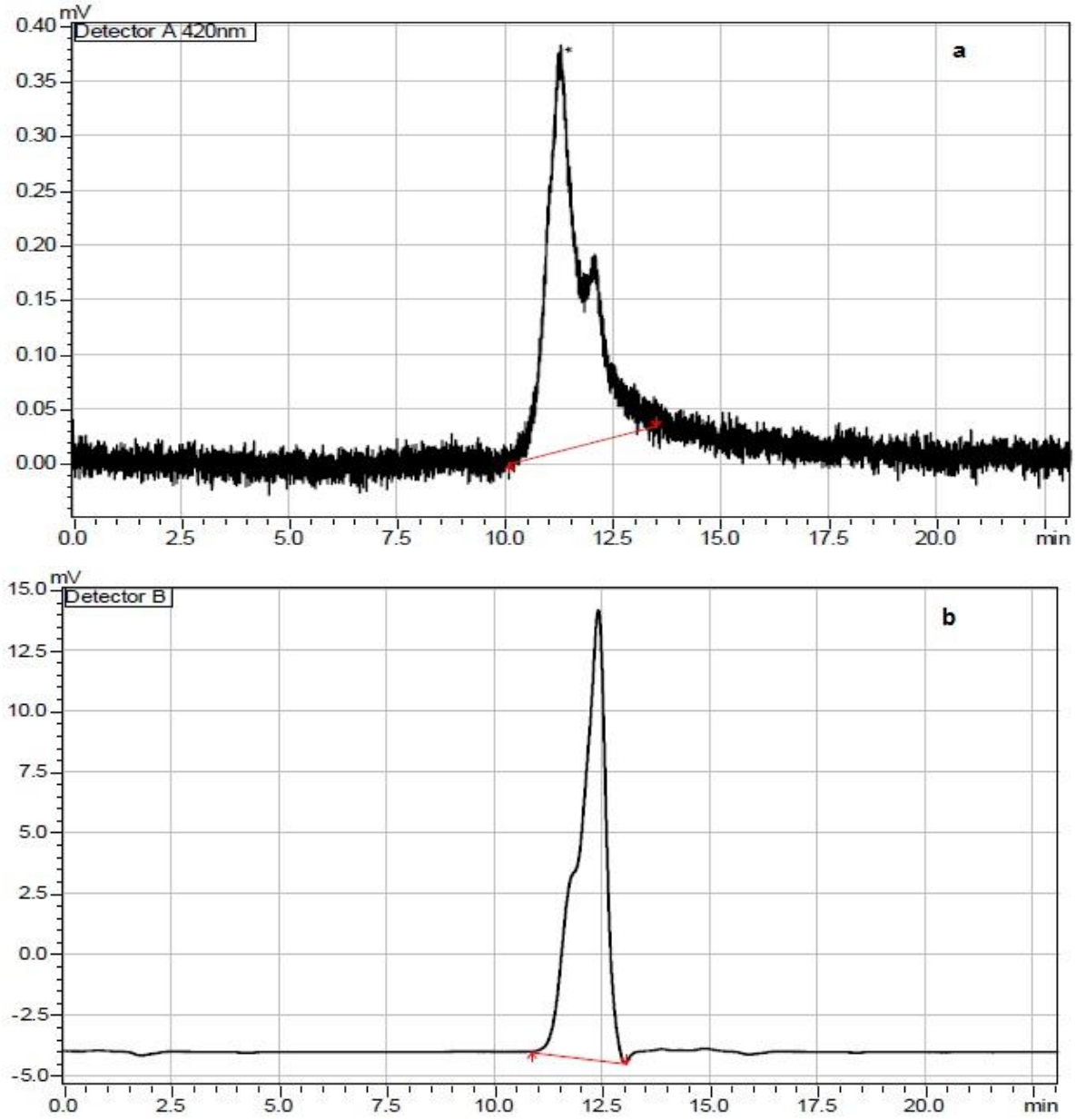

Fig. 1. Chromatograms for raw juice using UV (a) and RI (b) detectors

chromatograms of raw juice indicate the presence of high concentrations of low molecular substances. The peaks observed by the UV detector with the retention times of 10.08 minute initiation and the final time of 11.31 and 13.47 minutes, respectively, corresponded to colorants with molecular mass up to 2kDa. Refractive index detector indicated the same chromatogram with a peak appearing at 12.42 min. Melanin is the important group of colorant that might take place in diffusion process. They are mostly formed between the slicing of the beet and the heating of the juice in the diffuser. Melanin which is formed in the enzymatic oxidation of polyphenols is a dark pigment insoluble in aqueous solutions (Godshall and Baunsgaard, 2000). It is formed from phenolic compounds such as tyrosine and dopa in beet diffusion juice and from

chlorogenic acid in cane dilute juice, i.e. in the initial processing steps when the enzymes are still active (Godshall *et al*., 2002; Robinson and Smyth, 1997). Catechol amines have been isolated from beet diffusion juice and the three amino acids were found in beet process samples from diffusion juice to molasses are known to color precursor in raw juices (Godshall, 1996; Godshall and Baunsgaard, 2000; Robinson and Smyth, 1997). Color precursors are participating in color forming reactions during processing and it is therefore as important to characterize color precursors as colorants. Most of these colorants precipitate with lime and are removed during purification.

- *GPC analysis of thin juice*

Regarding the molecular size distribution of thin juice, three peaks were observed at RI detector (Figure 2). The first peak, appearing at 10.56 min, corresponded to colored components with molecular mass up to 5 kDa. The initiation time of the peak was 5.3 minutes; therefore the high concentration of molecular size distribution in this range was established. It has been shown that thin juices of beet sugar contain several melanoidins and HADPs colorant in the range of 0.4 -10kDa (Godshall and Baunsgaard, 2000; Mersad *et al*., 2003; Shore *et al*., 1984). Shore *et al*. (1984) found that melanoidin-type colorants were of relatively high molecular weight (from 1 to 5 kDa) whereas the HADPs and caramels were of considerably lower molecular weight (mostly up to 1 kDa). The colorants of higher molecular weight have a tendency to be occluded into sugar crystals whereas lower molecular weight colorants reside on the crystal surface. Nevertheless, some researcher reported that the colorants with molecular mass of 20 kDa, with anionic character, may be related to melanoidins (Coca *et al*., 2008).

Most of the colorants formed by the Maillard reaction are removed during purification, but because the high temperature is the driving force of their formation, they are again formed during evaporation and crystallization (Asadi, 2006).

- *GPC analysis of thick juice*

Evaporation is the beet processing step in which the formation of color due to the Maillard reaction is more important due to the high temperatures, up to 120˚C (Agudo *et al*., 2002). Another colorant which is important in this situation is caramel. Caramel is a brown-color, pleasant-tasting product formed from heating of sucrose (sugar) solutions. In terms of chemistry, caramel is the product of the caramelization reaction formed by the decomposition of sucrose to glucose, fructose, and finally to caramel at temperatures close to its melting point (the melting point of sucrose is about 185ºC). This reaction is called the caramelization of sugar, which does not occur to an appreciable extent during normal operations of the sugar factory. Caramel is produced commercially by boiling fermentable sugars, such as sucrose and fructose, in the presence of ammonia. It is used in food products as a flavor and color enhancer (Agudo *et al*., 2002; Coca *et al*., 2008).

Chromatogram of thick juice was shown in Figure 3. The result indicates a peak at the retention times of 11.05min for UV detector, and at 11.96 for refractive index. These peaks probably are related to the colorants in the range 1232Da the caramels and melanoidins.

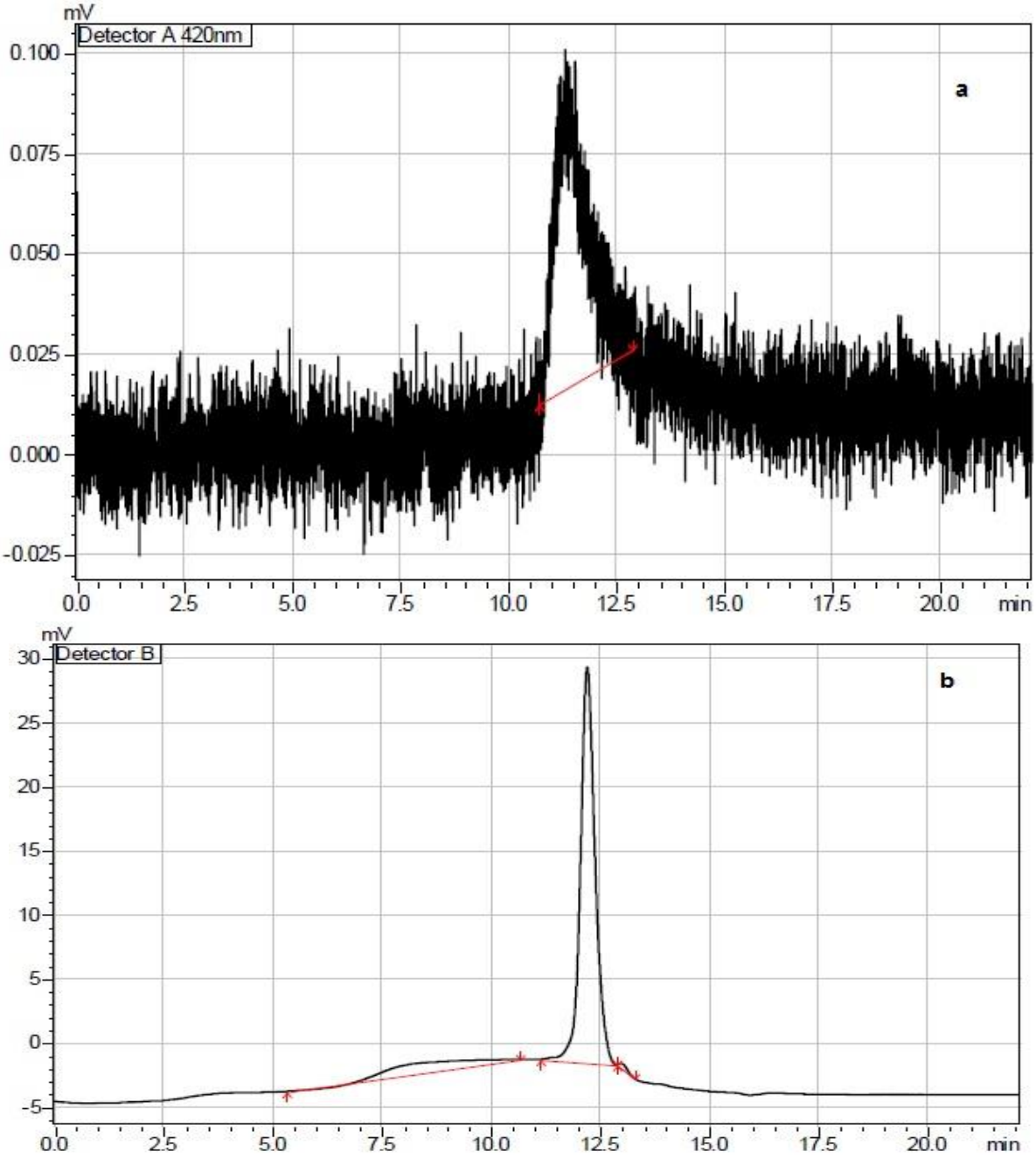

Fig. 2. Chromatogram for thin juice using UV (a) and RI (b) detectors

Conclusion

Colorants (coloring substances) are not present in beet juice but are formed during the processing (sugar beet is an off-white color but processed beet juice is colored). This paper summarizes the results of the study on the molecular size distribution of beet sugar colorants. GPC chromatography, using UV and RI as detectors is the analytical method proposed to study the nature of colored impurities and to characterize the stages of the process where colorants are generated. UV analysis indicated the presence of melanoidins, melanins, caramels and HADPs. Several of the substances in this study were low

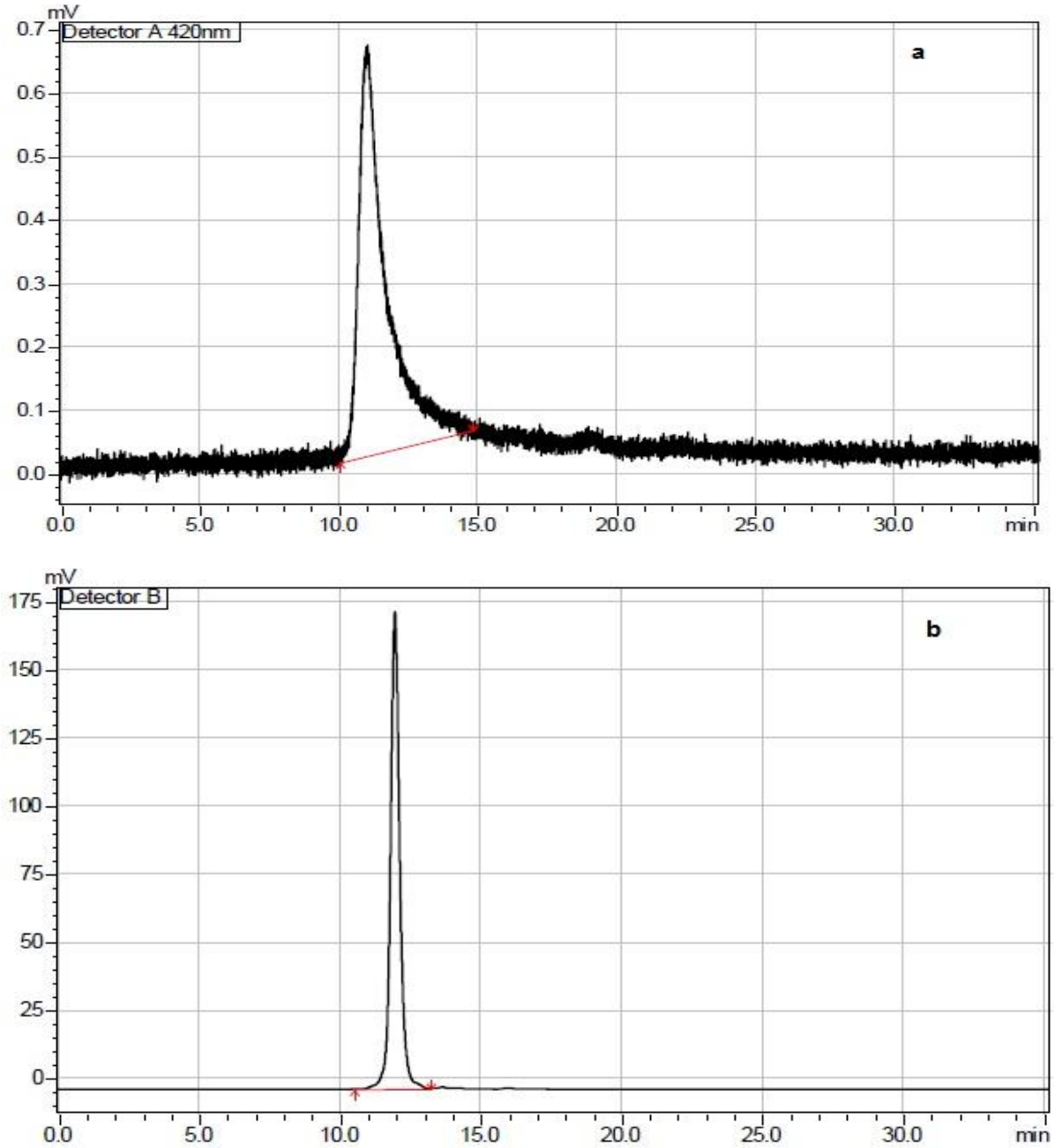

Fig. 3. Chromatograms for thick juice using UV (a) and RI (b) detectors

molecular weight colorants, that might increase on the application of heat due to the continued reaction and formation of new colorants from the precursors.

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