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Stability and quality of a bioactive peptide fraction incorporated orange juice

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ABSTRACT

The objective of this study was to evaluate the stability of a bioactive <5 kDa sized peptide fraction in orange juice and its effect on the organoleptic properties. A concentration of 3000 μg/mL of <5 kDa peptide fraction in orange juice was prepared, the samples were stored at 4 °C along with the control without hydrolysates. Samples were drawn at 0, 1, 3, 7, 10, 14, 21, 28, 35, and 42 days to monitor pH, color, total soluble solids, ascorbic acid, and peptide fraction concentration. Organoleptic properties were assessed at 0 and 14 days using a triangle test. The orange juice with the peptide fraction showed similar pH, color, and ascorbic acid content when compared to control throughout the study. The concentration of the peptide fraction in orange juice remained stable throughout the study. Analysis of sensory data showed no significant difference between the freshly prepared control and peptide fraction incorporated orange juice ($p = 0.0583$). However, the orange juice stored for 14 days at 4 °C showed varying acceptance levels from the panelists which was significant. Overall, the study demonstrated that orange juice can be a potential vehicle for application of bioactive peptide fractions.

1. Introduction

Functional foods offer consumers the added benefits beyond the essential nutritional support from the product (Gray, Armstrong, & Farley, 2003; Granato, Branco, Nazzaro, Cruz, & Faria, 2010). The global functional food market witnessed an annual growth of 6% in the last five years (Statista, 2015) and is expected to reach $54 billion by 2017 (Bonar, 2014). The retails sales of functional beverages in the United States is approximately $18 billion, with half of adult buyers consuming functional drinks (Statista, 2015). Hence, there is a great demand from the consumers for incorporated nutraceutical and functional ingredients in food products. Previously, scientists have studied new sources of functional ingredients, specifically proteins and peptides (Chandi & Sogi, 2007). Rice bran is a low cost by-product of rice milling industry but is rich in health beneficial components like proteins, vitamins, minerals, soluble fiber, and lipids (Hamada, 2000; Piyaratne, Atapattu, Mendis, & Amarasinghe, 2009). Kannan, Hettiarachchy, Johnson, and Nannapaneni (2008) demonstrated that protein hydrolysates isolated from rice bran protein have anticancer activities on multiple human cancer cells. Other researchers have demonstrated breast cancer cell inhibition by a rice bran peptide using molecular studies (Li, Hettiarachchy, & Mahadevan, 2014a, 2014b) and incorporated a nano-encapsulated peptide in fruit juice (Alessa et al., 2014). Although FDA has strict regulation for pure peptides (as biologics) under the label of 'drugs' (FDA, 2012), incorporation of hydrolyzed protein into beverages such as protein and sports drinks has become more common in the food industry (Pennings et al., 2011; Sinha, Radha, Prakash, & Kaul, 2007).

Functional ingredients added to foods or beverages become part of the system where they are prone to interact with other components and cause detrimental effect in overall product quality. The storage stability of the nutraceutical ingredient incorporated in the food product is an important attribute that influences its bioactive viability (Day, Seymour, Pitts, Konczak, & Lundin, 2009). Therefore, testing the stability and quality is a primary step while developing functional food products. The loss of one or more product constituents such as nutrients or flavors, or the formation of off-flavors are the limits that set the shelf life. Several food and beverage products, including fruit juices and health drinks have been incorporated with functional ingredients for their unique wellness promoting role along with consumer appeal and marketability (Harbourne,

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Marete, Jacquier, & O'Riordan, 2013; Ron, Zimet, Bargarum, & Livney, 2010).

Fruit juices are thought to be a good medium for serving as functional beverages (Tuorila & Cardello, 2002). They are positioned as healthy products with high acceptance in the rapidly growing market which drives the beverage product design (Luckow & Delahunty, 2004; Sorenson & Bogue, 2005). Orange juice is ranked number one in the United States and is considered as a functional beverage due to the accrued health benefits (Pollack, Lin, & Allshouse, 2003; Sloan, 2014). Beverages are conducive for modifications since they have minimum interactions among their components in comparison to other solid foods (Day et al., 2009). Acidity of orange juice influences other components including added nutraceutical or functional ingredients. Ascorbic acid or Vitamin C is the vital ingredient in natural fruit juices that is considered as a potent antioxidant, but is prone to interactions during processing and storage (Choi, Kim, & Lee, 2002; Kaack & Austed, 1998). Stability of ascorbic acid, with minimum required amounts (≥200 mg/L) in orange juice over the storage period is essential for preserving its quality (Polydera, Stoforos, & Taoukis, 2003). Several researchers have successfully demonstrated fortification of orange juice with functional ingredients for enhanced nutrition and health-promotion without deterring its quality (Biancuzzo et al., 2010; Devaraj, Jialal, & Vega-López, 2004). However, incorporation of functional protein products for enhancing the quality through value-addition has not been reported. Hence, the overall goal of the study was to develop a functional orange juice beverage using the <5 kDa peptide fraction derived from rice bran, which has been previously demonstrated as a bioactive ingredient (Kannan et al., 2008). The objective was to determine the stability of the peptide fraction incorporated in orange juice over storage.

2. Materials and methods

2.1. Materials

Rice bran peptide fraction (<5 kDa) was prepared in the protein research laboratory, University of Arkansas, under optimal conditions using food grade enzyme Alcalase 2.4L and fractionated using ultrafiltration columns (Kannan et al., 2008). Orange juice concentrate was provided by Southern Gardens Citrus Processing, Florida. Series 1050 of Hewlett Packard High-Performance Liquid Chromatography system (GMI Inc., Ramsey, MN) with biopore C-18 semi-preparative affinity chromatographic column (Shimadzu, Tokyo, Japan) was used to quantify the peptide fraction. An HPLC system with C-18 column (TOSOH Bioscience, King of Prussia, PA) was used to quantify Ascorbic acid in orange juice. Other instruments included a refractometer (Bausch & Lomb, Bridgewater, New Jersey) for determining the soluble solids content (°brix value) and a CR-300 Minolta Chroma meter (Minolta Inc. Osaka, Japan) for determining the color. All other materials and supplies were purchased from VWR International Inc. (Bridgeport, NJ) while the HPLC grade chemicals were purchased from Sigma (St. Louis, MO).

2.2. Methods

2.2.1. Determination of <5 kDa peptide fraction concentration

The <5 kDa peptide fraction was separated from other rice bran protein hydrolysates using ultrafiltration and quantified using an HPLC procedure by Kannan, Hettiarachchy, Lay, and Liyanage (2010). The standard curve for the hydrolysates was determined using solutions of peptide fraction in deionized water with the effective concentration as the median. The standards were injected (1000 μL) into a C18 affinity chromatographic column attached to an HPLC system with a flow rate of 2 mL/min using two solvents. Solvent A consists of 0.1% (v/v) Trifluoro Acetic acid (TFA) in deionized water and solvent B has 0.1% (v/v) TFA in 50% acetonitrile in water. Solvent gradient used was as follows: 100% solvent A for 0–5 min which was reduced to 90% from 5 to 60 min, 30% from 60 to 75 min, and 0% A from 75 to 80 min. Absorbance was monitored at 215 nm and the peak areas were used to prepare a standard curve using the three difference concentrations of the peptide fraction in water. The equation to calculate concentration based on the standard curve was:

$$\text{Concentration}(\mu g/mL) = (\text{Area} + 163006)/902.6 \quad (1)$$

2.2.2. Ascorbic acid quantification in orange juice

The ascorbic acid concentration was quantified with the HPLC method as described by the procedure from TOSOH Bioscience (2011) for the HPLC column. Single solvent system with acetonitrile in water (1% v/v) + 0.1% TFA was used for running the sample for 10 min with a 10 μL injection volume at a flow rate of 1 mL/min. The elution peaks were detected at 280 nm and a calibration curve was prepared using ascorbic acid standard obtained from Sigma (St. Louis, MO). The equation to calculate the concentration of ascorbic acid (mg/L) is:

$$\text{Ascorbic acid concentration} = (\text{Peak Area} - 21.518)/0.4453 \quad (2)$$

2.2.3. Experimental design

The experiment followed the statistical model of a repeated measure over time. The between treatment effect in this experiment were tested for orange juice with and without peptide fraction. Time (days of storage) was considered as the within treatment effect as the study was conducted for 42 days. Measurements of pH, peptide concentration, color, total soluble solids, and ascorbic acid concentration were taken at 0, 1, 3, 7, 10, 14, 17, 21, 28, 35, and 42 days of storage. The triplicates of samples for HPLC analysis were randomized to minimize experimental error.

2.2.4. Peptide fraction stability determination in orange juice based on physic-chemical properties

2.2.4.1. Preparation of protein fraction (<5 kDa) incorporated orange juice. Orange juice was prepared by mixing one part juice concentrate with 6.5 parts of water to achieve a solids content of 11.8 °brix, which is the recommended soluble solids for orange juice consistency according to the Code of Federal Regulations under Title 21, Part 146.145 (Johnson, 2000). The prepared orange juice was filled into 18, 1 L PET bottles for the experiment. Half the numbers (9 bottles) were incorporated with 3 g of the peptide fraction (3 mg/mL). The other nine Pet bottles with orange juice without peptide fraction were treated as controls for the study. All 18 bottles were pasteurized at 90 °C for 10 s, cooled to ambient temperature, head space was flushed with nitrogen and stored in the dark at 5 °C.

2.2.4.2. Testing for juice quality. Three replicates of each treatment; control orange juice and orange juice with peptide fraction were tested for the following quality attributes.

The pH meter was calibrated with standard pH buffer solutions (pH = 4.01, and pH = 7.00) and the values for the two samples were measured.

The soluble solids were determined using a refractometer.

Color was measured using a chroma-meter. The chroma-meter was calibrated using a white tile and L*, a*, b* color attributes