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EFFECT OF XANTHAN GUM AND PECTIN ON STABILIZATION AND ANTIOXIDANT ACTIVITY OF *MONASCUS*-PIGMENT EXTRACT BEVERAGE

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Abstract: This research aimed to study the effect of pectin and xanthan gum on the *Monascus* pigment turbidity, DPPH radical scavenging ability, pH and sensory evaluation of *Monascus*-pigment extract beverage (MPEB). The pectin and xanthan gum contents used in the beverage were 0.50 – 0.70 % w/v and 0.01 – 0.03 % w/v, respectively. The results showed that the addition of 0.60 % w/v of pectin in MPEB showed the highest values of the turbidity of *Monascus* pigment ($OD_{660} = 2.59$), DPPH radical scavenging ability (45.68 %) and sensory evaluation (>8 of all attributes).

Keywords: Pectin; Xanthan gum; *Monascus*-pigment extract beverage

1. Introduction

Presently, functional foods are highly popular products on the market such as baby foods, baked goods and cereals, dairy foods, confectionery, snacks, meat products, spreads, and beverages [1]. In particular, the advantages of functional beverages are the following: (1) a lot of bioactive compounds; (2) convenience and possibility to meet consumers' demands for container contents, sizes, shapes, and appearances; (3) ease of distribution and better storage for refrigerated and shelf-stable products [2]. There are beverages produced from herbs and fruit pulps indicating highly potential antioxidant activities. For examples, Awe et al. [3] documented the antioxidant benefits of a novel functional beverage obtained from *Hibiscus sabdariffa* extract (HSE), cocoa

and ginger since HSE extracts contain the high amounts of protein and other nutrients required for good health [4]. Moreover, Lu et al. [5] suggested that a durian pulp drink obtained from a combination of fermentation of *Bifidobacterium animalis* subsp. lactis or *Lactobacillus casei* and yeast *Williopsis saturnus* could be a novel non-dairy durian-based functional beverage to deliver probiotics. To date, *Monascus*-pigment extract from monascal rice has never been used as a functional drink. Thus, the research of a *Monascus*-pigment extract beverage (MPEB) is interesting; this beverage is expected to be an alternative drink to lower cholesterol in blood and a novel anti-oxidative drink. *Monascus* pigment is comprised of many kinds of cholesterol-lowering statins and pigments produced by *Monascus* sp. This

pigment is used as a medicine to the potent cholesterol-lowering, antiatherosclerotic drug lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor [6]. *Monascus* red pigment, obtained from *Monascus* fermentation is interesting as indicated by high antioxidant activities from *in vitro* experiments, such as inhibition of peroxidation, reducing power, scavenging ability on DPPH radicals and chelating ability on Fe^{2+} as well as used in food as a coloring agent in fish and meat [7, 8, 9, 10].

Commonly, the destabilizing problems of beverages are the coalescence and the sedimentation of food particles or bioactive compounds which are causes of unacceptable consumer [11]. The pigments are well known as low water solubility [12]. From this standpoint, the expected drawbacks found in MPEB are the phenomena of the coalescence and the sedimentation of water insoluble compositions around neck of beverage bottle. There are the researches applying the hydrocolloids, such as xanthan gum and pectin, to solve the dispersion in many products. Zecher and Van Coillie [13] reported that the contents of xanthan gums in the range of 0.001 – 0.5 % were used in drinks made with citrus and in fruit-flavored drinks to create a satisfactory texture and as a stabilizer for odor and flavor. Xanthan gum improved the insoluble components suspended in the sample at low pH, which the components would be dissolved quickly and completely. Lucey et al. [14] also mentioned that whey separation was significantly decreased by increased pectin concentration. Therefore, the effects of pectin and xanthan gum might be expected to occur as the functional properties on the stability of MPEB.

The aims of this work were to evaluate the turbidity, DPPH radical scavenging ability, pH, and sensory evaluation of MPEB formulated by pectin and xanthan gum.

2. Materials and methods

2.1 Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH) and ethanol were analytical reagent grade and obtained from Sigma–Aldrich (St. Louis, MO). Pectin and xanthan gum were of food grade and purchased from Daejung (Shiheung, South Korea).

2.2 Monascus rice preparation

About 100 g of rice seeds (*Oryza sativa*) and 10 mL of distilled water were transferred into a 500 mL flask. The mixture was sterilized by an autoclave at 121 °C for 15 min and then left until cool down. A 5 mL of 10^6 spores/mL spore suspension of *M. purpureus* TISTR 3090 obtained from actively growing slants in sterile water was inoculated into sterilized waxy corn and incubated at 25 °C for 12 days [9]. Then, the product was dried in an oven at 40 °C for 24 h and its moisture content was 7.12 ± 1.22 %. A fine powder (20 mesh) was obtained using a mill (Retsch ultracentrifugal mill and sieving machine, Haan, Germany).

2.3 Preparation of *Monascus* extract from angkak

The extraction method described by Yang et al. [7] was used with some modifications. A 10 g sample was extracted in a shaker with 100 mL of ethanol at 170 rpm for 24 h and the solution was filtered through Whatman no.4 filter paper. The residue was then extracted with two additional 100 mL portions of ethanol as described above. The combined ethanolic extracts were then evaporated at 40°C to dryness. The dried

extract was kept in -20°C until used for *Monascus*-extract beverage preparation.

2.4 *Monascus*-extract beverage preparation (MPEB)

The *Monascus* extract was diluted with drinking water at a ratio of 0.5 : 100 (w/v). Then, the contents of pectin (0.50, 0.60 and 0.70 % w/v) and xanthan gum (0.01, 0.02 and 0.03 % w/v) were added in the mixtures and mixed for 5 min using a mixer. MPEBs containing both pectin and xanthan gum were pasteurized at 60 °C for 20 min., whereas pectin and xanthan gum were not added to the control sample. MPEBs added with pectin and xanthan gum after pasteurization were then poured and packed in polypropylene bottles, and they were kept in 4°C until the analysis.

2.5 Turbidity

The turbidity of MPEB was determined in duplicate by diluting each emulsion in deionized water (1:1000) and measuring the absorbance at 660 nm against distilled water using a spectrophotometer (UV 2100, Unico, England) [15].

2.6 pH measurements

The pH of the samples was measured by using a pH meter (Thermo Orion model 420, USA). The measurement was carried out in triplicate [16].

2.7 DPPH Free Radical Scavenging Assay

The DPPH free-radical scavenging activity was determined by the method described by Kraboun et al. [9] Two hundred and fifty microliters of the beverages in ethanol solution (5 mg/mL) was added to 250 µL of 5.07×10^{-4} M DPPH ethanol solution. The reaction mixture was incubated in the dark at room temperature; the absorbance was measured at 517 nm after 15 min. The absorbance of a blank sample containing

the same amount of solvent was also measured. DPPH radical scavenging activity was calculated by using the following equation:

$$\text{Scavenging activity (\%)} =$$

$$\frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \times 100\%$$

2.8 Sensory evaluation

Twenty-six males and forty-four females of undergraduates from the Division of Food Safety Management and Technology, between the ages of 18 and 24, were panel participants. The samples were coded with three digits and panelists were instructed to evaluate the appearance, odor, texture, flavor and overall score using a nine-point hedonic scale ranging from "1=extremely dislike" to "9=extremely like" according to the method of Sudha et al. [17].

2.9 Statistical analysis

All determinations were performed in triplicate and results were expressed as the mean \pm standard deviation calculated using spreadsheet software Microsoft Excel. This was carried out in a completely randomized design (CRD) which the data were analyzed by an analysis of variance ($p \leq 0.05$) and means were compared with Duncan's multiple range test. The results were processed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows.

3. Results and discussion

3.1 Turbidity and pH of *Monascus*-pigment extract beverage (MPEB)

Turbidity is an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample. For turbidity analysis for a cloudy beverage, the beverage should have high turbidities due to indicating the

stability of beverage or absence of ringing resulting from raised flocculation and coalescence of water insoluble compositions around the neck of the beverage bottle [18]. According to Figure 1, the results showed that the turbidity of MPEBs increased with percentage of pectin which MPEB supplemented with 0.60 % w/v of pectin gave the highest turbidity ($OD_{660} = 2.59$). However, increasing percentages of xanthan gum affected decreased turbidity of the beverages. This is in agreement with Mirhosseini et al. [19], who reported that the addition of pectin to the orange beverages affected more physical stability and cloudiness than those added with other hydrocolloids. Buffo et al. [20] demonstrated the balance of Van der Waals, electrostatic and polymeric sterics as the main droplet interactions playing a

key role in the stability of beverage. This aggregation phenomenon of the water insoluble pigments of *Monascus* may be due to the change in refractive index of the pigment phase and aqueous phase. Therefore, addition of a high content of pectin is needed in the formulation in order to reduce ring formation phenomenon [21]. The changes of pH in MPEBs are shown in Figure 1 and were significant difference ($p \leq 0.05$), which were in the range of 3.92 – 5.43. The addition of xanthan gum contributed to higher pH values of MPEBs than that of pectin. This is agreement with Rothschild and Karsenty [22], who studied the low turbidity of pasteurized beverages during storage. It indicated that the loss of turbidity during storage was from a high acidity condition. Hence, this factor would be resolved by storing the products in near-neutral pH.

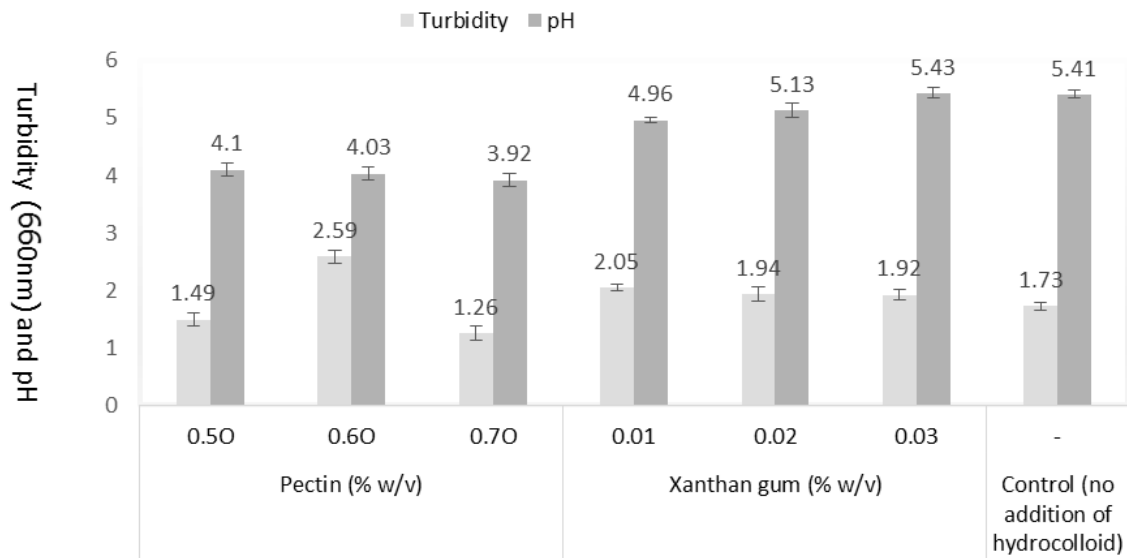


Fig. 1 Turbidity and pH of MPEB added with various concentrations of pectin and xanthan gum

3.2 DPPH free-radical scavenging ability of *Monascus*-pigment extract beverage (MPEB)

The DPPH free-radical scavenging ability of MPEB is shown in Figure 2. There were a relationship between DPPH free-radical scavenging ability and turbidity of MPEBs

(Figure 1 and 2). As pectin results, the higher 0.60 % w/v of pectin indicated decreased DPPH free-radical scavenging ability; however, increasing the contents of xanthan gum affected decreasing DPPH free-radical scavenging ability. It might be possible that the better dispersion of the pigment in MPEB added with 0.60 % w/v of pectin led to the better dispersion of monacolin K and/or other antioxidants (phenolic compounds) throughout within the bottle, which this cause affected the good detection for DPPH free-radical scavenging ability. Moreover, the addition of high pectin contents in MPEB (0.60 % w/v) increased the antioxidant activity by the nonpectins (phenols and proteins) obtained from pectin extraction [23]. Therefore, investigating the capacity might be from antioxidant crude pectic

polysaccharides from plant cell wall matrix (i.e., bioaccessibility). Raw polysaccharide extracts have been shown to possess the higher antioxidant activity than purified extracts [24]. These results agree with those of Wang and Lu [25], who found that the pectin has been shown to exhibit higher anti-DPPH activities (greater than 50%) at a concentration of 1 mg/mL. Pectin fractions showed to inhibit Xanthine Oxidase (XO) and scavenge Reactive oxygen species (ROS). Inhibition of XO may be mediated by pectic polysaccharide chains, whereas protein and phenolic contaminants were suggested to provide the scavenging of ROS. These data demonstrated each constituent of pectin fractions to be important for implementation of antioxidant effect in the gastrointestinal milieu.

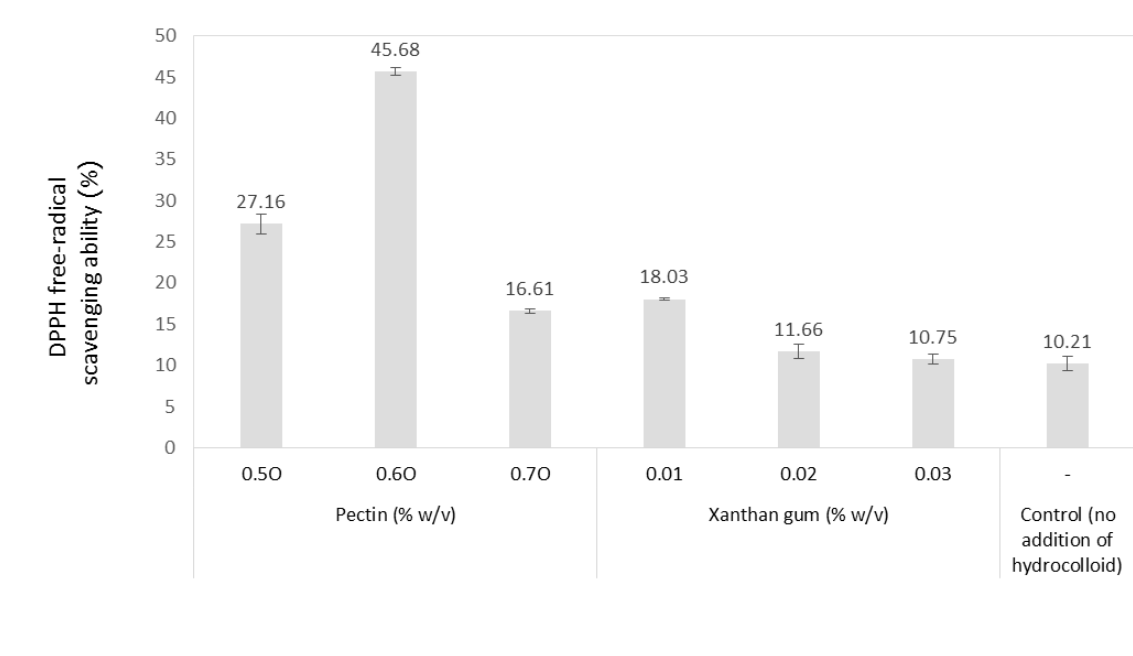


Fig. 2 DPPH free radical scavenging ability of MPEBs added with various concentrations of pectin and xanthan gum

3.3 Sensory evaluation of *Monascus*-pigment extract beverage (MPEB)

The results of a sensory evaluation using the 9-point hedonic scale are shown in Table 1. The results of sensory testing for

appearance, color, taste, odor and overall acceptability showed significant difference ($p\leq0.05$) between MPEBs containing pectin and xanthan gum. Therefore, the types of stabilizer affected these sensory

parameters. The sensory quality of MPEB added with pectin at 0.60 % w/v reached the highest scores of all attributes. Whereas, the scores of all attributes decreased when concentration of xanthan

gum increased. This is in agreement with Enriquez and Flick [26] affirmations, who reported that pectin was used as an odor and flavor stabilizer in the drinks because it provides good texture and odor.

Table 1
Sensory evaluation of *Monascus*-pigment extract beverage (MPEB) added with various concentrations of pectin and xanthan gum

| Hydrocolloid | Concentration (% w/v) | Attribute | | | | |
|---|--------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | | Appearance | Color | Odor | Taste | Overall acceptability |
| Pectin | 0.50 | 5.67±1.47 ^c | 5.83±1.20 ^c | 6.33±1.60 ^c | 6.87±1.63 ^c | 5.57±1.67 ^c |
| | 0.60 | 8.57±1.65 ^d | 8.70±1.57 ^d | 8.70±0.91 ^f | 8.97±1.18 ^f | 8.83±1.08 ^d |
| | 0.70 | 4.50±1.07 ^b | 5.07±1.38 ^b | 5.87±0.97 ^d | 6.83±2.05 ^e | 5.17±1.45 ^b |
| Xanthan gum | 0.01 | 5.50±1.77 ^c | 5.17±1.55 ^b | 5.27±2.16 ^c | 5.40±1.52 ^d | 5.13±1.22 ^b |
| | 0.02 | 4.77±1.30 ^b | 4.23±1.73 ^a | 4.43±0.93 ^b | 4.57±1.61 ^c | 5.14±.79 ^b |
| | 0.03 | 4.67±1.07 ^b | 4.13±1.35 ^a | 4.07±1.41 ^a | 4.10±1.71 ^b | 5.10±1.44 ^b |
| Control (no addition of - hydrocolloid) | - | 4.10±1.39 ^a | 4.03±1.74 ^a | 4.02±1.27 ^a | 3.47±1.85 ^a | 3.93±1.63 ^a |

Different letters indicate significant differences in treatment means from three determinations ($p \leq 0.05$).

4. Conclusion

The MPEB supplemented with 0.60 % w/v of pectin gave the highest turbidity, DPPH free-radical scavenging ability and sensory attributes. However, the changes of pH in all MPEB formulae were significantly different ($p \leq 0.05$), which were in the range of 3.92 – 5.43. Therefore, the use of pectin and xanthan gum could play an important role in the dispersion of *Monascus* pigment in the beverage product.

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