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Optimization of reactions between reducing sugars and 1-phenyl-3-methyl-5-pyrazolone (PMP) by response surface methodology☆

Weizheng Wang[a,b], Feng Chen[a,b,*], Yaqi Wang[b], Lina Wang[b], Haiyan Fu[b,c], Fuping Zheng[a,b], Lance Beecher[d]

[a] Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing 100048, China
[b] Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC 29634, USA
[c] School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan 430074, China
[d] Clemson Cooperative Extension Service, Clemson University, Clemson, SC 29634, USA

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ABSTRACT

Reducing sugars have strong reactivity with 1-phenyl-3-methyl-5-pyrazolone (PMP) to form the sugar-PMP derivatives, which can be more accurately analyzed by HPLC-UV at 248 nm. Glucose and glucosamine reacted with the PMP based on the response surface methodology within a range of temperature between 60 °C and 80 °C, and time between 60 and 180 min. The optimal conditions for the glucose-PMP and glucosamine-PMP reactions were obtained at 71 °C for 129 min, and 73 °C for 96 min, respectively. Subsequently, other sugars and their derivatives, including xylose, ribose, fructose, galactose, mannose, lactose, maltose, sucrose, glucuronic acid, sorbitol, mannitol, xylitol, and cyclodextrins were investigated and compared under the optimized condition for glucose. All of the above compounds, except the fructose, sugar alcohols and non-reducing sugars, could form the sugar-PMP derivatives. This study demonstrated that different chemical structures of sugars and their derivatives could significantly influence the rate and yield of the PMP derivatization.

1. Introduction

Carbohydrates are one of the major nutrients for human beings, and the primary metabolites of plants, which have various biological activities (Becker, et al., 2013). Although some analytical methods, such as NMR (Kazalaki, Misiak, Spyros & Dais, 2015), Fourier Infrared spectrometer (Tong et al., 2013), HPLC-RI (Zielinsk et al., 2014), HPLC-ELSD (Ma, Sun, Chen, Zhang, & Zhu, 2014; Shanmugavelan et al., 2013; Dvořáčková, Šnóblová, & Hrdlička, 2014) and LC-MS (Sun, Wang, Xie & Su, 2016) were used to characterize and/or measure carbohydrates, they are either too expensive, or less sensitive and accurate than HPLC-UV. In addition, gas chromatography mass spectrometer (GC–MS) is not perfectly desirable for carbohydrate detection due to their various end-product derivatives that will lead the analytical results to be complex and ambiguous (Galant, Kaufman, & Wilson, 2015). Therefore, developing a more accurate and sensitive method for the carbohydrate analysis is still a challenge and an attractive topic to many researchers.

Since natural carbohydrates that are lack of chromophore and luminophore groups cannot be detected by common UV–Vis spectrometers and fluorescence detectors, carbohydrates are often chemically derivatized to fit the UV and fluorescent determination with higher detective sensitivities, as well as to meet the huge analytical demands in academia and industry. Among the carbohydrate derivative reagents, 1-phenyl-3-methyl-5-pyrazolone (PMP) that was first proposed in 1989 (See Fig. 1) (Honda, et al. 1989) is considered to be one of the most satisfied reagents, which make the detection of sugar-PMP derivatives with many advantages in light of their strong absorbance under UV light at 245 nm, relatively lower detection limits as low as to 1 pmol or 100 fmol, and no side effects such as desialylation or desulfation on special sugars such as sialylated N-glycans (Harvey, 2011; Lamari, Kuhn & Karamanos, 2003). More importantly, the PMP derivatization products degrade very slowly under the storage at 4 °C even after 60 days (Xian et al., 2015). However, although some studies have reported the PMP derivatization for analysis of reducing sugars (Bai, et al., 2015; Rühmann, Schmid, & Sieber, 2014; Zhang, et al., 2016), none of them reported the optimization of the reaction conditions, let alone comparison of optimal conditions for different sugars.

In regards to the derivatization process, several factors, such as the reaction time, temperature and solvent pH, could remarkably influence the yields of reducing sugar-PMP derivatives. However, if just a single

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* Corresponding author at: Department of Food, Nutrition and Packaging Sciences, Clemson University, Clemson, SC 29634, USA.
E-mail address: fchen@clemson.edu (F. Chen).

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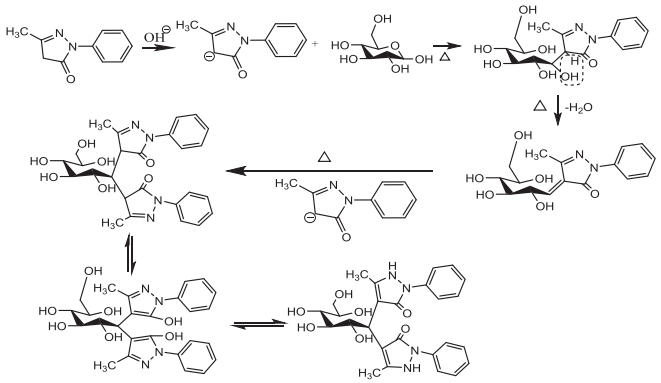

Fig. 1. Principle of reducing sugar-PMP derivatization.

variable of the aforementioned factors is analyzed, cross-impact of the variables would usually be neglected. On the other hand, a complete experimental design to explore the relationships of the exploratory variables is often time-consuming. In this context, response surface methodology (RSM) has been suggested to find the most valuable point, or called optimization to simplify the experimental design and, simultaneously, maximize the production, or minimize the cost, side reactions, etc,. RSM has been applied in many fields, such as extraction process, enzymatic catalysis, bacterial cultivation, and many other chemical reactions (Kong, He, Chen, & Chen, 2004; Lee, Yusof, Hamid, & Baharin, 2006; Zhao, et al., 2011). In regards to the RSM, central composite design (CCD) is often used to support the optimization of a two-level factorial or fraction factorial design, which is usually coded as −1 and 1 so as to simplify the experimental expression and calculation (Bezerra, Santelli, Oliveira, Villar & Escaleira, 2008).

To explore the optimized conditions for various sugar-PMP derivatives, three different aldehyde hexoses and one pentose, including glucose, glucosamine, glucuronic acid and ribose, were initially investigated as the models for the RSM optimization of their PMP derivatizations. Although these monosaccharides have different delicate structures, their reactions with PMP still follow the same chemical principle (shown in Fig. 1). Moreover, this study has investigated other aldehyde hexoses, pentoses, ketose, alditols, disaccharides, and oligosaccharides, in order to compare the efficiencies of their reactions with PMP.

In this context, this research aimed to (1) investigate the optimized conditions for different sugar-PMP derivatives; and (2) study the efficiency and yields of the chemical derivatizations of different sugars with PMP.

2. Experimental design

2.1. Chemicals and reagents

The following chemical standards were purchased from Sigma-Aldrich (Milwaukee, USA), including D (+)-glucose, D-(−)-ribose, D-(+)-xylose, D (+)-glucosamine, D-(+)-lactose, D-(−)-fructose, D-(+)-mannose, D-(+)-maltose monohydrate, D-sorbitol, D-mannitol, α-, β- and γ-cyclodextrins, 1-phenyl-3-methyl-5pyrazolone (PMP) and ammonium acetate. Galactose was purchased from Fisher Scientific (New Jersey, USA). Analytical grade of hydrochloric acid (37%) was obtained from Acros Organics (part of Thermo Fisher Scientific). HPLC-grade chloroform with 0.75% ethanol, as well as HPLC-grade acetonitrile, acetic acid (glacial) and sodium hydroxide, were provided by the Fisher Scientific (Atlanta, GA, USA).

2.2. Pretreatment of sugar standard

Approximate 5 mg of glucose was weighed using an XS-200D analytical balance, and transferred into a glass tube, followed by an addition of 10 mL of distilled water. The glucose was dissolved in water facilitated by a Fisher vortex mixer until no observable particles were observed. The aqueous solution was then filtered through 0.45 μm nylon filter (MACHEREY-NAGEL Co.). Other sugar standards were pretreated as glucose.

2.3. Preparation of reactants and buffer solutions

An amount of 87 mg of PMP powder was weighed accurately and transferred into a clean glass tube, which was mixed with 1 mL of methanol to prepare 0.5 M PMP-methanol solution. Meanwhile, 6 g of sodium hydroxide (NaOH) was dissolved in 500 mL of distilled water to prepare a fresh 0.3 M NaOH solution, while 0.3 M hydrochloric acid (HCl) solution was prepared via 37% HCl dilution. A buffer solution composed of ammonium acetate (7.7 g/L) was adjusted to pH 5.51 by acetic acid, which was used as the mobile phase A of the reverse phase HPLC. However, in order to rapidly and efficiently elute the glucuronic acid from the same HPLC column under the same chromatographic program, the original mobile phase A prepared by the ammonium acetate (7.7 g/L) was adjusted to pH 8.0 by triethylamine rather than the acetic acid.

2.4. Preparation of sugar-PMP derivatives

The preparation of reducing sugar derivatives with PMP was achieved based on the experimental modification of a previous report (Dai et al., 2010). In detail, an aliquot of 100 μL of sugar standard solution (500 μg/mL) was mixed with 100 μL of 0.3 M NaOH solution to approach its pH value at 13 in a 1.5 mL micro-centrifuge tube (VW, North American Co). Then, this mixture containing 200 μL of sugar-NaOH solution was added with 100 μL of 0.5 M PMP-methanol solution, mixed by a vortex at the fourth level-shaking rate for 1 min, followed by incubation in a water bath at 70 °C for 120 min. After the reaction was completed, the sample was neutralized with 100 μL of 0.3 M HCl solution. Then, HPLC-grade chloroform was added into the tube as an extraction solvent for clearance of the PMP residues. The mixture was shaken vigorously for 1 min before abandoning the bottom layer, the chloroform layer. This extraction procedure was repeated three times to remove the PMP residues. Finally, the supernatant was filtered through a 0.45 μm filter into a HPLC auto-sampler vial (12 × 32 mm, Sigma-Aldrich Co) for chemical determination.

2.5. Experimental design

There are some variables in regards to the chemical derivatization, including solution temperature, pH and reaction time. According to our preliminary experiment (shown in Supplemental Fig. 1), high pH value (pH = 13) was necessary for the sugar-PMP derivatization and could significantly increase its yield from pH 11 to 13, but continuous increase of the reaction pH value could impede the reaction. This result was in consistence with the proposed value in the previous reports (Honda, et al., 1989; Strydon, 1994). In this context, when the pH value of the reaction was fixed at pH 13, the reaction time and temperature were selected as the primary factors for the primary RSM design, for which a two-factor central composite design (CCD) was performed in order to fulfil an orthogonal analysis. Herein, two independent variables were depicted as time (X_1) and temperature (X_2), respectively, which were coded in two levels as −1 and 1, and one center point as 0. The experimental design of RSM for glucose, glucosamine, glucuronic acid and ribose are shown in Table 1, and Supplementary Tables 1, 2, 3, respectively, where the experimental response (ER) represents the area of the respective sugar-PMP peak shown in the HPLC-DAD chromatogram. The function of Y was used for predicting the optimal condition, expressed as the following Eq. (1)