Development of a novel fermented pumpkin-based beverage inoculated with water kefir grains: a response surface methodology approach

Wee Yin Koh[1] · Uthumporn Utra[1] · Ahmad Rosma[2] · Mohd. Esah Effarizah[1] · Wan Ishak Wan Rosli[3] · Yong-Ha Park[4]

Abstract: Pumpkin (Cucurbita pepo) is well known for its health and nutritional benefits and is recommended for daily consumption. This is the first report on optimization and development of fermented pumpkin-based water kefir beverage. Optimum pumpkin puree and brown sugar concentrations were found at 22.28 and 9.07% w/v, respectively, were made into a pumpkin-based beverage and fermented with water kefir grains for 24 h at 32 C. The optimized fermented pumpkin-based water kefir beverage was found to be non-alcoholic, achieved good overall acceptability and high Lactobacillus, acetic acid bacteria and yeast cell viability of approximately 10^{12},10^{9} and 10^{9} CFU mL^{-1}, respectively. Overall, the optimized product attained superb technological characteristics and has the potential for industrial exploitation as a refreshing water kefir drink.

Keywords: Box–Behnken design
Cucurbita pepo
Lactobacillus
Water kefir
Yeast

Introduction

Pumpkin (Cucurbita pepo), plant of the family Cucurbitaceae is widely cultivated and used as an ingredient in savory culinary and pastry preparations [1]. C. pepo contains steroidal saponins (tetracyclic triterpenoids), proteins, fibers, polysaccharides, minerals (iron, zinc, manganese, phosphorous, etc.), antioxidants (tocopherols), carotenoids and vitamins [2, 3]. C. pepo has been reviewed as antioxidative, antiviral, antiulcer, lipid-lowering, hepatoprotective, anticarcinogenic, antimicrobial and antidiabetic fruit [4]. Despite superb therapeutic and nutritional benefit in C. pepo, its unmodified fruit pulp or puree has a very short shelf life regarding nutritional as well as microbial quality [5]. Fermentation can increase the shelf life of the food commodities and process them into value-added products which can meet the market demand [6]. The original flat taste of the pumpkin fruit could be improved by the fermentation process which contributes to a ‘‘fermented flavor’’ with high sensory acceptability among consumers [7]. Besides, pumpkin fruit favored the growth of friendly bacteria (Lactobacillus)[7, 8], suggesting that it could be used as a potential fermentation substrate.

Water kefir emerged from the Caucasus Mountains and cultured by translucent irregular granules known as water kefir grains [9]. Water kefir grains contain a variety of beneficial live microorganisms (bacteria and yeasts) depending on their source and geographic region [10]. Several of the different live microorganisms in water kefir grains have probiotic properties that exert beneficial health effects to the host when sufficiently consumed [11]. The water kefir grains are commonly utilized to ferment nondairy substrates such as sugar, fruit or vegetable [12, 13]. Water kefir preparations must meet strict criteria related to quality, safety, and functionality. A key quality criterion is

that kefir contains accurately defined numbers of viable cells as stated in the international food standards (Codex Alimentarius): a minimum of 10^7 CFU mL^{-1} bacteria and 10^4 CFU mL^{-1} yeasts [14].

Most of the investigators had reported on fermented dairy-based kefir products which contain lactose sugar and cholesterol that may pose problems to those with lactose intolerance and cholesterol issues [15–17], thus people are more likely to use lactose-free water kefir with zero cholesterol and less calorie content [18]. Due to water kefir is dairy-free, and possessed consortium of friendly bacteria and yeasts that have high adaptability to various substrates as well as its economic efficiency, water kefir could be a good alternative to the fermented dairy-based product [18].

Response surface methodology (RSM) enables us to consider the interactional effects between multiple variables and this method is more cost-effective, reliable and accurate in comparison to one-at-a-time single factor processes [19]. For instance, to the best of authors' knowledge, only one study has exclusively dealt with the optimization of fermentation condition of apple juice and whey based novel beverage fermented by milk kefir grains (dairybased) [20]. However, no study has dealt with multilateral statistical modeling of factors affecting pumpkin-based beverage fermentation by non-dairy water kefir grains.

Therefore, this study aimed at investigating, by using RSM approach, the influences of ingredients concentration and temperature on the targeted responses of PWK beverage fermented with water kefir grains. This study also focused on the identification of the optimum condition for their fermentation time, overall acceptability, ethanol content, Lactobacillus, acetic acid bacteria (AAB) and yeast cell viability. The proximate, mineral, physicochemical, antioxidants and sensory quality of the optimized PWK beverage were evaluated.

Materials and methods

Water kefir grains

The water kefir grains involved in this research were sourced from a cottage industry located in Cheras, Kuala Lumpur, Malaysia. The water kefir grains were nurtured at room temperature in a brown sugar solution. Brown sugar water was changed every 3 days to maintain the grains viability.

Production of PWK beverage

Pumpkin fruits (C. pepo) was collected from the commercial plantation at Balik Pulau, Penang, Malaysia. The pumpkin fruits were cleaned, deseeded, sliced and steamed for 12 min, dried by absorbent paper and then blended into puree (Inochi Commercial Blender, USA).

In experimental runs, 17 batches containing triplicates of PWK beverage were prepared according to Table 1 experimental levels of ingredients concentrations in glass bottles. Each sample was inoculated with 5% (w/v) water kefir grains. The samples were then incubated at experimental temperature until the sample reached pH 4.5. Fermented samples were stored at 4 C for further characterization.

pH value measurement during fermentation

The pH value of PWK beverage samples was monitored at a 10-min interval (SevenEasy pH meter; Mettler-Toledo, Griefensee, Switzerland) during the fermentation process.

Sensory evaluation

A total of fifty sensory panels were recruited from among the students in the Food Technology Department, School of Industrial Technology, Universiti Sains Malaysia for the hedonic sensory acceptance evaluation of PWK beverage samples using a 7-Point Hedonic Scale. Mean scores of overall acceptability of each sample were used as responses for optimization.

Ethanol content

Ethanol concentrations of PWK beverage samples were measured by a headspace gas chromatography-flame ionized detector (SHIMADZU, HS-GC-FID Ultra, Japan) adhering to protocol laid out by Braga et al. [21]. Data were presented as ethanol content (% v/v).

Determination of Lactobacillus, yeast, and acetic acid bacteria cell viability

Log_{10} CFU mL^{-1} (colony-forming units per mL) was determined by standard spread plate method incubated for 72 h: (1) Lactobacillus cell viability on acidified De Man– Rogosa–Sharpe agar (Merck, Darmstadt, Germany) at 37 C (2) yeast cell viability on yeast–extract–peptone–glucose agar (Himedia, Mumbai, India) at 28 C (3) AAB cell viability on Acetobacter agar (Himedia, Mumbai, India) at 30 C.

Experimental design

RSM coupled with Box Behnken design (RSM-BBD) consisted of 17 experimental runs which include the central point (five replicates) was used for optimization. The ranges of the independent variables in the design were

prescribed into three levels, coded -1, 0, ? 1 (i.e., pumpkin puree concentration (X_1) 20, 25, 30% w/v, brown sugar concentration (X_2) 0, 5, 10% w/v and fermentation temperature (X_3) 22, 27, 32 C) as depicted in Table 1, using the equation as shown below: