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Food Chemistry 88 (2004) 299–303
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Biostrip technique for detection of galactose in dairy foods

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Received 18 June 2003; received in revised form 24 March 2004; accepted 24 March 2004

Abstract

A quick and simple biostrip technique for detection of galactose in food was developed by immobilizing galactose oxidase, peroxidase and chromogens on to a polymeric support. The biostrip changes its colour from yellowish white to dark green depending on the concentrations of galactose in milk or milk products. The developed colour on the strip is compared with the colour chart and the concentration of galactose in the sample is estimated. The working range of the strip is between 10 and 50 g galactose l^{-1} and the response time is 2 min. The technology can be used in dairies, hospitals and remote areas where sophisticated instruments are not available.

Keywords: Biostrip; Chromogen; Galactose; Galactose oxidase; Galactosemia

1. Introduction

Galactosemia is genetically inherited metabolic disorder characterized by an inability of the body to utilize galactose (Rhode, Elei, Taube, Podskarbi, & Horn, 1998; Suzuki, West, & Beutler, 2001; Suzanne, Robert, Jie, Yager, & Segal, 2002; Wang, Xu, Won, & Wong, 1998). Galactose is a type of food sugar found mainly in dairy products as well as produced within the human body. The main source of galactose in the diet is milk products (Pritzwald, 1986). Milk contains a disaccharide sugar called lactose, which is hydrolyzed into monosaccharides glucose and galactose on digestion (Paige, Bayless, Huang, & Wexler, 1975). Glucose is utilized as a source of energy by the body whereas galactose needs to be metabolized further by certain enzymes viz. galactokinase, galactose-1-phosphate uridyl transferase and galactose-6-phosphate epimerase (Petry & Reichardt, 1998). Deficiencies of one or more of these enzymes cause galactosemia. Infants may develop cataracts, liver disease and kidney problems if galactosemia is not treated (Berry et al., 2001). High level of galactose and galactose 1-phosphate can cause brain damage and in some cases it can lead to death. Galactosemia is treated by avoiding foods that contain galactose/lactose in the diet (Acosta & Gross, 1995; Gropper, Gross, & Olds, 1993; Gross & Acosta, 1991; Pritzwald, 1986; Robert & Meyer, 1993). Therefore, all persons with galactosemia should limit galactose intake from food (Guerrero et al., 2000; Winder et al., 1982; Weese, Gosnell, West, & Gropper, 2003). Infants can be fed with soya, protein hydrolysate or other lactose/galactose free formula. Various expensive and time-consuming methods have been reported for the determination of galactose in milk and milk products (Bayliss, 1998; Hansen, 1975; Kempen, 2003; Watanbe & Kawasaki, 1987). Different types of biosensors are also reported for detection of galactose (Cheng & Christain, 1979; Neng-Quin, Zong-Rang, Jiang-Zhong, & Guo-Xiong, 2003; Rajendran & Lrudayaraj, 2002; Schumacher, Vogel, & Lerde, 1994; Stoecker, Manowitz, Harvey, & Yacynych, 1998; Szabo, Adanyi, & Varadi, 1996). These biosensors are expensive and based on complicated techniques. The most common enzymatic method for determination of galactose is based on oxidation of galactose by galactose oxidase to form D-galacto-hexodialdose and hydrogen peroxide. The latter, in the presence of peroxidase form coloured complex with *o*-dianisidine or *o*-tolidine (Frings & Pardue, 1964;

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0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.03.052

Worthington, 2003). Different methods of immobilization have been attempted in our laboratory for development of biostrips for various tests (Sharma, Sehgal, & Kumar, 2002a; Sharma, Bala, Sehgal, Tulsani, & Kumar, 2002b; Sharma, Sehgal, & Kumar, 2003). Therefore, our attention was drawn to develop a quick, simple and economical immobilized enzyme based biostrip technique for estimation of galactose in food products. Galactose oxidase and peroxidase along with chromogen were immobilized on to a suitable matrix for development of colorimetric based galactose biostrip. Here, we have reported a simple enzyme based biostrip technique for quick detection of galactose in dairy products.

2. Materials and methods

2.1. Chemicals and dairy samples

Galactose, galactose oxidase, lactase, *o*-dianisidine, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and glutaraldehyde were from Sigma–Aldrich (USA). Horseradish peroxidase was from Boehringer (Germany). Citric acid and sodium citrate were from Qualigens (India). Nitrocellulose, polycarbonate, polyester membranes and filter papers were from Whatman International Ltd. (UK). Plastic sheets and non-reactive Vamical adhesive were obtained from the local market. Soya formula (Nusobee, Zerolac), milk and dairy product samples were collected from local market.

2.2. Preparation of enzymes

Galactose oxidase (12 U) and horseradish peroxidase (50 U) were dissolved in 230 μl 0.1 M phosphate buffer, pH 7.0. The mixture was vigorously shaken and stored at 4 °C.

2.3. Preparation of chromogens

The chromogen solution was prepared in 0.1 M phosphate buffer, pH 7.0 by mixing *o*-dianisidine (10 $mg\,ml^{-1}$) and ABTS (50 $mg\,ml^{-1}$) in 1:1 ratio. The chromogen solution (20 μl) and enzyme mixture (230 μl) were mixed together for immobilization on to different matrices viz. nitrocellulose, polycarbonate, polyester membranes and filter papers.

2.4. Preparation of buffers

0.1 M phosphate and citrate buffers of different pH were made to standardize the method for development of biostrips. The response time of the strip was kept uniform throughout the standardization.

2.5. Preparation of galactose biostrips

The mixture of enzymes and chromogen solution was immobilized on to different matrices using 0.25% glutaraldehyde (crosslinking reagent) in a humidity free chamber. After complete drying, the paper was cut into pieces of 5 mm width using paper cutter. Polyvinyl chloride sheet (1mm thickness) was cut into the size of 9.0×90 cm^2 with a strip cutter. The immobilized enzyme papers were pasted on to one edge of the plastic sheet using a non-reactive adhesive. The sheet was dried in a humidity free chamber for 1–2 h. After complete drying, the sheet was cut into 0.5×9.0 cm^2 pieces in such a manner that one end of the strip has an immobilized enzymatic pad and the other end is free for handling. The strips were packed in dark brown bottles containing desiccant and stored in refrigerator (4 °C) and at room temperature (25 °C).

2.6. Principle of the biostrip

The basic principle of the biostrip reactions are as follows:

$$\text{Galactose} + O_2 \xrightarrow{\text{Galactose oxidase}} \text{D-galacto-hexodialdose} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + \frac{1}{2}O_2$$

$$\frac{1}{2}O_2 + \text{Chromogens} \rightarrow \text{Coloured complex}$$

The biostrip is dipped in the sample and colour developed on the strip was compared after 2 min with colour chart, which was made using different known concentrations of galactose.

2.7. Preparation of samples

Milk samples are mostly undiluted used for testing galactose content by biostrips. A measured amount of liquid samples and powders were diluted to a known volume of water. Dry solids are ground to a fine powder and dissolved (w/v) in water. Wet solids such as soft cheeses are homogenized with water in a blender and then quantitatively diluted with water. After dilution, the samples are tested with biostrips.

3. Results and discussion

The galactose content in lactose hydrolyzed milk and its products become half of the lactose content and varies from 10 to 50 $g\,l^{-1}$ (Aseha, 2003; Sharma et al., 2002a). On the other hand, in non-hydrolyzed milk the galactose content may vary from 0.02 to 3.5 $g\,l^{-1}$ (Acosta, 1993). There are various analytical methods for the

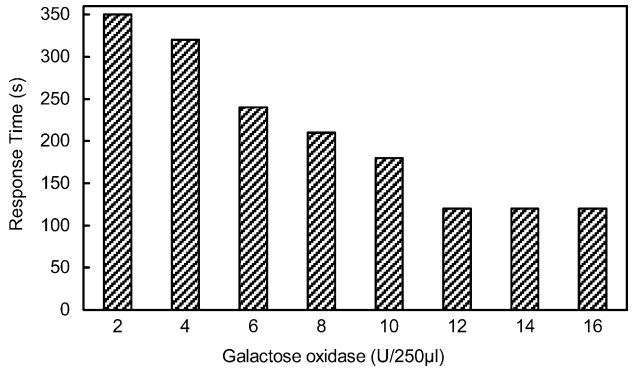

Fig. 1. Effect of galactose oxidase concentration on response time for development of colour gradient. Peroxidase (50 U) and chromogens (20 µl) were dissolved in 230 µl 0.1 M sodium phosphate buffer, pH 7.0. The response time of the strips were recorded by developing the colour.

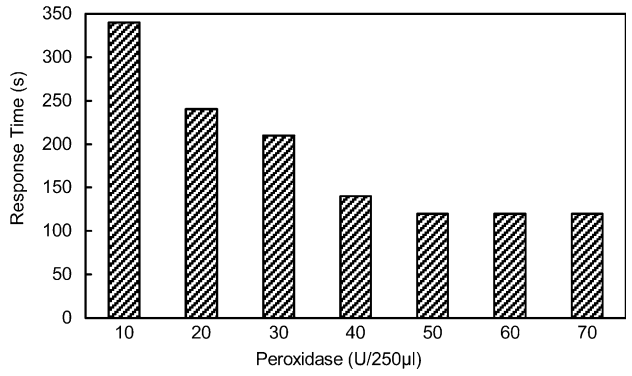

Fig. 2. Effect of peroxidase concentration on response time for development of colour gradient. Galactose oxidase (12 U) and chromogens (20 µl) were dissolved in 230 µl 0.1 M sodium phosphate buffer, pH 7.0. The response time of the strips were recorded by developing the colour.

detection of galactose in milk and other food products. The biostrip, based on colorimetric response is a quick and simple technique for the detection of analytes in the given samples. To develop galactose biostrip different parameters were standardized keeping others constant at fixed response time 120 s (Figs. 1 and 2). Galactose oxidase (12 U/250 µl) and peroxidase (50 U/250 µl) were found optimum units required for showing colour at different concentrations of galactose at fixed response time (120 s). Different buffers were also tried to achieve better gradient of the colour. Phosphate buffer (0.1 M, pH 7.0) was found the most appropriate buffer for galactose strip. All reported analytical methods require trained person and sophisticated instruments such as colorimeter, spectrophotometer, biosensor etc. while the immobilized enzyme strip developed in our laboratory offers quick detection of galactose in samples. The enzymatic pad region of the strip is dipped in the milk or milk products solution and developed colour can be compared with the colour chart (Fig. 3). The strip gives different shades of colour from yellowish white to dark

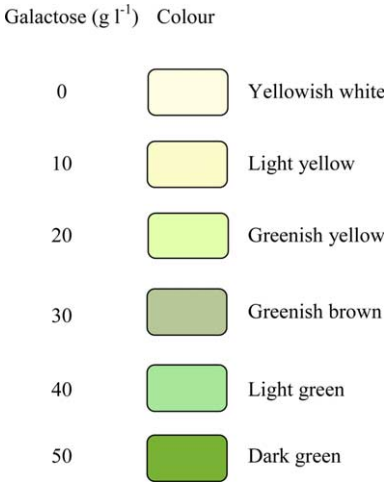

Fig. 3. Colour chart for galactose detection in milk and milk products. Colour gradient was made by dipping the strip in different galactose concentrations in galactose/lactose free milk samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

green depending on the concentration of galactose present in the sample. The test strip has the sensitivity of 10 g galactose l^{-1}. The colour of the strip does not show any significant change with galactose concentration lower than 10 g galactose l^{-1}. Galactose free milk was used as control and different concentrations of galactose were made with this milk. The response time of the strip was found to be 120 s. In galactose biostrip, co-immobilized enzymes (viz. galactose oxidase and peroxidase) and chromogens react with galactose present in the sample resulting in colour formation on the enzymatic pad of the strip. The intensity of the colour is proportional to the concentration of galactose present in the sample.

Different types of matrices (nitrocellulose, polycarbonate, polyester membranes and filter papers) were tried for the immobilization of enzymes. Whatman No.1 filter paper was only found to be more suitable for immobilization of enzymes since it did not react with the enzymes and chromogens and showed better gradient of colours.

Certain interferents like ascorbic acid, calcium chloride and uric acid may affect the response of galactose estimation. These are the major interferents found in the milk. Hence, the response in development of colour on biostrip in the presence of these substances were studied at their physiological normal levels with 20 g galactose l^{-1}. There was no significant effect of the interferents on the galactose estimation. Cross-reaction studies with disaccharides (lactose, sucrose) and monosaccharides (glucose, fructose) at 10–20 g l^{-1} have no significant effect due to strong specificity of galactose oxidase (immobilized in strips) with galactose.

The galactose content in some dairy samples was estimated using biostrip method and comparison was

Table 1
Estimation of galactose content in dairy samples by biostrips and its comparison with reported methods

Sources	Reference method[a] (g galactose l^{-1})	Biostrip method[b] (g galactose l^{-1})
Cow's milk (NH)	2.20	Light yellow
Cow's milk (H)	23.00	Greenish yellow
Buffalo's milk (H)	21.00	Greenish yellow
Human milk (NH)	3.50	Light yellow
Human milk (H)	34.00	Greenish brown
Goat milk (H)	23.00	Greenish yellow
Yogurt natural	23.00	Greenish yellow
Ice cream	23.00–29.00	Greenish brown
Chocolate milk	45.00	Dark green
Nusobee (Casein containing soya formula)	0.02	Yellowish white
Zerolac (soya formula)	0.02	Yellowish white

[a] Acosta (1993), Aseha (2003) and Osiecki (1990).
[b] The developed colour on to the reaction pad of the strips were compared with colour chart (Fig. 3). NH, non-hydrolyzed milk samples and H, hydrolyzed milk samples. The milk samples were hydrolyzed in the presence of lactase (0.1–0.5 units ml^{-1}) for 1–2 h at room temperature. The solid sample 1 mg was dissolved in 1 ml water to make uniform concentration (g galactose l^{-1}).

made with reported values found in different samples of dairy products. The results are shown in Table 1. The biostrip method is semi-quantitative and exact content of galactose in the samples cannot be measured but shows a good correlation of galactose content with reported values. The measured value may vary ±10 of the exact quantity.

The strips were stable at room temperature (25 °C) as well as in refrigerator (4 °C) for 1 year when stored under dry conditions. The stability of the strip was found more when stored in refrigerator (Fig. 4). The immobilized enzymes galactose oxidase and peroxidase are less inactivated at 4 °C as compared to room temperature (25 °C). The use of strip can prevent galactosemia symptoms by avoiding lactose and galactose containing milk and milk products. Being a visual assessment technique, it does not require any specialized training or sophisticated instruments. The test is quick, simple as well as economical and can be used in dairies, hospitals, home and remote places where sophisticated instruments may not be available.

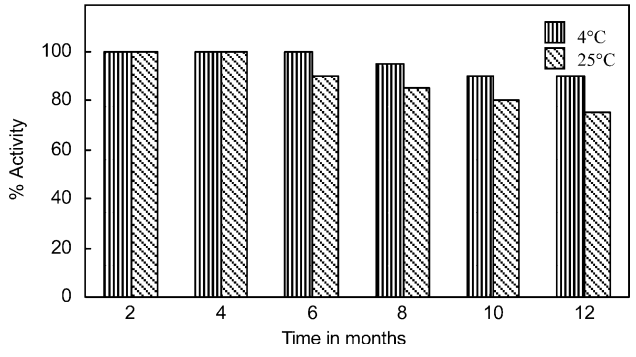

Fig. 4. Stability of galactose biostrips at different temperatures. The strips were stored in refrigerator (4 °C) and at room temperature (25 °C). The strips were tested at different periods by dipping in standard galactose solutions prepared in milk. Freshly made strip as well as stored strips at different temperatures were dipped in different dilutions of galactose solution. The developed colour on stored strips was compared with the colour of fresh strips keeping response time constant at 2 min.

Acknowledgements

The authors are thankful to Prof. S.K. Brahmachari, Director, Institute of Genomics and Integrative Biology, for providing necessary facilities and encouragement to carry out the research work. This work was supported by CSIR fellowship to Sandeep K. Sharma.

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