A POTENTIAL SOURCE OF HONEY BEE ALTERNATIVE FEED ; BANANA PEEL WASTES

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Abstract:Sugar is the main commodity to feed honey bees during off-season as nectar substitute. Due to the high cost of sugar to feed honey bees, it is required to find an alternative bee feed.Banana is one of the most widely consumed fruits in the world. However, banana peel is normally disposed in municipal landfills, which contribute to the existing environmental problems. The large quantities of banana peel waste (BPW) have the potential of being used for production of an alternative source of honey bee feed. Therefore, we investigated the potential of an alternative bee feed from renewable resources based on banana peel waste. The BPW can produce an alternative feed from cellulase and pectinase at a 61.86 % conversion rate. Also, total phenolic and flavonoid contents of banana peel waste based alternative bee fee (BPW-ABF) were found to be 0.93 mg/ g and 164.81 mg/ g of BPW-ABF, respectively. In addition, the combination of BPW-ABFwith fructose and pollen has reduced the mortality rate to almost 9 times.

Keywords: Alternative feed, Honey bee, Banana peel waste, enzymatic hydrolysis

I. INTRODUCTION

Honey bees are critical pollinators of many economically important agricultural crops [1, 2]. Due to their importance, the worldwide honey bee population decline continues to cause significant alarm [3]. This decline not only endangers an essential pollinator, but also puts food security at risk, as one third of the food humans consume is produced through partial or total honey bee pollination [4].

Honeybees need several nutrients, like carbohydrates, proteins, lipids, vitamins, and minerals for theirgrowth and development [5]. Nectar is the main source of carbohydrates in the natural diet of honey bees. Availability of natural nectar becomes scarce for honeybees during winter season in Korea. Therefore, beekeepers must feed honeybees artificially during off-season. Sugar is the main commodity to feed honeybees during off-season. However, the price for sugar is increasing every year making beekeeping an expensive enterprise. Therefore, the aim of this work is to investigate the suitable and cheaper feeding material to reduce the cost of feeding and keep bees viable during offseason.

A huge quantity of fruit wastes are produced around the world annually. This wastes are either uneconomically utilized or disposed of as they are, therefore causing serious pollution problems [6-7]. Thus, there is an urgent need for reuse of fruit waste. At present, interest in the recovery, bioconversion, recycling, and utilization of valuable constituents from fruit waste have markedly increased.

Banana is second largest produced fruit of total world's fruits. According to Food and Agricultural

Organization (FAO), approximately 126 million tons of banana was produced globally[8]. Banana peels are sometimes used as feedstock for cattle, goats, pigs, monkeys, poultry, fish, zebras and several other species, typically on small farms in regions where bananas are grown [9]. There are some concerns over the impact of tannins ontained in the peels on animals that consume them [10, 11]. In addition, banana peel contains high concentration of carbohydrates (59%) and crude fibre (31.7%), which makes it a good source of carbohydrates. Most of the carbohydrates are in the form of starch which is a polysaccharide of glucose [12]. Starch can be advantageously converted into glucose by enzymatic hydrolysis. Therefore, the objective of this study is to investigate the potential of an alternative honey bee feed from renewable resources based onbanana peel waste (BPW). To the best of our knowledge, this is the first study to demonstrate the effective applications of BPW.Our integrated approach for reusing BPW is described in Figure 1.

II. DETAILS EXPERIMENTAL

2.1. Materials

Banana was obtained from a local fruit shop. Only the banana peel was used in the experiments. First, the banana peels were thoroughly washed with water to remove all adhering substances. Banana peels were cut onto small pieces using a knife, and these were shade-dried on trays. Then the dried banana peel wastes were ground into powder (20 mesh size) using a mechanical grinder. The powder was stored in closed containers until further use.

Cellulase (Celluclast 1.5 L) and Pectinase (Pectinex SP-L) werepurchased from Novozyme A/S (Bagsvaerd, Denmark).



Figure 1. The steps involved in the conversion of BPW to BPW-ABF

2.2. BPW-ABF production by enzymatic hydrolysis

To produce the banana peel waste based alternative bee fee (BPW-ABF), enzymatic hydrolysis of BPW was performed in a 100 mL of citrate buffer (0.05 M, pH 4.8) containing 1% (w/v) dry matter, cellulase (8.4 mg/g BPW), and pectinase (8.4 mg/ g BPW), and incubated at 45 °C for 24 h in a 500-mL Erlenmeyer flask. At the end, the soluble sugar contents were analyzed by high-performance liquid chromatography (HPLC). HPLC was performed using a refractive index detector (2414, Waters, Milford, CT, USA) equipped with a Rezex RPM column (4.6 × 300 mm; Phenomenex, CA, USA). HPLC-grade water was supplied at a flow rate of 0.6 mL/min as a mobile phase at a controlled temperature of 80°C.

2.3. HMF analysis

To investigate the HMF contents in the bio-sugar solution, the soluble sugars in the enzymatic hydrolysis were measured using HPLC. ARezex ROA organic acid column $(300 \times 7.8 \text{ mm};$ Phenomenex, Torrance, CA) was used for HMF identification. The temperatures of the column and detector were maintained at 65 and 40°C, respectively, and 5 mM sulfuric acid was added to the mobile phase at a flow rate of 0.6 mL per min.

2.4. Determination of total phenol content

Total phenol content was measured using Folin– Ciocalteu colorimetric method described by Singleton et al [13]. BPW-ABF(20 μ l) was mixed with 100 μ l of Folin–Ciocalteu reagent and 1.58 ml of H₂O, and placed at room temperature for 3 min. After the addition of 300 μ l of 20% sodium carbonate to the mixture, total phenol was estimated after 2 h of incubation at room temperature. The absorbance of the mixture was measured at 765 nm with a Biochrom UV–VIS spectrophotometer. It was quantified with respect to the standard curve of gallic acid expressed as gallic acid equivalents (GAE).

2.5. Determination of total flavonoid content

Total flavonoid contents were estimated by aluminum chloride colorimetric methods of Chang et al [14].BPW-ABF(0.5 ml) was mixed with 1.5 ml of methanol. Following, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water were added and mixed completely after each addition. The mixture was incubated for 30 minutes at ambient temperature. The absorbance was measured at 415 nm with methanol as blank by the Biochrom UV–VIS spectrophotometer. Quercetin was used as standard for the standard curve and total flavonoids were expressed as quercetin equivalents (QUE).



Figure 2. The effect of enzymatic hydrolysis of BPW

III. RESULTS AND DISCUSSION

3.1. Production of BPW-ABF

The aim of hydrolysis is to further degrade the polysaccharides present in the banana peel waste into monosaccharides subunits. The enzymatic hydrolysis of BPW was carried out using cellulase and pectinase, and the results were examined for glucose and fructose. After enzymatic hydrolysis, the yield of glucose (7.96 mg/ml) and fructose (4.72 mg/ml) were increased. The result indicated that enzymatic hydrolysis had significant effect in BPW.

3.2. Toxicity assessment

5-Hydroxymethylfurfural (HMF) can be produced from all hexoses and also from those oligo- and polysaccharides that can yield hexoses upon hydrolysis. This substance is toxic to honey bees. We confirmed that no HMF existed in BPW-ABF using HPLC (Figure 3).



3.3. TPC and TFC of BPW-ABF

Among the components of honey products, the total phenolic acid (caffeic acid, ferulic acid) and flavonoid contents are determined as the indication of several biological activities which are attributed to these products (i.e. antimicrobial, antioxidant activity). As shown in Table 1, total phenolic and flavonoid contents of BPW-ABF were found to be 0.93 and 164.81 mg/g of BPW-ABF, which were estimated as gallic acid and quercetin equivalents, respectively. These results revealed that BPW-ABFpossesses phenolic and flavonoid compounds.

	Total phenolic Content (TPC) mg/g	Total flavonoid content (TFC) mg/g
BPW- ABF	0.93±0.2	461.81±1.9

Table 1. Total phenolic and flavonoid contents of BPW-ABF

3.4. Honey bee mortality rate

As shown in figure 4, the honey bee mortality rate of BPW-ABF was investigated. Compared with control (sucrose), the mortality rate of BPW-ABF was increased from 0.7% to 96%. In order to reduce the mortality, mono sugars (glucose, fructose) and pollen were added in BPW-ABF. In case of fructose, the mortality rate was lower than glucose. In addition, the lowest mortality rate was showed in the BPW-ABF with fructose and pollen. Results have clearly demonstrated that combination of BPW-ABF with





CONCLUSIONS

In summary, we have demonstrated for the first time that BPW is a novel potential raw material for the production of an alternative honey bee feed. The amount of glucose produced from the BPW accounted for approximately 61.86% of the total glucose in the original material. Also,total phenolic and flavonoid contents of BPW-ABF were found to be 0.93 mg/g and 164.81 mg/g of BPW-ABF, respectively. In addition, the combination of BPW-ABF with fructose and pollen has reduced the honey bee mortality rateto almost 9 times. We predict that economically beneficial processes could be developed from the reuse of these waste materials.

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