PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

DESENVOLVIMENTO DE MISTURAS DE GÁS DE CALIBRAÇÃO (DIÓXIDO DE CARBONO E OXIGÊNIO EM MATRIZ DE NITROGÊNIO) EM UMA FAIXA DE CONCENTRAÇÃO TÍPICA DE EMBALAGEM DE ATMOSFERA MODIFICADA

DEVELOPMENT OF CALIBRATION GAS MIXTURES (CARBON DIOXIDE AND OXYGEN IN NITROGEN MATRIX) AT A TYPICAL CONCENTRATION RANGE OF MODIFIED ATMOSPHERE PACKAGING

PENGEMBANGAN CAMPURAN GAS KALIBRASI (KARBON DIOKSIDA DAN OKSIGEN DALAM MATRIKS NITROGEN) PADA KISARAN KONSENTRASI TIPIKAL UNTUK PENGEMASAN ATMOSFER TERMODIFIKASI

HINDAYANI, Ayu[1]; MULYANA, Muhammad Rizky[2]; BUDIMAN, Harry[3]; DARMAYANTI, Nur Tjahyo Eka[4]; ZUAS, Oman[5]*

[1,2,3,4,5] Center for Research and Human Resources Development (PUSRISBANG-SDM), National Standardization Agency of Indonesia (BSN), Kawasan PUSPIPTEK Building 420, Serpong 15314, Tangerang Selatan - Banten, Indonesia

** Corresponding author*
e-mail: oman@bsn.go.id

Received 30 July 2020; received in revised form 27 August 2020; accepted 22 October 2020

RESUMO

A medição da concentração de dióxido de carbono (CO_2), oxigênio (O_2) e nitrogênio (N_2) em alimentos embalados em atmosfera modificada (MAP) é crítica para ser realizada pela indústria de alimentos. Uma ligeira variação nas concentrações de CO_2, O_2 e N_2 nas embalagens de alimentos pode ter um impacto significativo na qualidade do produto e na segurança para a saúde humana. A medição precisa e confiável das concentrações de CO_2, O_2 e N_2 em embalagens de alimentos é crucial e só pode ser obtida calibrando o analisador de gás. Este estudo teve como objetivo desenvolver misturas de gases para calibração de analisadores de gás CO_2, O_2 e N_2 em uma faixa de concentração típica de embalagens com atmosfera modificada. As misturas de gás de calibração foram preparadas gravimetricamente seguindo ISO 6142. A faixa de concentração de CO_2, O_2 e N_2 para misturas de gás de calibração foi definida em 9-19% mol/mol, 1-5% mol/mol e 74-88% mol/mol, respectivamente. Cada gás original foi identificado por suas impurezas usando cromatografia gasosa com um detector de ionização de hélio de descarga pulsada (GC-PDHID). As composições de CO_2, O_2 e N_2 nas misturas foram verificadas por meio da avaliação da consistência interna das misturas gasosas preparadas por meio de cromatografia gasosa com detector de condutividade térmica (GC-TCD). A estabilidade a curto prazo das misturas de gases preparadas foi avaliada usando um método de divisão igual. O resultado mostrou que foi obtida boa consistência interna entre os valores gravimétricos e de verificação do GC, havendo regressão do coeficiente linear (R2) ≥ 0,999. O resultado do teste t mostrou que o CO_2 tem melhor estabilidade de curto prazo do que O_2 e N_2. Em conclusão, as misturas de gases de calibração desenvolvidas em uma faixa de concentração típica de embalagens de atmosfera modificada têm mostrado resultados satisfatórios para o componente CO_2. No entanto, avaliações adicionais ainda são necessárias para minimizar a instabilidade dos componentes O_2 e N_2.

Palavras-Chave: *calibração de misturas de gás padrão, embalagem de atmosfera modificada, analisador de gás*

ABSTRACT

Measurement of carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2) concentration in modified atmosphere packaging (MAP) food is critical to be carried out by the food industry. A slight variation in concentrations of CO_2, O_2, and N_2 in food packaging may have a significant impact on product quality and safety for human health. Accurate and reliable measurement of CO_2, O_2, and N_2 concentrations in food packaging is crucial, and it can only be achieved by calibrating the gas analyzer. This study aimed to develop gas mixtures for the calibration of CO_2, O_2, and N_2 gas analyzers at a typical concentration range of modified atmosphere packaging. The calibration gas mixtures were prepared gravimetrically by following ISO 6142. The concentration ranges of CO_2, O_2, and N_2 for calibration gas mixtures were set at 9-19% mol/mol, 1-5% mol/mol, and 74-88%

DOI: 10.52571/PTQ.v17.n36.2020.689_Periodico36_pgs_674_687.pdf

mol/mol, respectively. Each parent gas was identified for its impurities using gas chromatography with a pulsed discharge helium ionization detector (GC-PDHID). The compositions of CO_2, O_2, and N_2 in the mixtures were verified by evaluating the internal consistency within the prepared gas mixtures using gas chromatography with a thermal conductivity detector (GC-TCD). The short term stability of the prepared gas mixtures was evaluated using an equal division method. The result showed that good internal consistency was achieved between the gravimetrical and GC's verification values, having linear regression coefficient (R^2) ≥ 0.999. The t-test result has shown that CO_2 has better short term stability than O_2 and N_2. In conclusion, the developed calibration gas mixtures at a typical concentration range of modified atmosphere packaging have shown satisfying results for CO_2 component. However, further evaluation is still required to minimize the instability of O_2 and N_2 components.

Keywords: *calibration standard gas mixtures, modified atmosphere packaging, gas analyzer*

ABSTRAK

Pengukuran konsentrasi karbon dioksida (CO_2), oksigen (O_2), dan nitrogen (N_2) untuk pengemasan pangan atmosfer termodifikasi (MAP) sangat penting dilakukan oleh industri pangan. Sedikit perubahan konsentrasi CO_2, O_2, dan N_2 dalam pengemasan makanan dapat berdampak signifikan terhadap kualitas dan keamanan produk bagi kesehatan manusia. Pengukuran konsentrasi CO_2, O_2 dan N_2 yang akurat dan handal dalam pengemasan makanan menjadi sangat penting dan hanya dapat dicapai dengan mengkalibrasi peralatan analisa gas tersebut. Studi ini bertujuan untuk mengembangkan campuran gas untuk kalibrasi alat analisa gas CO_2, O_2 dan N_2 pada kisaran konsentrasi tipikal dari pengemasan atmosfer termodifikasi. Campuran gas kalibrasi dibuat secara gravimetri sesuai dengan ISO 6142. Kisaran konsentrasi CO_2, O_2, dan N_2 untuk campuran gas kalibrasi ditentukan masing-masing pada 9-19% mol/mol, 1-5% mol/mol, dan 74-88% mol/mol. Tiap gas induk diidentifikasi pengotornya menggunakan kromatografi gas dengan detektor *pulsed discharge helium ionization* (GC-PDHID). Komposisi CO_2, O_2, dan N_2 dalam campuran gas diverifikasi dengan mengevaluasi konsistensi internal menggunakan kromatografi gas dengan detektor keterhantaran panas (GC-TCD). Kestabilan campuran gas jangka pendek dievaluasi dengan menggunakan metode pembagian setara. Hasil studi menunjukkan bahwa terdapat konsistensi internal yang baik antara nilai gravimetri dan nilai verifikasi GC, dengan koefisien regresi linier (R^2) ≥ 0,999. Hasil uji-t menunjukkan bahwa CO_2 memiliki stabilitas jangka pendek yang lebih baik dari pada O_2 dan N_2. Sebagai kesimpulan, campuran gas kalibrasi yang dikembangkan pada kisaran konsentrasi tipikal dari pengemasan atmosfir termodifikasi telah menunjukkan hasil yang memuaskan untuk komponen CO_2, namun diperlukan penanganan lebih lanjut untuk meminimalkan ketidakstabilan komponen O_2 dan N_2.

Kata Kunci: *campuran gas standar kalibrasi, pengemasan atmosfer termodifikasi, penganalisa gas*

1. INTRODUCTION:

Food is an essential element in the human body besides air and water (De and De, 2019; Aversa *et al.*, 2016). In general, food consists of essential nutrients, such as carbohydrates (Marinangeli *et al.*, 2020), proteins (Beals *et al.*, 2017), fats (Utyanov *et al.*, 2018), minerals, and vitamins (Kodentsova and Vrzhesinskaya, 2018) for human on providing energy, developing and maintaining life and stimulating growth (Millward, 2017; Dorgan *et al.*, 2019). Most of these nutritious foods are originated from natural products, such as vegetables, fruit, fish, and meat. However, the self-life of natural products is limited because of spoilage. Food spoilage is defined as a change in food quality, such as objectionable odor, texture, and appearance. Therefore, this food is unfit for consumption (Odeyemi *et al*., 2020; Amit *et al.,* 2017). Storage temperature, pH, moisture loss, the action of enzymes, microorganism (molds, yeasts, and bacteria) contamination, processing operation, transportation, and food handlers influence the rate of spoilage (Franceschini *et al.*, 2020; Wagner *et al.*, 2020; Shwaiki *et al.*, 2019; Tsang *et al.*, 2018). Food spoilage can affect economic loss, such as in Australia. The cost of food losses related to spoilage was estimated at $10,000,000 annually (Pitt and Hocking, 2009). Other than that, spoiled food contributes to food waste, in which approximately 1.3 billion tons of food is wasted every year (Odeyemi *et al*., 2020; Rawat, 2015; Ishangulyyev *et al.,* 2019). Consequently, food preservation is needed to resolve the spoilage.

There are some possible ways to slow down the spoilage processes and to keep food edible as long as possible. It may include drying, refrigeration, fermentation, chilling, irradiation, pasteurization, canning, and the addition of synthetic chemicals (Vaclavik and Christian, 2008). However, this method has a limitation, such as significant loss of flavor, aroma, and nutrients, a decrease of some vitamin and mineral availability, crispiness reduction of selected food items, the deformation of the original color, and the formation of undesirable taste and appearance

(Cachon and Alwazeer, 2019; Kharobe, 2018; Amit *et al.,* 2017). Certain synthetic chemicals are used as a food preservative because they are the most effective longer shelf life (Linke *et al.,* 2018; Sharma, 2015). However, several synthetic chemicals have negative and potentially life-threatening side effects. Nitrates can cause loss of consciousness and death, especially in an infant; sulfite can cause severe allergic reactions and asthma; benzoates can cause skin rashes, and sorbates can contact dermatitis (Sharma, 2015; Anand and Sati, 2013; Mirza *et al.*, 2017; Dwivedi *et al.*, 2017).

A good packaging system for storage, transportation, and end-use of food is another preferable way to keep the quality of natural products. Practically, the good packaging system could prevent deterioration of the food quality because the environment may influence and contribute to the efficiency of distribution, sales, and consumption (Regattieri and Santarelli, 2013; Han *et al.*, 2018). Nowadays, the application of food packaging systems has been proven as an effective method for reducing food waste and spoilage (Opara and Mditshwa, 2013; Verghese, *et al*., 2015). The food packaging systems have several advantages, including reducing the costs of preservation, extend the shelf life of foods, provide safe and convenient foods to consumers, maintain the quality of the food product, and contribute to sales and marketing efforts, as well as the address to environmental issues (Han *et al.*, 2018; Han, 2014; Montero-Calderón, *et al.*, 2010). Several factors of the package itself should be carefully tested because of the possible migration of harmful content into food products reported by previous studies (Gavriil, *et al*., 2018; Lin, Q-B., et al., 2017).

One of the most interesting food packaging systems is the modified atmosphere packaging (MAP). It is an effective preservation method for maintaining quality and extending the shelf-life of various foods (Hyun and Lee, 2017). The MAP can maintain the visual, textural, and nutritional appeal of natural products (Sandhya, 2010). It can provide an extended shelf life without the addition of chemical preservatives or stabilizers by slowing chemical and biochemical deteriorative reactions and inhibiting spoilage organisms (Abdulmumeen, *et al*., 2012; Sandhya, 2010; Mullan and McDowell, 2003).

The MAP is defined as the packaging of a perishable product in an atmosphere by which its composition is other than air (Mullan and McDowell, 2003). Normal air has a composition of 78 % nitrogen (N_2), 21 % oxygen (O_2) and 1 % argon (Ar). In a MAP, the food packaging is flushed with a gas or a mixture of gases. The most common gases used are N_2, O_2, and CO_2. The choice of gas or mixture of gases is dependent on the food product being packed (Sandhya, 2010). An N_2 is an inert gas with a primary function as a filler gas to avoid the collapse when CO2 dissolves in the food product and minimize the respiration rate in fruit and vegetables. An O_2 with a concentration below normal air (<21%) can inhibit the growth of aerobic microorganisms (Meredith *et al*., 2014). A CO_2 with a high concentration (20% or greater) effectively inhibits spoilage bacteria and molds (Kotsianis *et al.,* 2002; Devlieghere *et al.,* 2003; Embleni, 2013).

Even though the MAP is the best available technology for food packaging, a failure such as leaks of packaging is inevitable, resulting in a severe effect from the loss of nutrients, taste, color, or structure to a foul smell and also risk to the consumer health (Wen *et al.*, 2018; Smolander *et al.,* 1997). Therefore, comprehensive quality assurance activities are essential to be carried out during and after the packaging process. It is required to monitor the correct composition gas mixture of the modified atmosphere and the leaks of packaging because variations of N_2, O_2, and CO_2 composition can significantly impact food product quality (Pu *et al.*, 2020; Javanmard, 2017).

In this regard, gas analyzers are used for quality control of MAP process to measure the modified atmosphere gas levels inside product packages (Ali *et al.*, 2019; Ozturk *et al.*, 2019; Scarabottolo, 2020). They can detect the leaks of the package to keep the quality of the product. The accurate and reliable result of measured gas levels can be achieved by calibrating the gas analyzer regularly using a calibration standard gas mixture (Budiman and Zuas, 2015). Besides, the calibration of the gas analyzer is required by ISO 17025 to establish the traceability of the measurement results (Jacksier and Weterings, 2017; Brown *et al.*, 2017; ISO, 2017b)

The availability of calibration gas mixtures in Indonesia, especially for the calibration of the gas analyzer in food packaging, is imported from overseas. This process is relatively costly and time-consuming, leading to an increase in production cost. Related to this issue, the National Institute of Standards and Technology (NIST) has studied the economic impact of their Gas Mixture NIST-Traceable Reference Materials Program on the local industry in the U.S. They have reported several financial benefits that could be gained from their calibration gas standards (Gallaher *et al*., 2002).

Therefore, this study aimed to provide an insight into the development of calibration gas mixtures which are required as quality control in modified atmosphere packaging. In this article, relevant contributing factors involved in the preparation of calibration gas mixtures (CO_2 and O_2 in N_2 matrix) using the gravimetric method following ISO 6142 were discussed. It is expected that this study can be of help to potential stakeholders who need to develop their gas reference material for MAP of food products.

2. MATERIALS AND METHODS:

A preparation of calibration gas mixtures (CO_2 and O_2 in N_2 matrix) using the gravimetric method following ISO 6142 has been conducted. In the gravimetric method, the parent gases (target gases: CO_2 and O_2) were diluted by the high purity gases (matrix gas: N_2) in high pressurized cylinders. The composition of calibration gas mixtures was calculated from the weighing amount of parent and matrix gases, the amount fraction, and the molar mass of each component gases according to equation 1.

$$x_i = \frac{\sum_{A=1}^{P}\left(\frac{x_{i,A}.m_A}{\sum_{i=1}^{n} x_{i,A}.M_i}\right)}{\sum_{A=1}^{P}\left(\frac{m_A}{\sum_{i=1}^{n} x_{i,A}.M_i}\right)} \quad \text{(Eq.1)}$$

where x_i is the mole fraction of component *i* in the final mixtures. *P* is the total number of parent gases. *n* is the total number of the component in the final mixture. m_A is the mass of parent gas *A* determined by weighing. Mi is the molar mass of component *i.* $x_{i,A}$ is the mole fraction of component *i*, in parent gas *A* (ISO, 2001).

The brief preparation of calibration gas mixtures was taken from our previous work (Budiman *et al.,* 2017) and described as follows. Firstly, 4 L of the empty aluminum cylinder was cleaned by evacuating until 10^{-7} mbar and heating at 60 °C for 24 hours. After that, a specified amount of the parent gases (CO_2, O_2) were filled into the cleaned cylinders and diluted with high purity N_2 as matrix gas. Subsequently, the exact amount of parent gases and matrix gas were precisely weighed by a mass comparator located at a room with controlled temperature (22 ± 1 °C) and humidity (50 ± 5%). The weighing process was performed by weighing the mass of the sample and reference cylinder. The amounts of parent gases and matrix gas-filled were calculated by the difference between the sample and reference cylinder mass. This calibration gas mixture is used for assuring the traceability of the measurement results. During the preparation process, the CO_2, O_2, and N_2 as parent gases were assessed for their impurities. For short term stability of the calibration, gas mixtures was evaluated using an equal division method.

2.1. Materials

Ultra-high purity grade of gases, such as CO_2 (Grade Alphagaz 2, claimed by the manufacturer as 99.9995% mol/mol of purity) and O_2 (Grade Alphagaz 2, claimed by the manufacturer as 99.9995% mol/mol of purity) from Air Liquide, Indonesia, and N_2 gas (Grade UHP, claimed by the manufacturer as 99.9995% mol/mol of purity) from Surya Indotim Imex-Indonesia, was used as the parent gases for each target of calibration standard gas mixtures concentration. The impurities of each gas were analyzed using GC-PDHID (Agilent Technologies, USA). The NET company, China, supplied the aluminum gas cylinders (capacity 4 Liter, 12 cm in diameter) equipped with stainless steel valve. All gas cylinders were checked for possible leakage by filling the gas cylinder with N_2 gas until its pressure reached 100 bar. The leakage test was conducted for 1 month. The gas cylinder is free from leakage if there is no difference in pressure between the initial and final tests. Subsequently, the gas cylinder was evacuated using a vacuum system until a pressure of 10^{-7} mbar while heated for 24 hours at 60 °C by using a heating mantle.

2.2. Equipment

A GC (7890B, Agilent Technologies, USA) equipped with Thermal Conductivity Detector (TCD) was used for the verification composition of the calibration gas mixtures. Analysis of the gas sample was conducted under optimum operating conditions (Table 1). Mass comparator XP10003S (Mettler Toledo, Switzerland) was used for the gravimetric preparation of the calibration gas mixture.

2.3. Procedure

2.3.1 Determination of Impurities in Parent Gas

The impurities in the parent gases have a significant effect on the composition of the calibration gas mixtures, because the impurities may react with other component gases in the gas mixtures (Mulyana *et al.,* 2019; ISO, 2001). Gas Chromatography can determine the impurities, such as CO_2, Argon (Ar), O_2, N_2, methane (CH_4)

and carbon monoxide (CO) at trace level - Pulsed Discharged Helium Ionization Detector (GC-PDHID) under the optimum operating condition as described in our previous work (Hindayani *et al.,* 2019, Mulyana *et al.,* 2019).

The optimum operating conditions of GC-PDHID were described as follows: the parent gas samples with flow 40 mL/min were maintained by a thermal mass flow controller and introduced to 1 mL of the GC-system loop. The gas samples were injected with a split ratio 1:1 to the column of GC-system. Two capillary columns were used to separate the gas component such as Pora PLOT Q column (50 m length, 530 µm OD, 20 µm film thickness) and Molsieve 5A column (50 m length, 530 µm OD, 20 µm film thickness). The getter instrument initially purified the helium carrier gas before it was flown to the GC-system. The programmed flow rate of helium carrier gas was performed with the following condition: initial 10 ml/min (hold for 11.5 min), ramp down 60 ml/min by each minute to 5 ml/min (hold for 3.4 min), ramped up 60 ml/min by each minute to 10 ml/min (hold for 0 min). The oven column was set to programmed temperature as follows: Initial 40 °C (hold for 6.5 min), first ramp down 100 °C/min to 30 °C (hold for 8.4 min), second ramp up 6 °C/min to 75 °C (hold for 0 min), third ramp up 12 °C/min to 160 °C (hold for 0 min). The gas sampling box and detector temperature were set to 100 ºC and 250 ºC, respectively.

2.3.2 Preparation of Calibration Standard Gas Mixtures of CO_2 and O_2 in N_2 Matrix

Firstly, a pre-mixture gas containing 10.042% mol/mol O_2 in N_2 balance (89.958% mol/mol) was gravimetrically prepared. After that, the calibration gas mixtures for MAP measurement were prepared by mixing the pre-mixture gas and pure CO_2 gas, followed by diluting the mixture with pure N_2 gas as a gas matrix. Three different calibration gas mixtures were prepared, and each calibration gas mixtures were having a composition, as shown in Table 2. The gravimetric concentration of the prepared gas mixtures was calculated according to ISO 6142. The transferring process of the gases was carried out using the gas filling station, as described in the previous report (Budiman *et al.,* 2017). Then, the filled gases in the cylinder were homogenized by rotating the cylinder using a cylinder homogenization system for 12 hours.

Table 2.*Three cylinders of the calibration gas mixtures and their composition*

Code of Cylinder	Gravimetric concentration (%mol/mol)		
	CO_2	O_2	N_2
ADK 004	9.949	1.089	88.962
ADK 009	16.852	3.115	80.033
ADK 005	19.885	5.206	74.908

2.3.3 Verification of Internal Consistency between Calibration Standard Gas Mixtures

The prepared calibration gas mixtures were verified for their composition using GC-TCD. The linear regression curves were generated for each component gases (CO_2, O_2, and N_2) in calibration gas mixtures to investigate the consistency among the prepared calibration gas mixtures. The detail of the analysis procedure using GC-TCD is briefly explained in the following experimental condition (Budiman and Zuas, 2015).

The 30 mL/min of a constant flow of calibration gas mixtures samples were introduced to the 500 µL loop of the GC-system using thermal mass flow controller. In the GC-system, the injector and detector temperature were set to 100 ºC and 250 ºC, respectively. The target gas components (CO_2, O_2, and N_2) were separated from their mixtures using two packed columns stainless steel, such as Porapak Q column (1/8 inch OD, 6 feet, 80-100 mesh) and Molsieve 5A (1/8 inch OD, 9 feet, 80-100 mesh) that were connected in series. The elution of gas components from the column was performed using the helium carrier gas 28 mL/min and isothermal condition (40 ºC). This procedure aims to determine the consistency of the composition between calibration standard gas mixtures in each cylinder.

2.3.4 Short Term Stability of Calibration Standard Gas Mixtures

The short term stability of the prepared calibration gas mixtures was investigated and evaluated by the equal division method. The amount of gas from the ADK009 cylinder was equally divided into the AH06004 cylinder, and the concentration of each gas component was checked using GC-TCD. After that, a significant difference between the concentration of calibration gas mixtures in AH06004 and ADK009 were statistically tested using *t-test* (two-sample

assuming equal variances, two-tailed) method. If t_{stat} is less than $t_{critical}$, the concentration of each component in the two cylinders is not significantly different and can be categorized as a stable gas mixture (Minitab, 2019).

3. RESULTS AND DISCUSSION:

A calibration gas mixture can be defined as a mixture containing multiple gases with well-established and certified composition for measurement calibration, including gas measurement (ISO, 2017a). Establishing the response of a gas analyzer to a known concentration of a gas component through a calibration process is necessary (Shaw, 2020). In this regards, a certified standard gas mixture having traceability to the International Standard unit (SI) with precisely defined composition is required. In this work, a set of calibration gas mixtures was prepared by a gravimetric method following ISO 6142. The ISO 6142 specifies a gravimetric method to prepare calibration gas mixtures in cylinders with traceable values for the amount fraction of one or more components (ISO, 2015). The gravimetric method is one of the most precise and accurate methods for preparing a traceable calibration gas mixture through the mass unit (Shimosaka *et al*., 2011).

3.1. Purity analysis of parent gas

The accuracy of the calibration gas mixtures composition depends significantly on the purity of parent gases (CO_2, O_2 and N_2) used. The purity analysis of parent gases can be obtained by identifying and quantifying of the impurities in parent gases.

Table 3. *The purity analysis data of CO_2 parent gas*

Components	Concentration (µmol/mol)
Ar	0.49
O_2	0.85
N_2	2.25
CH_4	0.31
CO	0.85
CO_2	999994.25

The purity analysis data of the parent gases of CO_2, O_2, and N_2 were obtained by using GC-PDHID. For purity analysis of N_2 has been conducted in the previous study (Mulyana *et al.,* 2019). The results are shown in Tables 3, 4, and 5, respectively.

Table 4. *The purity analysis data of O_2 parent gas*

Components	Concentration (µmol/mol)
CO_2	0.75
Ar	n.d.*
N_2	9.10
CH_4	n.d.*
CO	2.39
O_2	999987.77

note: n.d.* = not detected

Table 5. *The purity analysis data of N_2 parent gas*

Components	Concentration (µmol/mol)
CO_2	0.02
Ar	0.99
O_2	5.82
CH_4	3.16
CO	1.29
N_2	999987.0

Table 6. *The comparison of the concentration of calibration standard gas mixtures from GC measurement and gravimetric calculation*

Code of Cylinder	Gas	Concentration (%mol/mol)	
		GC-TCD	Gravimetric
ADK 004	CO_2	9.960	9.949
	O_2	0.523	1.089
	N_2	89.515	88.962
ADK 009	CO_2	17.012	16.852
	O_2	1.505	3.115
	N_2	81.491	80.033
ADK 005	CO_2	20.086	19.885
	O_2	2.525	5.206
	N_2	77.393	74.908

All impurities data above were used in calculating the composition of the prepared calibration gas mixtures. The concentrations of the

composition of prepared calibration standard gas mixtures are listed in Table 6. These values were obtained by calculating the mass of each transferred gas in the cylinders based on ISO 6142. The concentration from gravimetric, however, can be occasionally incorrect because of accidents in the preparation. The errors could be contamination from air, faulty of weighing, adsorption or desorption of gases like O_2 and N_2 from air to the inner wall of the cylinder, or other unknown factors (Shimosaka *et al.*, 2011). Therefore, this concentration needs to be verified further.

3.2. Verification of calibration standard gas mixtures

The composition of a calibration gas mixture must be verified experimentally by using GC. This verification is used to check the consistency of mixture composition between GC measured value and calculated value from the gravimetric preparation process (ISO, 2015). The peak areas of each component (O_2, CO_2, and N_2) from the GC were plotted as a function of the mole fraction of the calibration gas mixtures.

The results show that linear regression coefficients (R^2) were found to be ≥ 0.999 were generated for each component in the gas mixtures, as depicted in Figures 1, 2, and 3. These findings indicate a good consistency in the concentration of the prepared calibration standard gas mixtures for all mixture cylinders.

3.3. Short term stability of calibration standard gas mixtures

The short term stability was carried out by transferring the calibration gas mixture in ADK009 into another empty cylinder (AH06004) until the equilibrium of gas pressure in both cylinders was achieved. The concentrations of gases in the mixture from both cylinders were determined using GC-TCD. The significant difference in concentrations between the two cylinders was statistically evaluated using *t*-test: two-sample assuming equal variances two-tailed. If t_{stat} is less than $t_{critical}$, the concentration of two cylinders is not significantly different, and it can be categorized as stable gas mixtures (Minitab, 2019). The *t*-test results for CO_2 concentration in ADK009 and AH06004 are listed in Table 7.

From Table 7, it can be seen that t_{stat} (1.33) is less than $t_{critical}$ (2.18), indicating that the CO_2 in two cylinders is not significantly different. Thus, the CO_2 component in the calibration gas mixture is categorized as stable.

Besides, the *t*-test of O_2 and N_2 concentration are listed in Tables 8 and 9, respectively. It can be evaluated that t_{stat} of O_2 and N_2 values are higher than $t_{critical}$ indicating that the concentrations of O_2 and N_2 in ADK009 are different significantly compared to that of AH06004. Therefore, it can be concluded that O_2 and N_2 components are categorized as unstable components in the gas mixtures.

4. CONCLUSIONS:

1. The calibration gas mixtures prepared using the raw materials have shown satisfying results in general, judging from the good internal consistency and a high linear regression coefficient.
2. The purity of the parent gases used as raw materials is sufficient to prepare calibration gas mixtures in the typical concentration range of the modified atmosphere food package.
3. The good consistency also indicated that the concentration values from the gravimetric method were in good agreement with the concentration value verified by GC-TCD.
4. The stability of the gas mixture, however, shows a rather unsatisfying result for O_2 and N_2 components. Both are the major components in our ambient air with some probability to enter the systems during analysis or the equal division process. This might be considered as a possible cause of instability in the mixture composition.

Further treatment shall be taken into consideration to minimize the instability of the O_2 and N_2 components of the mixtures. Therefore, in further study, it is recommended to minimize ambient air effect by using very tight tubing system and gas cylinders that ensure no components could enter or escape the system.

5. ACKNOWLEDGMENTS:

The authors would like to express their acknowledgment to the Ministry of Research and Technology-BRIN for their support through the project INSINAS and Research Centre for Metrology - Indonesian Institute of Sciences (RCM - LIPI). Thanks to the Centre for Research and Human Resources and Development- National Standardization Agency of Indonesia (PUSRISBANG-BSN) for partial support of this study through a DIPA-BSN 2020 Project.

6. REFERENCES:

1. Abdulmumeen, H. A., Risikat, A. N., Sururah, A. R. (2012). Food: Its preservatives, additives and applications. *International Journal of Chemical and Biochemical Sciences*. *1, 36-47.* https://doi.org/10.13140/2.1.1623.5208
2. Ali, S., Khan, A. S., Malik, A. U., Anjum, M. A., Nawaz, A., and Shah, H. M. S. (2019). Modified atmosphere packaging delays enzymatic browning and maintains the quality of harvested litchi fruit during low-temperature storage. *Scientia Horticulturae*, *254*, 14-20. https://doi.org/10.1016/j.scienta.2019.04.065.
3. Amit, S.K.; Uddin, M.M.; Rahma, R.; Islam, S.M.R.; Khan, M.S. (2017). A Review on Mechanism and Commercial Aspect of Food Preservation and Processing. *Agric and Food Security*. 6, 1-22. https://doi.org/10.1186/s40066-017-0130-8.
4. Anand, S. P., and Sati, N. (2013). Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. *International journal of pharmaceutical sciences and research*, *4*(7), 2496. https://doi.org/10.13040/IJPSR.0975-8232.4(7).2496-01.
5. Aversa, R., Petrescu, R. V., Apicella, A., and Petrescu, F. I. (2016). The basic elements of life's. *American Journal of Engineering and Applied Sciences*, *9*(4), 1189-1197. https://doi.org/10.3844/ajeassp.2016.1189.1197.
6. Beals, J. W., Mackenzie, R. W., Van Vliet, S., Skinner, S. K., Pagni, B. A., Niemiro, G. M., ... and De Lisio, M. (2017). Protein-rich food ingestion stimulates mitochondrial protein synthesis in sedentary young adults of different BMIs. *The Journal of Clinical Endocrinology and Metabolism*, *102*(9), 3415-3424. https://doi.org/10.1210/jc.2017-00360.
7. Blackburn, C. D. W. (2006). Managing microbial food spoilage: an overview. In *Food spoilage microorganisms* (pp. 147-170). Woodhead Publishing.
8. Brown, R. J., Brewer, P. J., Harris, P. M., Davidson, S., van der Veen, A. M., and Ent, H. (2017). On the traceability of gaseous reference materials. *Metrologia*, *54*(3), L11. https://doi.org/10.1088/1681-7575/aa6ede.
9. Budiman, H., Mulyana, M. R., and Zuas, O. (2017, January). Gravimetric dilution of calibration gas mixtures (CO2, CO, and CH4 in He balance): toward their uncertainty estimation. In *AIP Conference Proceedings* (Vol. 1803, No. 1, p. 020056). AIP Publishing LLC. https://doi.org/10.1063/1.4973183.
10. Budiman, H., and Zuas, O. (2015). Validation of analytical method for determination of high level carbon dioxide (CO2) in nitrogen gas (N2) matrix using gas chromatography thermal conductivity detector. *Periódico Tchê Química*, *12*, 7-16.
11. Cachon, R.; Alwazeer, D. (2019). Quality Performance Assessment of Gas Injection During Juice Processing and Conventional Preservation Technologies. *The Science of Beverages.* 14, 465-485. https://doi.org/10.1016/B978-0-12-816687-1.00014-X.
12. Conte, A., Angiolillo, L., Mastromatteo, M., and Del Nobile, M. A. (2013). *Technological options of packaging to control food quality* (pp. 354-379). InTech.
13. De, L. C., and De, T. (2019). Healthy Food For Healthy Life. *Journal of Global Biosciences*, *8*(9), 6453-6468..
14. Devlieghere, F., Debevere, J., and Gil, M. I. (2003). MAP, product safety and nutritional quality. *Novel food packaging techniques*, 208e230.
15. Dorgan, V., Prodan, D., and Nastas, N. (2019). Food principles providing muscular activity energy in the sports training of students practicing powerlifting. *Physical Education, Sport and Kinetotherapy Journal*, 63-69. https://doi.org/10.35189/iphm.icpesk.2019.10.
16. Embleni, A. (2013). Modified atmosphere packaging and other active packaging systems for food, beverages and other fast-moving consumer goods. In *Trends in Packaging of Food, Beverages and Other Fast-Moving Consumer Goods (FMCG)* (pp. 22-34). Woodhead

Publishing.https://doi.org/10.1533/9780857098979.22.

17. Fiori, R., Pizzolato, T. M., Noreña, C. P., and Brandelli, A. (2009). Investigation of Pentachlorophenol in edible gelatin and its paperboard packaging in southern Brazil. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(7), 492-499.
18. Franceschini, B., Previdi, M. P., and Schianchi, I. (2020). Food Spoilage by Bacilli: Combined Effects of pH, aw and Storage Temperature on Spore Germination and Growth in Cultural Broth Added with Solutes and Organic Acids. *Journal of Advances in Microbiology*, 49-55. https://doi.org/10.9734/jamb/2020/v20i330227.
19. Gallaher, M.P., O'Connor, A.C., White, W.W., Wright, R.S. (2002). The Economic Impact of the GasMixture NIST-Traceable Reference Materials Program. *Economic Analysis.* National Institute of Standards and Technology.
20. Gavriil, G., Kanavouras, A., and Coutelieris, F. A. (2018). Food-packaging migration models: A critical discussion. *Critical Reviews in Food Science and Nutrition*, 58(13), 2262-2272. https://doi.org/10.1080/10408398.2017.1317630.
21. Han, J-W., Luis, R-G., Qian, J-P., and Yang, X-T., (2018). Jia-WeiFood Packaging: A Comprehensive Review and Future Trends. Comprehensive Reviews in Food Science and FoodSafety, 17(4), 860-877. https://doi.org/10.1111/1541-4337.12343
22. Han, J. H. (2014). A review of food packaging technologies and innovations. In *Innovations in food packaging* (pp. 3-12). Academic Press.
23. Hindayani, A., Zuas, O., Mulyana, M. R., Budiman, H., Styarini, D., Krismastuti, F. S. H., and Sirenden, B. H. (2019, February). Validated method for trace impurities analysis in bulk gas using Gas Chromatography with Pulse Discharge Helium Ionization Detection. In *Journal of Physics: Conference Series* (Vol. 1153, No. 1, p. 012033). IOP Publishing. https://doi.org/10.1088/1742-6596/1153/1/012033.
24. Hyun, J. E.; Lee, S.Y. (2017). Preservation Method for Extending The Shelf-Life of Various Foods. *J.Food.Saf.* 38, 1-9. https://doi.org/10.1111/jfs.12376.
25. International Organization for Standardization. (2017). ISO 16664:2017: Gas analysis - Handling of Calibration Gases and Gas Mixtures - Guidelines. Switzerland.
26. International Organization for Standardization. (2017). ISO/IEC 17025:2017: General Requirements for the Competence of Testing and Calibration Laboratories. Switzerland.
27. International Organization for Standardization. (2001). ISO 6142:2001: Gas Analysis - Preparation of Calibration Gas Mixtures - Gravimetric method. Switzerland.
28. International Organization for Standardization. (2015). ISO 6142-1:2015: Gas analysis - Preparation of Calibration Gas Mixtures - Part 1: Gravimetric Method for Class I Mixtures. Switzerland.
29. Ishangulyyev, R., Kim, S., and Lee, S. H. (2019). Understanding Food Loss and Waste-Why Are We Losing and Wasting Food. *Foods (Basel, Switzerland)*, *8*(8), 297. https://doi.org/10.3390/foods8080297.
30. Jacksier, A. T., and Weterings, W. (2017). Gaseous Calibration Standards: Manufacturing, Stability, Traceability and Uncertainty. In *Offshore Technology Conference*. Offshore Technology Conference. https://doi.org/10.4043/27614-MS.
31. Javanmard, M. (2017). Effect of Modified Atmosphere Packaging and Storage Temperatures on Quality of Shelled Raw Walnuts. *International Journal of Nutrition and Food Engineering*, *11*(7), 510-514. http://doi.org/10.5281/zenodo.1131405.
32. Kharobe, V.B. (2018). Advantages and Disadvantages of Traditional Fermentation of Dairy Products. *International Journal of Recent Trends in Science And Technology*, Special issue. 52-55.
33. Kodentsova, V. M., and Vrzhesinskaya, O. A. (2018). Vitamin-mineral complexes: forms and application. *AV Skalny, MD, Prof.(Moscow, Russia)*, 21.

34. Kotsianis, I. S., Giannou, V., and Tzia, C. (2002). Production and packaging of bakery products using MAP technology. *Trends in Food Science and Technology*, *13*(9-10), 319-324. https://doi.org/10.1016/S0924-2244(02)00162-0.

35. Lin, Q-B., Song, X-c, Fang, H., Wu, Y-M., Wang, Z-W. (2017). Migration of styrene and ethylbenzene from virgin and recycled expanded polystyrene containers and discrimination of these two kinds of polystyrene by principal component analysis. *Food Additives and Contaminants: Part A*, 34(1), 126-132. https://doi.org/10.1080/19440049.2016.1253875.

36. Linke, B.G.O.; Casagrande, T.A.C.; Cardos, L.A.C. (2018). Food Additives and Their Health Effects: A Review On Preservative Sodium Benzoate. *Afr. J. Biotechnol*. 17, 306-310. https://doi.org/10.5897/AJB2017.16321

37. Marinangeli, C. P., Harding, S. V., Glenn, A. J., Chiavaroli, L., Zurbau, A., Jenkins, D. J., ... and Sievenpiper, J. L. (2020). Destigmatizing Carbohydrate with Food Labeling: The Use of Non-Mandatory Labelling to Highlight Quality Carbohydrate Foods. *Nutrients*, *12*(6), 1725. https://doi.org/10.3390/nu12061725.

38. Meredith, H., Valdramidis, V., Rotabakk, B. T., Sivertsvik, M., McDowell, D., and Bolton, D. J. (2014). Effect of different modified atmospheric packaging (MAP) gaseous combinations on Campylobacter and the shelf-life of chilled poultry fillets. *Food microbiology*, *44*, 196-203. https://doi.org/10.1016/j.fm.2014.06.005.

39. Millward, D. J. (2017). Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutrition research reviews*, *30*(1), 50. https://doi.org/10.1017/S0954422416000238.

40. Minitab. (2019). Using The t-value to Determine Whether to Reject The Null Hypothesis. Minitab. Retrieved 18/03/2020 from https://support.minitab.com/en-us/minitab-express/1/help-and-how-to/modeling-statistics/regression/supporting-topics/regression-models/using-the-t-value-to-determine-whether-to-reject-the-null-hypothesis/.

41. Mirza, S. K., Asema, U. K., and Kasim, S. S. (2017). To study the harmful effects of food preservatives on human health. *J Medicinal Chemist Drug Discovery*, *2*(2), 610-616.

42. Montero-Calderón, M., Rojas-Graü, M. A., Aguiló-Aguay, I., Martin-Belloso, O. (2010). Influence of Modified Atmosphere Packaging on Volatile Compounds and Physicochemical and Antioxidant Attributes of Fresh-Cut Pineapple (*Ananas comosus*). *Agriculture and Food Chemistry, 58(8),* 5042–5049. https://doi.org/10.1021/jf904585h.

43. Mullan, M., McDowell, D. (2003). Modified Atmosphere Packaging in Food Packaging Technology. *Food and Beverages Packaging Technology*. 303-339.

44. Mulyana, M. R., Budiman, H., Zuas, O., and Hindayani, A. (2019, February). Trace impurities measurement in ultra-high purity gases and their uncertainties: case study on permanent gas impurities in pure nitrogen. In *Journal of Physics: Conference Series* (Vol. 1153, No. 1, p. 012035). IOP Publishing. https://doi.org/10.1088/1742-6596/1153/1/012035.

45. Odeyemi, O. A., Alegbeleye, O. O., Strateva, M., and Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety*, *19*(2), 311-331. https://doi.org/10.1111/1541-4337.12526.

46. Opara, U. L., and Mditshwa, A. (2013). A review on the role of packaging in securing food system: Adding value to food products and reducing losses and waste. *African Journal of Agricultural Research*, 8(22), 2621-2630, https://doi.org/10.5897/AJAR2013.6931.

47. Ozturk, A., Yildiz, K., Ozturk, B., Karakaya, O., Gun, S., Uzun, S., and Gundogdu, M. (2019). Maintaining postharvest quality of medlar (Mespilus germanica) fruit using modified atmosphere packaging and methyl jasmonate. *LWT-Food Science and Technology*, *111*, 117-124..

48. Pitt, J. I., and Hocking, A. D. (2009). Fungi

and food spoilage (Vol. 519). New York: Springer.

49. Pu, Y., Zhou, Q., Yu, L., Li, C., Dong, Y., Yu, N., and Chen, X. (2020). Longitudinal analyses of lignin deposition in green asparagus by microscopy during high oxygen modified atmosphere packaging. *Food Packaging and Shelf Life*, *25*, 100536. https://doi.org/10.1016/j.fpsl.2020.100536.

50. Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research*. 5, 47-56.

51. Sandhya. (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *LWT-Food Science and Technology*, *43*(3), 381-392.

52. Scarabottolo, N., Fedel, M., Cocola, L., and Poletto, L. (2020, April). In-line inspecting device for leak detection from gas-filled food packages. In *Sensing for Agriculture and Food Quality and Safety XII* (Vol. 11421, p. 1142103). International Society for Optics and Photonics. https://doi.org/10.1117/12.2557784.

53. Sharma, S. (2015). Food Preservatives and their harmful effects. *International journal of scientific and research publications*, *5*(4), 1-2.

54. Shaw, M. (2020). Introduction to Calibration Gases. CAC Gas and Instrumentation. Retrieved 18/03/2020 fromhttps://www.cacgas.com.au/introduction-to-calibration-gases.

55. Shimosaka, T., Matsumoto, N., and Kato, K. (2011). High-precision GC-TCD for verification of gravimetrically prepared primary gas standards of oxygen in nitrogen. *Analytical Methods*, *3*(2), 280-287. https://doi.org/10.1039/C0AY00397B.

56. Shwaiki, L. N., Arendt, E. K., Lynch, K. M., and Thery, T. L. (2019). Inhibitory effect of four novel synthetic peptides on food spoilage yeasts. *International journal of food microbiology*, *300*, 43-52. https://doi.org/10.1016/j.ijfoodmicro.2019.04.005.

57. Smolander, M.; Hurme, E.; Ahvenainen, R. (1997). Leak indicators for modified-atmosphere packages. *Trends in Food Science and Technology*, 8, 101–106

58. Tsang, Y. P., Choy, K. L., Wu, C. H., Ho, G. T. S., Lam, H. Y., and Tang, V. (2018). An intelligent model for assuring food quality in managing a multi-temperature food distribution centre. *Food Control*, *90*, 81-97. https://doi.org/10.1016/j.foodcont.2018.02.030.

59. Utyanov, D. A., Kulikovskii, A. V., Vostrikova, N. L., and Ivankin, A. N. (2018). Determination of Vegetable Fats in Food Products. *Food systems*, *1*(4), 27-41. https://doi.org/10.21323/2618-9771-2018-1-4-27-41

60. Vaclavik, V. A., and Christian, E. W. (2008). Food preservation and processing. In *Essentials of food science* (pp. 425-446). Springer, New York.

61. Verghese, K., Lewis, H., Lockrey, S., and Williams, H. (2015). Packaging's Role in Minimizing Food Loss and Waste Across the Supply Chain: Packaging's Role in Minimizing Food Waste Across the Supply Chain. *Packaging Technology and Science*, 28(7), 603-620. https://doi.org/10.1002/pts.2127.

62. Wagner, E. M., Pracser, N., Thalguter, S., Fischel, K., Rammer, N., Pospíšilová, L., Alispahic, M., Wagner, M., and Rychli, K. (2020). Identification of biofilm hotspots in a meat processing environment: Detection of spoilage bacteria in multi-species biofilms. *International Journal of Food Microbiology*, 328, 108668. https://doi.org/10.1016/j.ijfoodmicro.2020.108668.

63. Wen, J., Huang, S., Sun, Y., Chen, Z., Wang, Y., Li, H., and Liu, X. (2018). Titanium Dioxide Nanotube-Based Oxygen Indicator for Modified Atmosphere Packaging: Efficiency and Accuracy. *Materials*, *11*(12), 2410. https://doi.org/10.3390/ma11122410.

Table 1. *The optimum operating condition of GC-TCD*

Parameters		Optimum condition
Injection	Flow rate sample	30 mL/min
	Loop	500 µL
	Valve box temp	100°C
	Valve 1	on (0.1 min),off (1 min)
Column	Column	Porapak Q column (1/8 inch OD, 6 feet, 80-100 mesh) and Molsieve 5A (1/8 inch OD, 9 feet, 80-100 mesh)
	Oven temp	isothermal, 40 °C
	Carrier gas	He, 41 psi (28 mL/min)
Detector	Detector	TCD
	Temp	250 °C
	Reference flow	He, 20 mL/min
	Make-up flow	He, 7 mL min
	Negative pol	On

Table 7. *The t-test result of CO_2 concentrationin ADK009 and AH06004*

	ADK009	**AH06004**
Mean	17.01	16.99
Variance	1.30E-03	3.60E-04
Observations	7	7
Pooled Variance	8.32E-04	
Hypothesized Mean Differ.	0	
df	12	
t_{stat}	1.33	
$t_{critical}$	2.18	

Table 8. *The t-test result of O_2 concentration in ADK009 and AH06004*

	ADK009	**AH06004**
Mean	1.51	1.50
Variance	3.68E-05	5.49E-05
Observations	6	6
Pooled Variance	4.59E-05	
Hypothesized Mean Differ.	0	
df	10	
t_{stat}	2.57	
$t_{critical}$	2.23	

Table 9. *The t-test result of N_2 concentration in ADK009 and AH06004*

	ADK009	**AH06004**
Mean	81.49	81.22
Variance	0.01	0.01
Observations	7	7
Pooled Variance	0.01	
Hypothesized Mean Difference	0	
df	12	
t_{stat}	5.41	
$t_{critical}$	2.18	

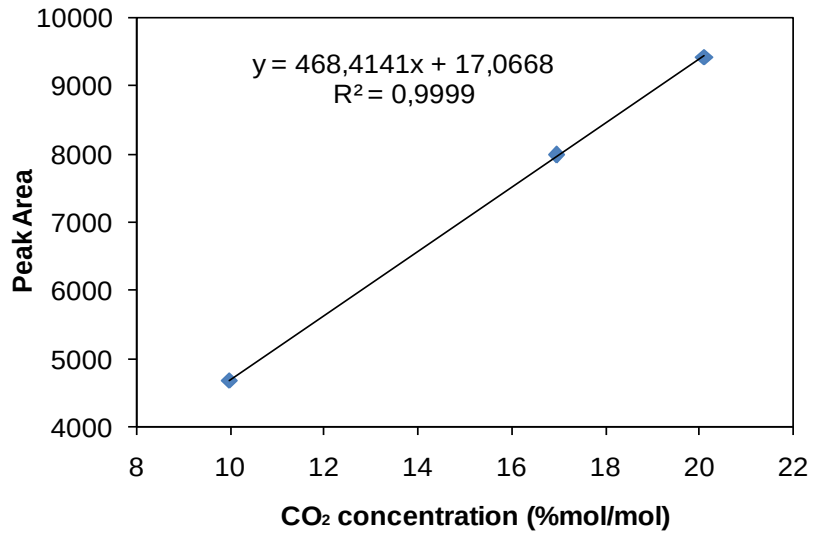

Figure 1. *Linear regression curve of CO_2*

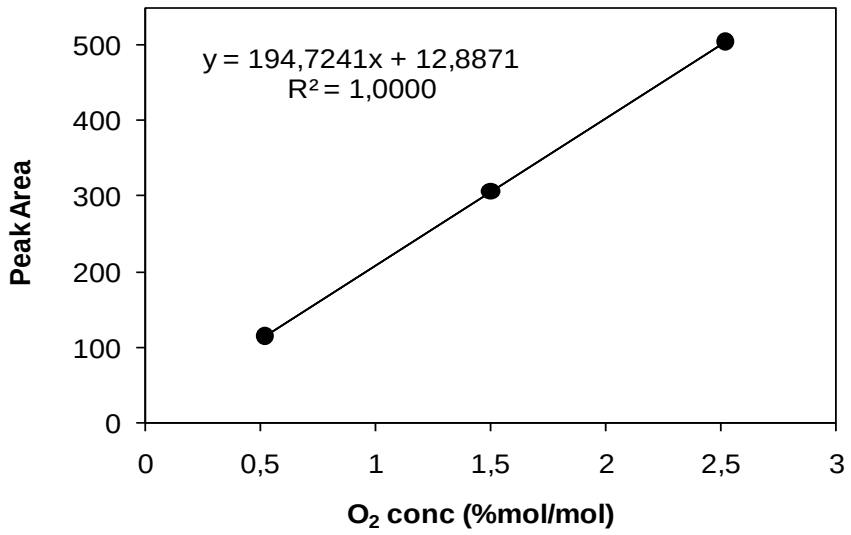

Figure 2. *Linear regression curve of O_2*

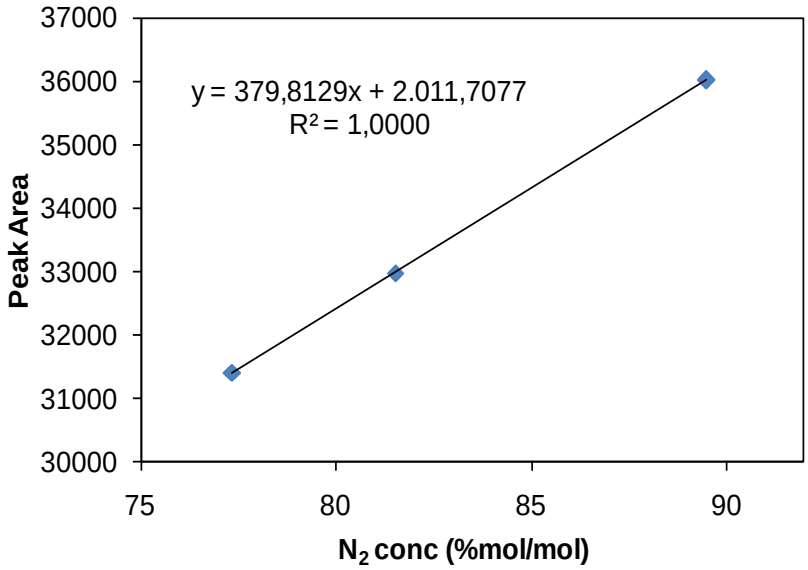

Figure 3. *Linear regression curve of N_2*