



Viscozyme L pretreatment on palm kernels improved the aroma of palm kernel oil after kernel roasting

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ABSTRACT

With an interest to enhance the aroma of palm kernel oil (PKO), Viscozyme L, an enzyme complex containing a wide range of carbohydrases, was applied to alter the carbohydrates in palm kernels (PK) to modulate the formation of volatiles upon kernel roasting. After Viscozyme treatment, the content of simple sugars and free amino acids in PK increased by 4.4-fold and 4.5-fold, respectively. After kernel roasting and oil extraction, significantly more 2,5-dimethylfuran, 2-[(methylthio)methyl]-furan, 1-(2-furanyl)-ethanone, 1-(2-furyl)-2-propanone, 5-methyl-2-furancarboxaldehyde and 2-acetyl-5-methylfuran but less 2-furanmethanol and 2-furanmethanol acetate were found in treated PKO; the correlation between their formation and simple sugar profile was estimated by using partial least square regression (PLS1). Obvious differences in pyrroles and Strecker aldehydes were also found between the control and treated PKOs. Principal component analysis (PCA) clearly discriminated the treated PKOs from that of control PKOs on the basis of all volatile compounds. Such changes in volatiles translated into distinct sensory attributes, whereby treated PKO was more caramelic and burnt after aqueous extraction and more nutty, roasty, caramelic and smoky after solvent extraction.

1. Introduction

Palm kernel oil (PKO) is the oil extracted from palm kernels (PK), the seeds of oil palm (*Elaeis guineensis* Jackqu), either by solvent extraction or through mechanical pressing or their combination (Cornelius, 1977). Due to the specific fatty acid composition which can afford it the unique melting characteristics, PKO has a wide range of applications in food industry as a specialty fat (Trautler & Dieffenbacher, 1985).

PKO that is commercially available is generally well deodorized, with a bland flavor. As such, its application in foods is non-aroma related, where it mainly functions as an odorless fat base (Trautler & Dieffenbacher, 1985). Therefore, it would be of value to enhance the aroma of PKO, which may expand its application beyond being a fat base, especially in bakery and confectionery products.

The PK roasting process in producing PKO is critical to aroma as it is the main aroma generating step. Previous research indicated that PKO extracted from roasted PK possessed a favorable and pleasant odor (Jayalekshmy, Narayanan, & Mathew, 1987; Zhang, Wang, Yuan, Yang, & Liu, 2016). Gas chromatography-olfactometry (GC-O) further identified that substituted pyrazines, furan and pyran derivatives are the

most aroma-active compounds in roasted PKO, most of which are thought to be originated from thermal reactions such as Maillard reaction and caramelization during the kernel roasting process (Zhang et al., 2016). Therefore, the aroma of PKO is expected to be enhanced if those thermal reactions could be promoted.

Simple reducing sugars, being part of the most important precursors in both Maillard reaction and caramelization, are a critical factor that can directly affect the thermal reactions and the ultimate aroma profile (Parker, 2014). However, fresh PK contains low levels of simple sugars, where only 2.4% of its total carbohydrate are simple sugars while 81% are present as non-starch polysaccharides; of which the majority are linear mannans (78%) with low levels of substituted galactose residues, followed by cellulose (12%) and small amounts of glucuronoxylans and arabinoxylans (3% each) (Daud & Jarvis, 1992; Düsterhöft, Posthumus, & Voragen, 1992).

Our previous study found that the commercial cellulase (Celluclast 1.5 L) and xylanase (Pentopan mono BG) could modify the soluble sugar profile of PK with more glucose being released by Celluclast and possibly more pento-oligosaccharides being released by Pentopan (Zhang, Zhao, Yang, Zhao, & Liu, 2017; Zhang, Zhao, Zhao, Yang, & Liu, 2017). Correspondingly, the volatile composition of PKO derived from

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those hydrolyzed PK (after roasting) changed evidently. Based on these findings, we further investigated the impact of Viscozyme L, a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β -glucanase, hemicellulase and xylanase, with a claimed activity of 100 fungal beta-glucanase units (FBGU)/g (Rosset, Prudencio, & Beléia, 2012), on the volatile modification of PKO. As Viscozyme is expected to release both hexoses and pentoses, we could examine how the volatile of PKO would be modified with the presence of both free hexoses and pentoses, and estimate the contribution of individual simple sugars to the generation of volatile compounds.

Viscozyme was reported to be able to increase the content of glucose and galactose in soy slurry, where treated tofu yielded a total reducing sugar content approximately five times that of untreated one (Rosset et al., 2012). Besides, due to its robust ability in hydrolyzing cell wall polysaccharides, it has been applied to increase the protein extraction in defatted soy flour, defatted rice bran and oat bran (Guan & Yao, 2008; Rosset & Beléia, 2014; Tang, Hettiarachchy, Eswaranandam, & Crandall, 2003). Thus, it is hypothesized that Viscozyme is able to increase the content of simple sugars by hydrolyzing polysaccharides in PK, which has not been studied yet. The objectives of this study were to assess the release of simple sugars from polysaccharides in PK treated with Viscozyme and to evaluate their consequential impact on the generation of flavor compounds and aroma in PK and PKO after three degrees of kernel roasting, with a view to develop a new way of improving PKO aroma.

2. Materials and methods

2.1. Materials, chemicals and solvents

Fresh oil palm nuts, from a hybrid of Dura and Pisifera variety of *Elaeis guineensis* species cultivated and harvested in North Sumatera Province of Indonesia in 2016, were provided by PT Wilmar Nabati, Indonesia. After manually removing the hulls, palm kernels were obtained with a moisture content of 200 g/kg and stored at -20°C before use.

Viscozyme L (100 FBGU/g, produced by *Aspergillus* sp.) was obtained from Novozymes (Bagsvaerd, Denmark). Sugar standards (xylose, arabinose, fructose, galactose, glucose, mannose, sucrose and maltose), sodium citrate and citric acid were purchased from Sigma Aldrich (St Louis, MO, USA). Acetonitrile acquired from Tedia (Fairfield, OH, USA) was of HPLC grade. Amino acid standards were procured from Thermo Scientific (Rockford, IL, USA), AccQ-Tag eluent A concentrate and AccQ-Fluor reagent kits were obtained from Waters (Dublin, Ireland). Petroleum ether (boiling point range of $35\text{--}60^{\circ}\text{C}$) and ethanol acquired from Merck (Darmstadt, Germany) were of ACS grade.

2.2. Palm kernel powder preparation

Fresh palm kernels were ground by using a food blender (Panasonic MX-J 210GN, Osaka, Japan) and sifted through a No.12 USA standard testing sieve (Fisher Scientific, Leicestershire, UK) to obtain a fine powder with a uniform particle size ($\leq 1.68\text{ mm}$).

2.3. Enzyme treatment of palm kernel powder

Viscozyme treatment on PK powder to release simple sugars was carried out in a water bath (Julabo SW22, Seelbach, German) at a constant shaking speed of 195 rpm, followed by inactivation of enzyme at 95°C for 10 min. The enzyme treatment procedure was optimized by single factor experiments on the following four factors: enzyme dosage (0, 2, 4, 6, 8, 10 FBGU/g PK), enzyme treatment temperature (30, 40, 50, 60°C), enzyme treatment duration (0, 0.5, 1.5, 3, 5.5, 9 h) and PK powder: citrate buffer ratio (1:2, 1:4, 1:6, 1:8, 1:10, w/v) and an optimized hydrolysis was obtained when treating PK with an enzyme dosage of 6 FBGU/g PK, PK powder: buffer ratio of 1:2 at 50°C for 5.5 h.

After enzyme treatment, PK powder was freeze-dried (Vir-Tis AdVantage, Genevac, SP Scientific, Ipswich, UK) and stored at -20°C until use. The same treatment process was applied to the control sample without adding the enzyme.

2.4. Roasting of palm kernel powder

The freeze-dried PK powder was firstly equilibrated at 50°C for 30 min in a drying oven (Memmert UM200, Apeldoorn, Netherlands) and then distributed on an aluminum tray in a layer of 5–6 mm thickness. The powder was roasted in a baking oven (Euroflours MS01T04-2, Gommegnies, France), preheated to 180°C , for 8 min, 14 min and 20 min to obtain light, medium and dark roasted PK powder, respectively.

2.5. Determination of moisture and lightness (L^* value) of palm kernel powder

The moisture content of PK powder was determined by oven-drying (Memmert UM200, Apeldoorn, Nederland) at 104°C to a constant weight. The L^* value of PK was determined by using a spectrophotometer (Konica Minolta CM-3500d, Osaka, Japan).

2.6. Oil extraction from palm kernel powder

2.6.1. Aqueous extraction

PK powder was mixed with distilled water at a ratio of 1:8 w/v for extraction. The extraction was carried out in a water bath with agitation (Julabo SW22, Seelbach, Germany) at a shaking speed of 170 rpm, 80°C for 1 h. The mixture was then centrifuged at $4629 \times g$ (Eppendorf 5804R, Hamburg, Germany) for 10 min at 30°C and the oil phase (the top layer of the mixture) was collected as the crude PKO.

2.6.2. Solvent extraction

Petroleum ether (boiling point range of $35\text{--}60^{\circ}\text{C}$) was used to extract PKO from PK by using an auto fat extraction system (FOSS SoxtecTM2050, Hillerød, Denmark) at a PK powder: solvent ratio of 1:14 (w/v). After extraction, the oil phase was left in the fume hood overnight to allow evaporation of the residual petroleum ether. The resultant oil was collected as the crude PKO.

2.7. Analysis of non-volatile compounds in palm kernels

2.7.1. Soluble sugar extraction and analysis

The PK powder with various roasting degrees was first defatted with petroleum ether using an auto fat extraction system (FOSS SoxtecTM 2050, Hillerød, Denmark). The defatted PK powder (2.5 g) that was mixed with 40 mL 80% (v/v) aqueous ethanol was shaken on a vortex shaker (Heidolph Rotamax 120, Schwabach, Germany) and sonicated for 30 min each. The extraction was repeated twice. The extracts were combined, filtered and concentrated to 5 mL using a rotary evaporator (EYELA N-1200, Tokyo, Japan) and stored at -20°C before analysis.

The concentration of sugars was determined using Shimadzu ultrafast liquid chromatography system (UFLC, Kyoto, Japan) coupled to a low temperature evaporative light scattering detector (ELSD) (Shimadzu, Kyoto, Japan). The column used was a Zorbax carbohydrate column ($150 \times 4.6\text{ mm}$; Agilent, Santa Clara, CA, USA). The column was eluted at 40°C with a mixture of acetonitrile and water (80:20, v/v) at a constant flow rate of 1 mL/min. Identification and quantification were carried out by using retention times and calibration curves of sugar standards with R^2 -values ≥ 0.99 .

2.7.2. Free amino acid extraction and analysis

Free amino acids in the defatted PK powder (1.0 g) were extracted with 1% trichloroacetic acid for 30 min using a vortex shaker (Heidolph Rotamax 120, Schwabach, Germany). After extraction, the mixture was

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