Quantitative Analysis of Polycyclic Aromatic Hydrocarbons in Heated Soybean Oil

by

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Graduate Program in Global Health Program
Duke Kunshan and Duke University

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Lijing Yan

Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Graduate Program
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2022

ABSTRACT

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are compounds ubiquitous in the environment and are harmful to human health. PAHs can easily enter the human body through the consumption of edible oils due to their high lipophilicity. Most of the published papers investigating PAH concentrations in edible oils focused on virgin (i.e., unheated) samples instead of those being used in cooking at temperatures higher than room temperature. This study aimed to provide a comprehensive landscape of PAH concentration in soybean oil by simulating a more realistic scenario of oil consumption—eating oil being cooked at different temperatures. I quantified the concentration of PAHs in soybean oil after being heated to four temperatures (100°C, 150°C, 190°C, and 222°C) with three durations (5 minutes, 30 minutes, and 60 minutes). Liquid liquid extraction (LLE), tandemly followed by solid phase extraction (SPE), were used to extract PAHs from the matrix and remove interferences from the extract. PAH concentrations were determined by a gas chromatography mass spectrometer (GC-MS). With extended heating time, concentrations of ACE and FLU showed a rising trend in 100°C and 150°C data groups but a falling trend at higher temperature data groups. Concentrations of PHEN and ANTH had a sharp increase when the oil was heated to 222°C and held one hour. Concentrations of B[a]A, CHRY, and B[a]P rose slightly with extended heating time at almost all temperatures. Concentrations of ACY, FLTH, B[b]F,

B[k]F, IND, D[ah]A, and B[ghi]P were low and nearly undetectable. Only NAP concentrations in 100°C-oil samples were over the maximum residue level (MRL) set by China's national standard. Diverse concentration changes of different PAHs after the oil was heated suggested that B[a]P alone or a combination of several PAHs are not sufficiently representative to be the marker or surrogate for PAH exposure. This diversity also resulted in an undetermined correlation between the PAH remaining in the oil and heating temperatures and hold time. The information of PAHs increment caused by heating soybean oil was incomplete because of data unavailability for the vaporized part.

Dedication

This thesis is dedicated to my daughter, Melody Chu, and my son, Mike Chu. They are my M&M's.

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Acknowledgments

When I decided to start my dream-pursuing journey in 2018, I did not expect it to be so challenging. Today, when I look back, I realize that I have got so much help from so many people to keep me from falling apart. I would like to express my gratitude hereby. First of all, I would like to thank Mr. Qixiang Yu, Ms. Josephine Bournon, and Mr. Zhihong Lu. They wrote recommendation letters for me and helped me to start my venturous journey. Second, I would like to thank all the teachers that have helped me to load professional skills to be a veritable science student. Third, I would like to thank all the faculty and staff of the Global Health Program, Department of Natural and Applied Science and Duke Kunshan University kitchen for providing resources to support me. Finally, are my special thanks to Mr. Maw-Rong Lee, Ms. Yen-Hsien Li, Ms. Sio-Leng Wong of National Chung Hsing University and Ms. Jing Peng, Mr. Hua Li of Yangzhou University for their generosity to me and professional guidance for me during a hard time of the pandemic. Without help from all these my 貴人, I would never have been even close to my dream.

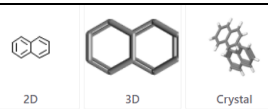
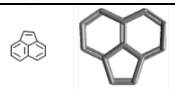
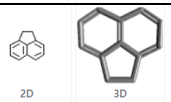
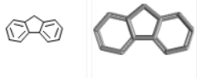
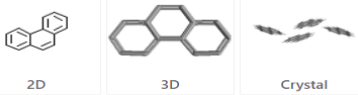
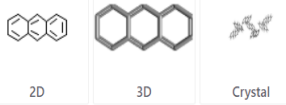
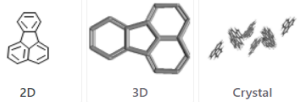
1. Introduction

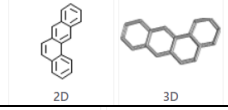
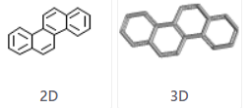
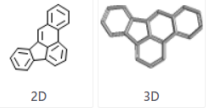
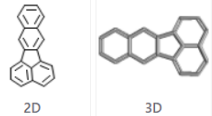
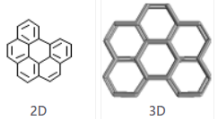
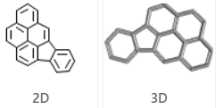
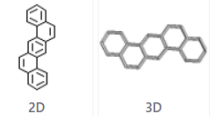
1.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds having similar compositions and structures. They have two or more aromatic ring structures and no atoms other than carbon and hydrogen. They are lipophilic and nonpolar. The number and arrangement of their aromatic rings (linear, angular, or clustered) is responsible for the difference in their physical and chemical properties (Abdel-Shafy & Mansour, 2016).

There are more than 100 compounds in the PAH group. Sixteen of them are considered as priority pollutant by the U.S. Environmental Protection Agency (USEPA) negatively affecting human health (Table 1): naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLTH), pyrene (PYR), benzo[a]anthracene (B[a]A), chrysene (CHRY), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), benzo[g,h,i]perylene (B[ghi]P), indeno[1,2,3-c,d]pyrene (IND), and dibenz[a,h]anthracene (D[ah]A) (Hussar, Richards, Lin, Dixon & Johnson, 2012).

Table 1: Name, abbreviation, molecular formula, structure, and boiling point of 16 PAHs.

Name	Abbreviation	Molecular formula	Structure	Boiling point
naphthalene	NAP	$C_{10}H_8$		217.97 °C
acenaphthylene	ACY	$C_{12}H_8$		280 ℃
acenaphthene	ACE	$C_{12}H_{10}$		279 °C
fluorene	FLU	$C_{13}H_{10}$		295 °C
phenanthrene	PHEN	$C_{14}H_{10}$		332 °C
anthracene	ANTH	$C_{14}H_{10}$ or $(C_6H_4CH)_2$		341.3 °C
fluoranthene	FLTH	$C_{16}H_{10}$		375 °C
pyrene	PYR	$C_{16}H_{10}$		404°C

benzo[a]anthracene	B[a]A	$C_{18}H_{12}$		438 °C
chrysene	CHRY	$C_{18}H_{12}$		448 °C
benzo[b]fluoranthene	B[b]F	$C_{20}H_{12}$		481 °C
benzo[k]fluoranthene	B[k]F	$C_{20}H_{12}$		480.0 °C
benzo[a]pyrene	B[a]P	$C_{20}H_{12}$		495 °C
benzo[g,h,i]perylene	B[ghi]P	$C_{22}H_{12}$		500 °C
indeno[1,2,3-c,d]pyrene	IND	$C_{22}H_{12}$		536.0 °C
dibenz[a,h]anthracene	D[ah]A	$C_{22}H_{14}$		524.0 °C

1.2 Adverse effects of PAHs and mechanism

The high lipophilicity of PAHs determines their significant bioavailability after entering the human body. PAHs can be detected in almost all organs especially those rich in adipose tissue. Description of their short-term and long-term effects on human health are all adverse: reproductive toxic, teratogenic, embryotoxic, neurotoxic, immune-toxic, genotoxic, cardiovascular toxic, mutagenic, developmental toxicity, and carcinogenic (Ramesh, Archibong, Hood, Guo & Loganathan, 2011; Abdel-Shafy & Mansour, 2016; Rengarajan et al., 2015). The mechanism of these effects, on the cellular level, is that they disrupt the function of cellular membranes and membrane associating enzyme systems. To further explain it, the process is described on the molecular level. When PAHs enter the body, they are metabolically activated into diol-epoxides, and bind to DNA covalently. PAH-DNA adducts can be found in various tissues and the concentration of these adducts has a positive correlation with the level of PAH exposure. When the body fails to eliminate these adducts, permanent mutations occur. And when these mutations appear on critical sites such as tumor suppressor genes or oncogenes, healthy cells transform into cancerous cells (Rengarajan et al., 2015)

PAHs have short-term and long-term health effects on the human body. Short-term health effects are caused by instantaneous exposure to high concentration PAH-containing pollutants. Such exposure usually happens in workplaces such as coal

industry chain enterprises, shale oil fields, metallurgical industry enterprises, infrastructure sites, diesel engine repair shops, and fire fields. As a result, acute symptoms such as eye irritation, nausea, vomiting, diarrhea, confusion (Bølling et al., 2009), skin irritation, and inflammation (Kim, Jahan, Kabir & Brown, 2013) were recorded.

In addition to short-term health effects, organs and tissues are affected by PAHs with long-term health effects. Symptoms are more complex and diverse due to the various degrees and times people are exposed to PAHs.

1.2.1 Reproductive toxicity

In *in vitro* studies, Russo, Troncoso, Sanchez, Garbarino & Vanella (2006) reported that B[a]P caused human spermatozoa DNA strand breakage, and Mukhopadhyay et al. (2010) found that human sperm hyperactivation and premature acrosome reaction were related to B[a]P. In an *in vivo* study, with adult male F-344 rats sub-chronically exposed to inhaled B[a]P, scientists found decapitated sperm in their semen (Ramesh et al., 2008). Epidemiology studies provided more evidence of a correlation between PAH exposure and harmful effects on male reproduction. Hsu et al. (2006) reported sperm DNA damage in coke-oven workers. An investigation among infertile males found that urinary PAH metabolites, indicators of PAH exposure, were correlated with sperm DNA damage (Ji et al., 2010). A study conducted in the Czech

Republic among people exposed to PAH-polluted air revealed that these people had abnormal chromatin/fragmented DNA in their sperm (Rubes, Selevan, Sram, Evenson & Perreault, 2007).

Evidence showed that PAHs damage female reproduction. Ramesh, Rorke & Niaz (2010) conducted an animal experiment exposing female Fischer-344 rats to a high dose of B[a]P. Reactive B[a]P metabolites and adducts were still able to be detected up to a month after exposure. Sizemore, Mukhtar, Couch & Howard (1998) found B[a]P metabolites in cervical cells and indicated that these metabolites exerted an inhibitory effect by enhancing terminal cell death. Cell adhesion proteins in human uterine cells were reported to have changed because of B[a]P exposure (McGarry et al., 2002). As a rich source of PAHs, cigarette smoke was considered not only to reduce the fertility of parents (Soares, Simon, Remohi & Pellicer, 2007), but also to increase the risk of offspring by transmitting altered DNA to preimplantation embryos and contributing to childhood cancers (Zenzes, Puy & Bielecki, 1998; Zenzes, 2000).

Research has shown that PAHs have multigenerational effects through fertilization from both parents. Zenzes, Bielecki & Reed (1999) reported B[a]P-DNA adducts in spermatozoa of smokers. Another team with several overlapping members reported preimplantation embryos from a non-smoker mother and smoker fathers carrying B[a]P-DNA adducts (Zenzes, Puy, Bielecki & Reed, 1999). Shamsuddin & Gan

(1988) indicated that mature ova with B[a]P-DNA adducts could carry defective genes. Those defective genes could either express or not express themselves. If expressed, it could be during intrauterine residence or post-partum.

1.2.2 Teratogenicity

In an animal experiment, some PAHs were found to cause deformities in the early life stages of fish (Wassenberg & Di Giulio, 2004). Another experiment on mice revealed that ingesting a high level of B[a]P during pregnancy resulted in birth defects and offspring underweight (Kristensen et al., 1995). Adverse birth outcomes such as low birth weight, premature delivery, and heart malformations were found in human infants when their mothers were exposed to PAH pollution during pregnancy. It was estimated that fetuses may be 10-fold more susceptible to the damage caused by PAHs than mothers (Perera, Tang, Whyatt, Lederman & Jedrychowski, 2005). In a prospective cohort study in Poland, a reduction in Raven's Colored Progressive Matrices (RCPM) score, a standardized test to obtain a non-verbal reasoning score for children, and an estimated average decrease of 3.8 IQ points at five years of age were found to be associated with high airborne PAH prenatal exposure (Edwards et al., 2010).

1.2.3 Neurotoxicity

Neuronal death, plasticity deficits and neurobehavioral deficits of laboratory animals caused by acute and subacute PAH exposures were recorded in several

experiments (La'Nissa et al., 2007; Bouayed et al., 2009; Dutta, Ghosh, Nazmi, Kumawat & Basu, 2010). Learning disorders of some Czech Republic children, born in the early 1980s, were presumed to result from pollution from local mining and the combustion of coal (Otto, Skalik, Bahboh, Huduell & Sram, 1997). The prevalence of neurotic syndromes with vegetative dysregulation and a loss of short-term memory among Poland coke workers was dependent on the exposure of B[a]P (Majchrzak, Sroczyński & Chełmecka, 1990). Firefighters were reported to have more multiple myeloma and brain cancer (Golden, Markowitz & Landrigan, 1995).

1.2.4 Immunotoxicity

Effects of PAHs on the immune system could be either immune suppression or immune-potentiation depending on different conditions designed in various protocols such as model, exposure, dose, and endpoint. Both immune suppression and immune-potentiation were observed, with immune suppression more frequently reported (Burchiel & Luster, 2001). Immune suppression is associated with cancer development and susceptibility to infectious diseases. Immune-potentiation is associated with inflammation (Abdel-Shafy & Mansour, 2016).

1.2.5 Genotoxicity, mutagenicity, carcinogenicity, and developmental toxicity

Evidence showed that it is the metabolites of PAHs instead of PAHs themselves that are genotoxic (Rengarajan et al., 2015). PAHs are metabolized to diol epoxides,

which interact with DNA to induce mutations. They are therefore thought to be mutagenic and genotoxic. Mutagenicity and genotoxicity are important parts of the process of carcinogenicity. They are also considered to be developmental toxic as they were found inhibiting metabolism. In an experiment using T-47D breast cancer cells, exposure to lower level (0.1 to 1 μM) B[k]F induced CYP1A1/1B1-catalyzed 17β-estradiol (E2) metabolism, whereas higher level (>1 μM) B[k]F inhibited E2 metabolism (Spink et al., 2008). Base-pair substitutions have been found induced by benzo[a]pyrene-7,8-diol-9,10-epoxide (BaPDE), a B[a]P metabolite, in mammalian cells *in vitro* (Yang, Mazur-Melnyk, de Boer & Glickman, 1999). The exact process was reported *in vivo* among transgenic mice (Miller et al., 2000). Besides base pair substitutions, DNA frameshifting, base deletion, S-phase arrest, and strand breakage were also reported by Jung et al. (2013) in their experiment on rat liver tissues.

1.2.6 Cancers

Dermal cancer. The evidence of considerable dermal toxicity and increased cancer risk upon exposure to PAHs was robust in animal bioassay studies (Knafla, Phillipps, Brecher, Petrovic & Richardson, 2006). However, results from different human experiments were not consistent. Evidence from human biomonitoring studies conducted on road pavers was not able to reach a statistically significant conclusion (Väänänen, Hämeilä, Kalliokoski, Nykyri & Heikkilä, 2005). But another recent study

showed that dermally applying coal tar accompanied with ultraviolet radiation to children with psoriasis caused mutagenic and temporary genotoxic effects (Borska et al., 2010).

Esophageal cancer. Northeast Iran had the highest rate of esophageal squamous cell carcinoma (ESCC) in the world, and this can be attributed to its widespread and very high level of PAHs from unknown sources (Kamangar et al., 2005). In China, a study measuring the expression of aryl hydrocarbon receptor and related genes in frozen esophageal cell samples from patients exposed to different levels of indoor air pollution (domestic heating with coal) implied that indoor air pollution was the causative factor for esophageal cancer (Roth et al., 2009).

Colorectal cancer. Sinha, Kulldorff, Gunter, Strickland & Rothman (2005) conducted a clinic-based case-control study by developing a food frequency questionnaire with detailed questions. As a surrogate for the total intake of carcinogenic PAHs, high intake of B[a]P was strongly associated with an increased risk of colorectal adenomas, both in the group considering total diet and the group considering meat only. Studies conducted in a mouse model revealed an increased prevalence ($P<0.05$) of colorectal cancers in the group ingesting B[a]P through saturated dietary fat (Harris, Washington, Hood, Roberts & Ramesh, 2009).

Breast cancer. An *in vitro* study revealed that PAHs contribute to the etiology of sporadic breast cancer by inhibiting cell proliferation and downregulating the expression of the BRCA-1 gene (a known tumor suppressor) (Jeffy et al., 1999). A review of the epidemiologic evidence stated that PAH-DNA adducts were formed when the human body responded to PAH exposure. Among populations with natural DNA repair mechanisms inadequacy and PAH-susceptibility, which are usually caused by certain genotype, breast cancer risk increased with different magnitude (Gammon & Santella, 2008).

Lung cancer. The conclusion that exposure to PAH via inhalation is likely to contribute to the development of lung cancer was drawn from studies conducted comparing smokers and nonsmokers (Goldman et al., 2001) and on a cohort of 16,431 aluminum smelter industry workers (Armstrong & Gibbs, 2009). This cohort study reported an increased risk with increased cumulative occupational exposure to PAH (measured as B[a]P), seen in at least 680 cases. This rising trend was not seen enhanced by smoking. Elovaara et al. (2007) observed wide variations in PAH metabolizing enzyme activities in lung cancer patients' lung tissues. Further study by this team revealed that PAH metabolizing enzyme activities were inducible by exposure to specific PAHs in the rat lung and liver.

1.2.7 Other adverse effects

Obesity. Hutcheon, Kantrowitz, Van Gelder & Flynn (1983) reported a positive correlation between plasma B[a]P level and body mass index (BMI). Irigaray et al., (2006) reported that when exposed to B[a]P (0 – 100 μM), adipose tissue lipolysis was inhibited in a dose-dependent manner in experimental mice. Consequently, consistent weight gain and increased fat mass occurred without detectable changes in food intake. These effects persisted even after B[a]P dosing was withdrawn.

Atherosclerosis. The fact that PAHs reside in adipose tissue draws attention to their health effect on the cardiovascular system. Curfs et al. (2004) reported larger and phenotypically different atherosclerotic plaques in experimental mice when they were exposed to 5 mg/kg B[a]P chronically (12-24 weeks) and the stages of plaques were correlated to the levels of B[a]P metabolites and B[a]P-DNA adducts. The mechanism was the enhanced expression of the AhR target gene in mouse aortic endothelial cells (Wang et al., 2009).

Osteoporosis. Data from animal studies documented the association between PAH exposure and bone damage. A PAH compound, 3-methylcholanthrene (3-MC), was reported to have an impact on the process of bone-forming by inhibiting the proliferation and differentiation of osteoblasts (bone-forming cells) (Naruse, Ishihara, Miyagawa-Tomita, Koyama & Hagiwara, 2002). 3-MC was also found to have an impact

on osteoclasts (bone destroying cells) by either affecting osteoclast supporting cells (Naruse, Otsuka, Ishihara, Miyagawa-Tomita & Hagiwara, 2004) or inhibiting osteoclast differentiation (Voronov, Heersche, Casper, Tenenbaum & Manolson, 2005).

1.3 Formation of PAHs and routes of human ingestion

In the long history of the earth, PAHs are formed through pyrogenic, petrogenic, and biological processes as the product of incomplete combustion of carbonaceous materials (Abdel-Shafy & Mansour, 2016). When examining PAHs in the smoke from heated oils, Chen & Chen (2001) stated that PAH formation is through degradation products, mostly compounds containing benzene rings, of heated oils. These benzene-like compounds undergo further reactions to form various PAHs.

Since PAHs are ubiquitous in the environment, humans will inevitably encounter PAHs. There are three main ways for PAHs to enter the human body: inhalation, skin absorption, and ingestion (Hao et al., 2016). For the general non-smoking population who are not accidentally or occupationally exposed to PAHs, ingestion is a more usual exposure than breathing and skin absorption (Ramesh et al., 2011). Therefore, as one of the primary sources of PAHs, foods have been widely investigated regarding the extent of contamination by PAHs. In the food category, oils and fats contribute almost half of the total PAHs of human food (Jira, 2004). Hence, numerous efforts have been made to develop valid methods to measure the concentration of PAHs in oils and fats.

1.4 Current methods to test PAHs in edible oils

A recent review summarized the development of strategies scientists used to extract PAHs from edible oils and to determine their concentration (Sánchez-Arévalo, Olmo-García, Fernández-Sánchez & Carrasco-Pancorbo, 2020). They concluded that two moves, pretreatment and detection, are necessary to determine the concentration of PAHs in edible oils.

1.4.1 Pretreatment

The purpose of pretreatment, also called sample treatment, is to extract PAHs out of the matrix and remove interferences from the extract. This procedure of isolating PAHs relies on multi-step approaches such as saponification, liquid-liquid extraction (LLE), solid-phase-extraction (SPE), donor–acceptor complex chromatography (DACC), and gel permeation chromatography (GPC). When saponification is included in a series of procedures, the aim is to reduce the lipidic content of the matrix. However, this aim is usually not well achieved since the removal of oil is generally not complete, and additional extraction steps are still required. Therefore, skipping saponification and starting directly from LLE is common (Sánchez-Arévalo, Olmo-García, Fernández-Sánchez & Carrasco-Pancorbo, 2020). The logic of LLE is to have an oil-immiscible solvent to fully contact with the oil and allow the PAHs to diffuse into the solvent. This process is mainly vortexing and often assisted by ultrasound bath. After LLE, SPE is often used

to clean up the extract by eliminating interfering co-extractants. The use of the SPE cartridge is widespread due to its efficiency and convenience. Some scientists reported that they apply oil samples directly onto SPE cartridges to avoid the time-consuming and solvent-consuming LLE (Sánchez-Arévalo, Olmo-García, Fernández-Sánchez & Carrasco-Pancorbo, 2020). Besides the above methods, DACC is an alternative isolation procedure. Because PAHs are electron acceptors, DACC solid-phase extraction cartridge uses electron acceptors to retain PAHs while lipids are eluted to the waste liquid. DACC saves solvent but is time-consuming. Another alternative isolation procedure is GPC, using the different molecular sizes of each component in the sample to separate. As a semi-automatic purification, GPC saves manpower and time, but consumes a lot of organic solvents.

1.4.2 Detection

After being isolated from the oil matrix, reverse-phase liquid chromatography (LC) and gas chromatography (GC) are most often employed to separate the PAHs. In an LC column, with the different polarity of each PAH, meaning diverse binding force to the stationary phase, PAHs are separated through the application of solvent gradient. Fluorescence or ultraviolet detector is often used to qualify and quantify these PAHs, while mass spectrometer (MS) is not an often-chosen detector to tandemly connecting to LC due to low ionization efficiency. The separation of PAHs in a GC column has a

similar principle as LC, only using gas as the mobile phase through the ramping of the oven temperature. The detector used to qualify and quantify the separated PAHs is mostly MS, regarding its sensitivity and precision. Other analytical strategies not entailing chromatographic separation is not common as such analyses can be considered to be time and solvent consuming (Sánchez-Arévalo, Olmo-García, Fernández-Sánchez & Carrasco-Pancorbo, 2020).

1.5 Research gap and study goal

With these ever-improving methods, monitoring the occurrence of PAHs in edible oils became possible. On this basis, many scientists conducted studies to investigate PAH concentrations in edible oils. Most of the published papers on this issue focused on fresh ones purchased from markets instead of those being cooked. For example, some of the studies investigated the distribution of PAHs in all categories of a particular oil, such as olive oil (Bertoz, Purcaro, Conchione & Moret, 2021), some surveyed PAHs in available edible oils in a particular market (Hao et al., 2016; Shi, Zhang & Liu, 2016). Only limited studies focused on the concentration of PAHs in edible oils after certain kinds of cooking, such as frying (Purcaro, Navas, Guardiola, Conte & Moret, 2006; Wu & Yu, 2012). Given human eating habits that we usually eat cooked food, the existing research probably underestimates the actual amounts of PAHs we ingest from edible oils.

In practice, heated oil must contain more PAHs than unheated oil, but no study has made a rigorous comparison. According to Purcaro, Moret & Conte (2013), PAH formation is related to temperature and is most efficiently produced at 500 - 700°C. Another paper, Rose (2010), reported that low temperature (100–150°C) aromatization produces PAHs through relatively more time. Moreover, although Abdel-Shafy & Mansour (2016) concluded in their review that foods cooked at high temperature are major sources of PAHs and even presented details of higher concentration of PAHs in some foods, they ascribed it to certain types of cooking methods such as grilling, roasting and frying instead of exploring the connection with heating temperatures and time. All these papers generally pointed out that high temperature has an association with the generation of PAHs. Still, none of them depicted a clear relationship between temperature, heating time, and the PAHs generated during the process, especially since none of them took the effect of vaporization into consideration.

This study aimed to understand the relationship between PAH concentration, heating temperature, and heating time. There are two specific aims:

1. Reveal the changes in PAH concentrations after the oil is heated.
2. Find out patterns of PAH concentration changes and provide explanations.

2. Methods

2.1 Overall design of the project

The PAH concentration of unheated oil was considered the reference for heated oil samples. Four portions of oil were proposed to be heated to four different temperatures. From each portion of oil, three oil samples were obtained after reaching and holding the target temperature (100°C, 150°C, 190°C, and 222°C) for three different lengths of time (5, 30, and 60 minutes). The PAH concentration of each sample was determined through the process of extracting PAHs from oil, removing interferences, and determining their concentrations.

2.2 Reagents and equipment

Acetonitrile, *n*-hexane, and dichloromethane: all HPLC grade, were obtained from Damas-beta (Shanghai, China).

Vortex: Thermo Scientific basic vortex mixer.

Heating equipment: Heidolph.

SPE cartridge: 500 mg Supelco Supelclean ™ LC-Si tube (Bellefonte, PA, USA)

A certified USEPA PAHs standard mixture (200 μg/ml, dissolved in acetonitrile), containing NAP, ACY, ACE, FLU, PHEN, ANTH, FLTH, PYR, B[a]A, CHRY, B[b]F, B[k]F, B[a]P, B[ghi]P, IND, and D[ah]A, with their guaranteed purity between 97.3% and 99.6% was obtained from O2si (Charleston, SC, USA).

GC-MS system: 8890 GC system & 5977B MSD from Agilent Technologies (Wilmington, NC, USA); equipped with a 7693A autosampler. The instrument was controlled by the MassHunter software.

Column: non-polar HP-5MS column (30 m × 0.25 mm × 0.25 μm) from Agilent Technologies (Wilmington, NC, USA).

2.3 Pretreatment

I used soybean oil (Arawana Brand), provided by Duke Kunshan University (DKU) kitchen, for this experiment. Four portions of oil, each 100 grams, were put in crystalizing dishes and heated, stirring with magnetic stir bars, to reach their target temperatures. It took 17 minutes to reach 100°C, 8 minutes to 150°C, 25 minutes to 190°C, and 22 minutes to 222°C. Oil samples were heated to each target temperatures for 5 minutes, 30 minutes, and 60 minutes. Subsequently, they were placed in glass bottles, allowed to cool, and then stored in a 4°C refrigerator.

For each sample, a total of 5.0 g (± 0.01 g) of oil sample was weighed in a 50 ml centrifuge tube (A) and 20 ml of acetonitrile was added. The sample was vortexed at 2500 rpm for 8 minutes. After standing to allow for stratification, the supernatant was transferred as much as possible to another centrifuge tube (B) by a pipette. The operation of adding solvent, vortexing, and transferring supernatant was repeated twice. The liquid in the centrifuge tube B was concentrated to 7.5 ml by a gentle flow of air

with the help of a 43°C water bath. Then, both centrifuge tubes A and B were stored in a -20°C refrigerator for one hour. After the oil was frozen, the upper liquid of both tubes was poured into a 100 ml round-bottom flask and air-blow to dry with the help of a 43°C water bath. The residue was dissolved by adding 2 ml of *n*-hexane and then loaded onto an SPE cartridge, preconditioned with 3 mL of *n*-hexane. Then, the SPE column was washed with 3 ml of *n*-hexane and 4 ml of *n*-hexane/dichloromethane (v/v = 9:1) at a flow rate of 1 drop per second, which was achieved by using a vacuum manifest. Eluents from both the load and the wash were collected and dried by airflow with the help of a 40°C water bath and then redissolved in 400 μl of acetonitrile and transferred to a vial for detecting.

2.4 Detecting and quantifying PAHs

A stock PAH standard solution (20 μg/ml of each PAH) was prepared in acetonitrile and stored at 4°C in a dark bottle. Each working PAH standard solution was prepared by diluting the stock standard solution. For each test, 1 μl of the sample was injected in splitless mode. Helium (99.999% purity) was used as carrier gas with a constant flow of 1.0 ml/min. The oven temperature was set initially at 60°C (1 min hold) before increasing to 180°C at 15°C/min. After reaching 180°C, the temperature was raised at a rate of 3°C/min to 200°C, followed by a rate of 6°C/min to 250°C (3 min hold). The final ramp stage was to 300°C at 3°C/min and held for 5 min. The temperatures of

the injector, transfer line and MS source were set at 250°C, 300°C and 230°C, respectively. The MS was operated in electron ionization (EI) mode at 70 eV. Scan mode was used to acquire a continuous range of ion fragments data. In addition, it was also used to determine whether all 16 target PAHs could be identified. Retention time data for all target PAHs obtained from the scan mode were the basis of the Selected Ion Monitoring (SIM). In SIM mode, the whole chromatography time was cut into time segments; each segment covers one or two PAH retention time so that only these one or two specific compounds were quantified by monitoring selected ions.

3. Results

3.1 Identification of PAHs

The 16 target PAHs in the standard solution were well separated by the GC program and detected by the MS. The total ion chromatogram of PAH standards is shown in Figure 1.

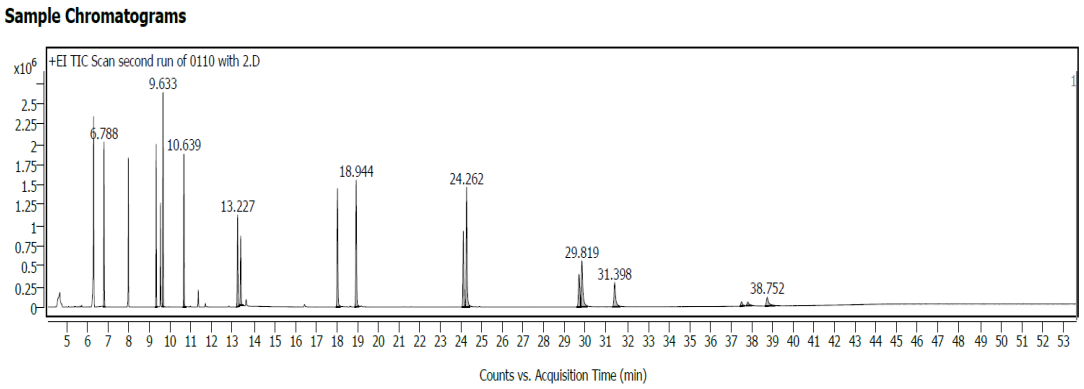


Figure 1: Total ion chromatogram of 16 PAHs standards.

The retention times, quantification ions and identification ions of 16 analytes are listed in Table 2.

Table 2: Retention time, quantification ion, and identification ions of 16 PAHs.

PAH	Retention time (min)	Quantification ion	Identification ion
NAP	6.79	128	129, 127, 102
ACY	9.31	152	150, 153, 151
ACE	9.63	153	154, 151, 152
FLU	10.64	166	165,167,163
PHEN	13.23	178	176 ,179 ,152
ANTH	13.38	178	176, 179, 152
FLTH	18.04	202	203, 200, 101
PYR	18.94	202	203, 200, 101
B[a]A	24.10	228	226, 229, 113
CHRY	24.26	228	226, 229, 113
B[b]F	29.68	252	253, 250, 116
B[k]F	29.82	252	253, 250, 116
B[a]P	31.40	252	253, 250, 116
IND	37.52	276	138, 274, 277
D[ah]A	37.81	278	276, 279, 138
B[ghi]P	38.75	276	138, 274, 277

3.2 Method Validation

Calibration curves of the 16 PAHs were obtained by measuring a group of PAH standard solutions at 0.03 μg/ml, 0.04 μg/ml, 0.05 μg/ml, 0.06 μg/ml, and 0.08 μg/ml. Coefficients of correlation (r^2) of calibration curves were provided by MassHunter software automatically. Limit of detection (LOD), meaning the lowest signal, or the lowest corresponding quantity that can be determined, was calculated by adding the mean of the limit of blank and three times of the signal-to-noise ratio (S/N) (LOD = blank

+ $3\sigma_{bl}$). Limit of quantification (LOQ), meaning the smallest concentration that can be reliably quantified was calculated by adding the mean of the limit of blank and 10 times the S/N (LOQ = blank + $10\sigma_{bl}$). Coefficients of correlation (r^2) of calibration curves and LODs and LOQs of both standard and matrix are provided in Table 3.

Intra-day precision was determined by performing seven analyses on the same sample on the same day and calculating their relative standard deviation (RSD). Inter-day precision data were obtained by performing five analyses on the same sample on five consecutive days. RSDs of the 16 PAHs for intra-day were between 1.6% and 10.1% and were between 21.6% and 44.3% for inter-day (Table 4).

Table 3: Coefficients of correlation (r^2), LODs and LOQs of standard, LODs and LOQs of matrix.

PAH	Coefficient of correlation (r^2)	LOD of standard (µg/ml)	LOD of matrix (µg/kg)	LOQ of standard (µg/ml)	LOQ of matrix (µg/kg)
NAP	0.9952	0.037	0.003	0.125	0.010
ACY	0.9746	0.036	0.003	0.121	0.010
ACE	0.9415	0.036	0.003	0.121	0.010
FLU	0.9562	0.036	0.029	0.119	0.010
PHEN	0.9510	0.036	0.003	0.120	0.010
ANTH	0.9384	0.036	0.003	0.121	0.010
FLTH	0.6658	0.045	0.004	0.150	0.012
PYR	0.8385	0.040	0.003	0.132	0.011
B[a]A	0.9648	0.038	0.003	0.127	0.010
CHRY	0.9388	0.037	0.003	0.124	0.010
B[b]F	0.9741	0.036	0.003	0.120	0.010
B[k]F	0.9469	0.038	0.003	0.128	0.010
B[a]P	0.9735	0.036	0.003	0.120	0.010
IND	0.9650	0.036	0.003	0.120	0.010
D[ah]A	0.8833	0.039	0.003	0.130	0.010
B[ghi]P	0.9509	0.036	0.003	0.120	0.010

Table 4: The intra- and inter-day precision of the 16 PAHs.

PAH	RSD of intra-day precision (%)	RSD of inter-day precision (%)
NAP	7.8	39.9
ACY	5.9	33.0
ACE	4.4	38.0
FLU	3.5	31.4
PHEN	3.3	34.5
ANTH	3.3	29.6
FLTH	4.7	39.8
PYR	3.1	32.9
B[a]A	6.7	30.1
CHRY	4.7	21.6
B[b]F	5.6	34.1
B[k]F	10.1	27.7
B[a]P	5.8	35.4
IND	1.6	42.2
D[ah]A	6.1	44.3
B[ghi]P	3.9	37.2

3.3 PAH concentration in heated oil

The methods described in 2.3 and 2.4 were used to determine the 16 PAHs in all the samples. To avoid the error from relatively high RSDs of inter-day precision, comparisons were made between data collected from samples treated on the same day: one from unheated oil, three from oil heated to an exact temperature while held for different lengths of time. All the raw data are listed in Appendix A.

Checking all the data provided by the software through examining the compound information, data of several PAHs, including ACY, FLTH, B[b]F, B[k]F, IND, D[ah]A, and B[ghi]P were found not suitable to analyze. Data of these compounds were considered not valid for two reasons: integrating on wrong peaks or integrating on images that should not be considered peaks. For example, Figure 2 shows different analyses of the same sample, oil heated to 100°C and held for 5 minutes. The software identified two different peaks for the same compound and provided data on these identifications. However, neither of these peaks matched the retention time well and were not considered compound ACY. Figure 3 shows examples of images that should not be considered peaks.

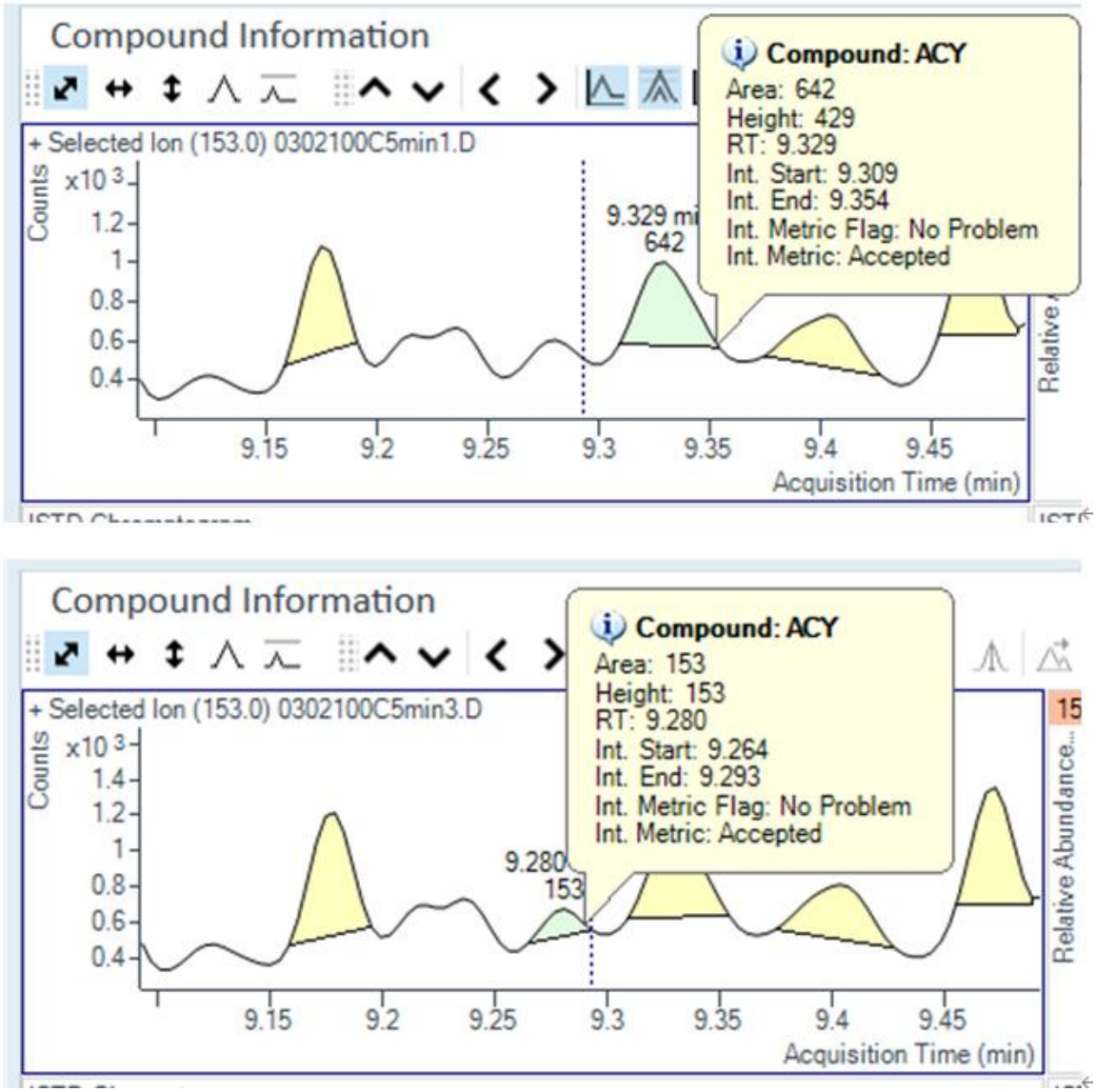


Figure 2: Compound information of ACY from different analyses of the same sample.

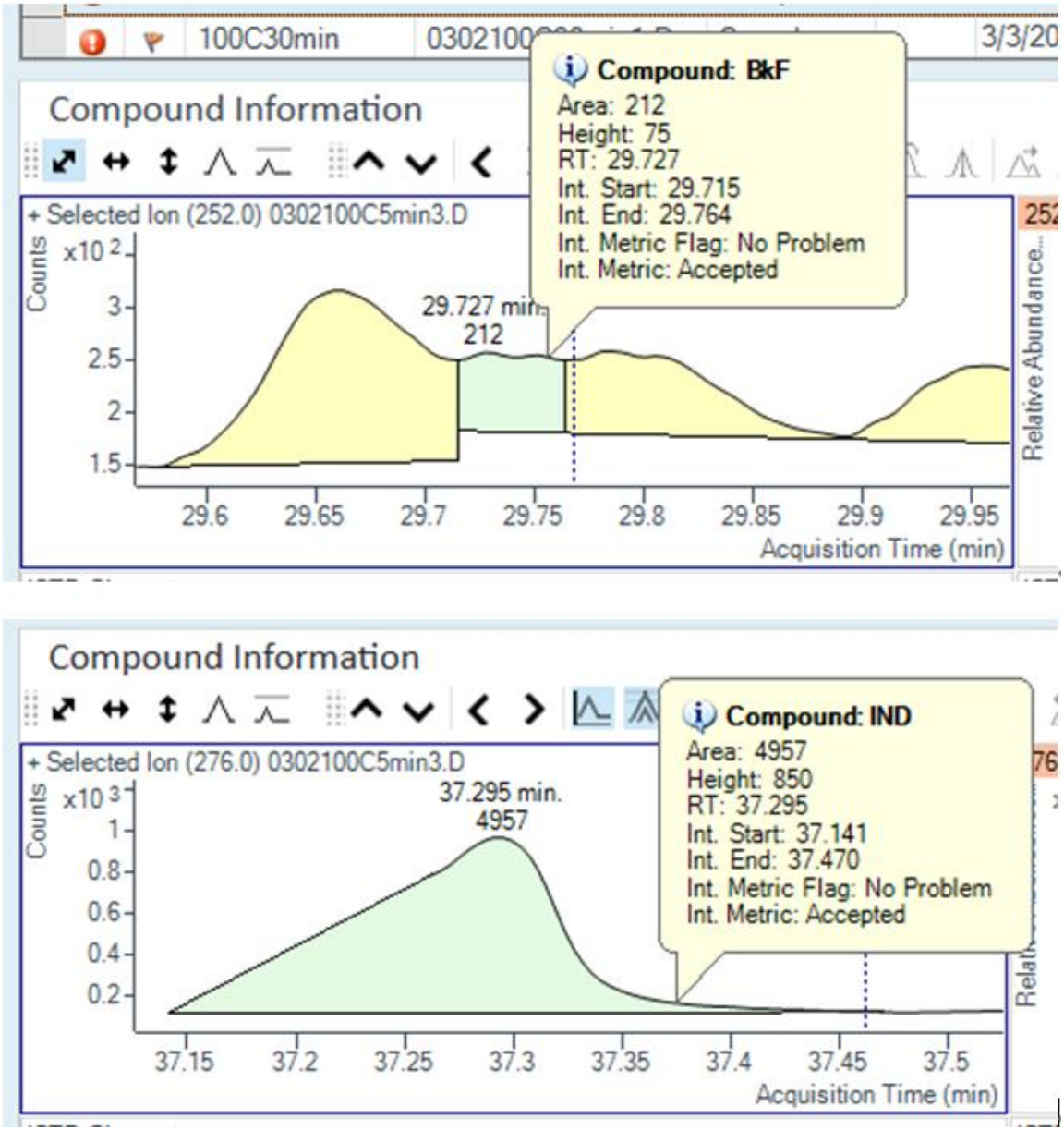


Figure 3: Images that should not be considered peaks.

A conversion calculation was conducted to translate the data provided by MassHunter, which are PAH concentrations of solutions (ng/ml), into PAH levels in oil samples (μg/kg). Suppose there are x μg of a PAH in 1 kg oil, then 5 g oil contains 0.005x μg of this PAH. When this amount of PAH is extracted to a 0.4 ml solution, which is detected to be y ng/ml, or 0.001y μg/ml of concentration, that means $0.005x = 0.4*0.001y$. Therefore, $x = 0.08y$ is the converting equation. So, after converting useful raw data to PAH levels in oil (three digits after the decimal point), comprehensive information on each PAH is shown in Tables 5 - 13. Bar charts showing their trends are shown in Figures 4 - 12.

Table 5: NAP levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	2.390	20.071	39.988	79.695
150°C	0.632	2.783	9.772	0.555
190°C	12.143	0.485	0.317	20.331
222°C	39.046	1.210	0.286	1.273

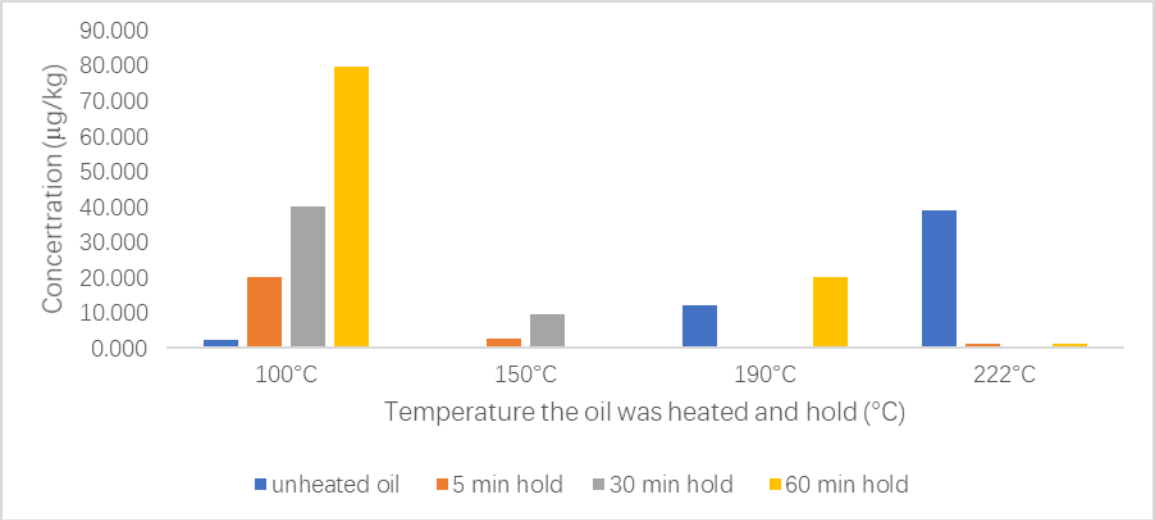


Figure 4: NAP level trends in Arawana Brand soybean oil after heated.

Table 6: ACE levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C		0.4113	0.538	0.580
150°C		0.330	0.573	0.460
190°C	0.074	0.404		0.230
222°C		0.057		0.188

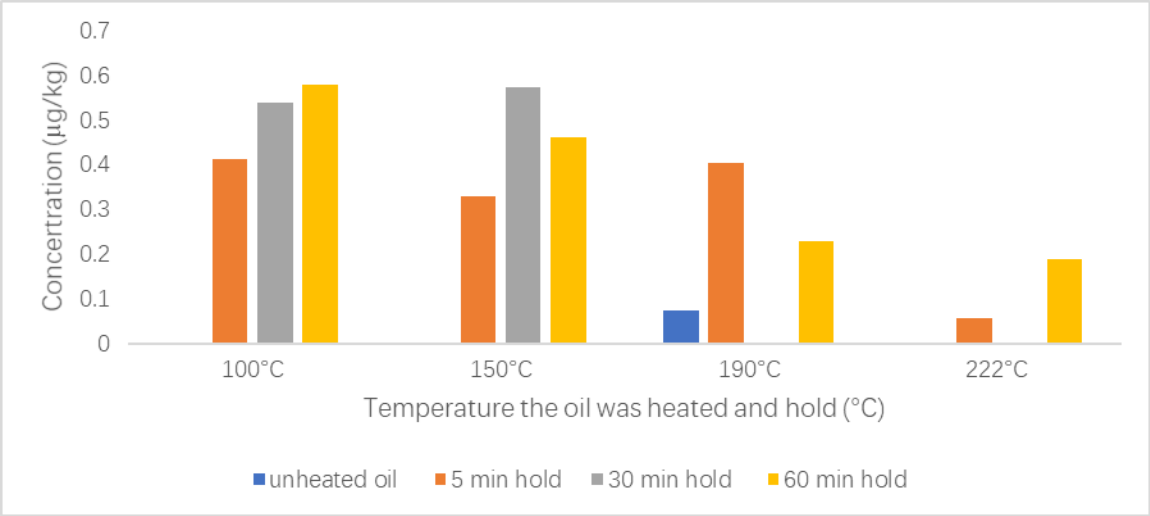


Figure 5: ACE level trends in Arawana Brand soybean oil after heated.

Table 7: FLU levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	1.290	1.803	1.895	1.997
150°C	0.481	1.294	1.480	1.370
190°C	2.171	1.838	1.217	1.070
222°C	1.664	1.113	1.066	

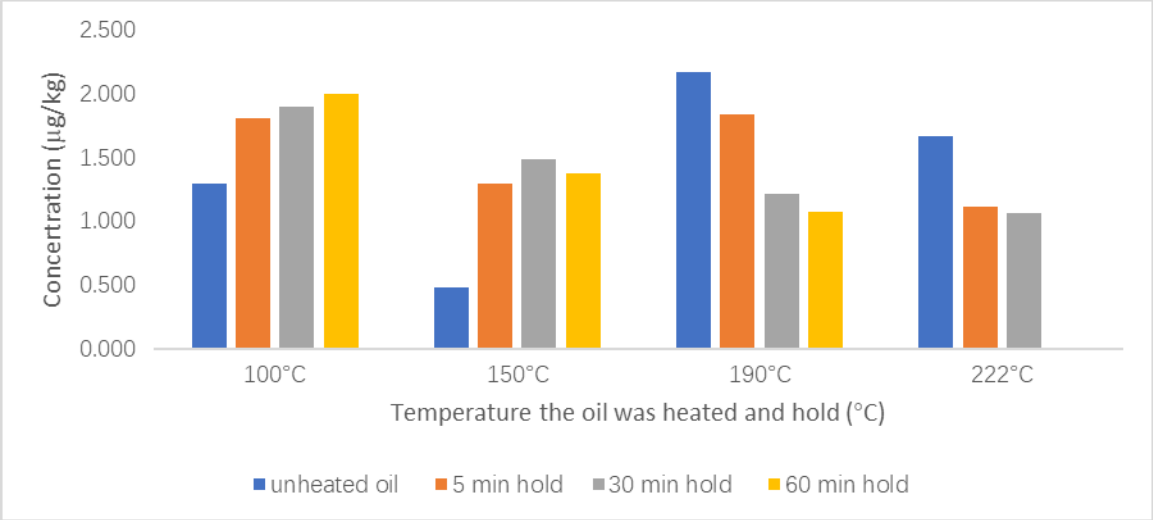


Figure 6: FLU level trends in Arawana Brand soybean oil after heated.

Table 8: PHEN levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	3.486	1.762	2.264	1.755
150°C	0.610	1.467	1.851	1.594
190°C	1.671	2.849	1.992	1.336
222°C	1.634	1.735		5.131

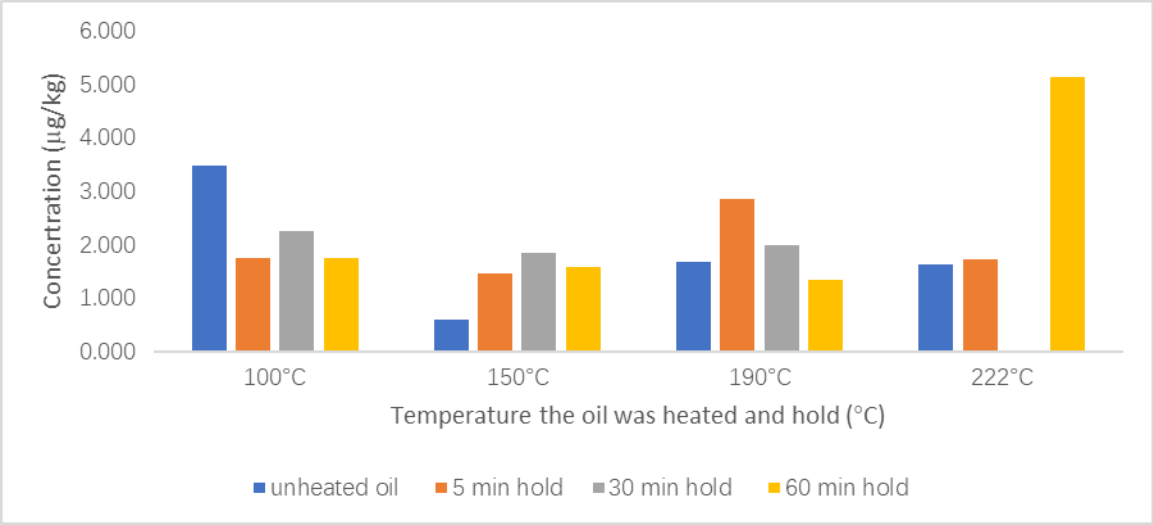


Figure 7: PHEN level trends in Arawana Brand soybean oil after heated.

Table 9: ANTH levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	0.776	0.494	0.499	0.500
150°C	0.391	0.453	0.484	0.505
190°C	0.478	0.533	0.505	0.484
222°C	0.480	0.541	0.592	1.778

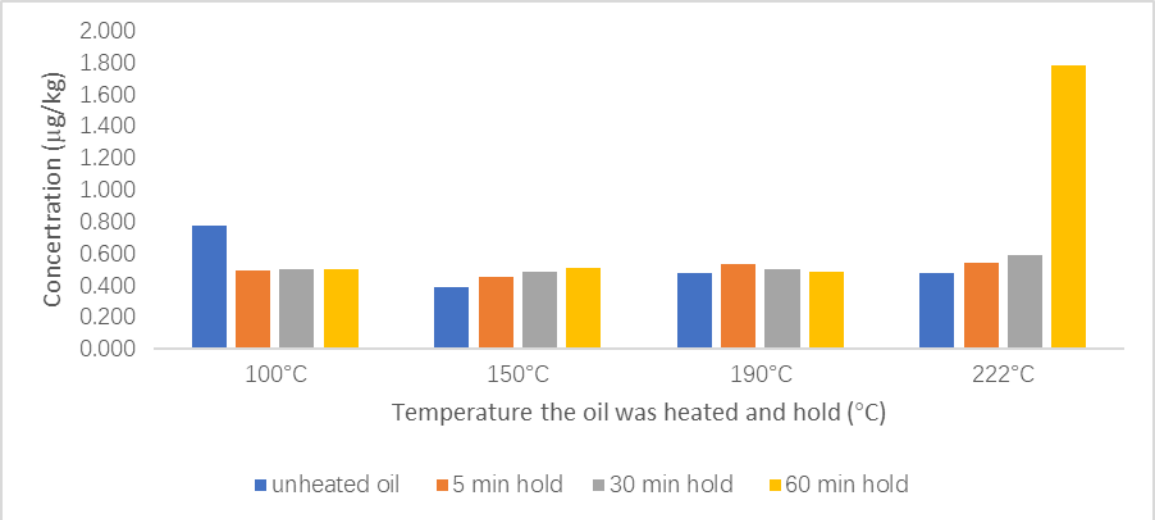


Figure 8: ANTH level trends in Arawana Brand soybean oil after heated.

Table 10: PYR levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	1.385	1.814	0.566	0.606
150°C	1.292	0.755	0.357	0.384
190°C	0.829	0.643	0.760	0.413
222°C	0.511	0.480	0.620	0.748

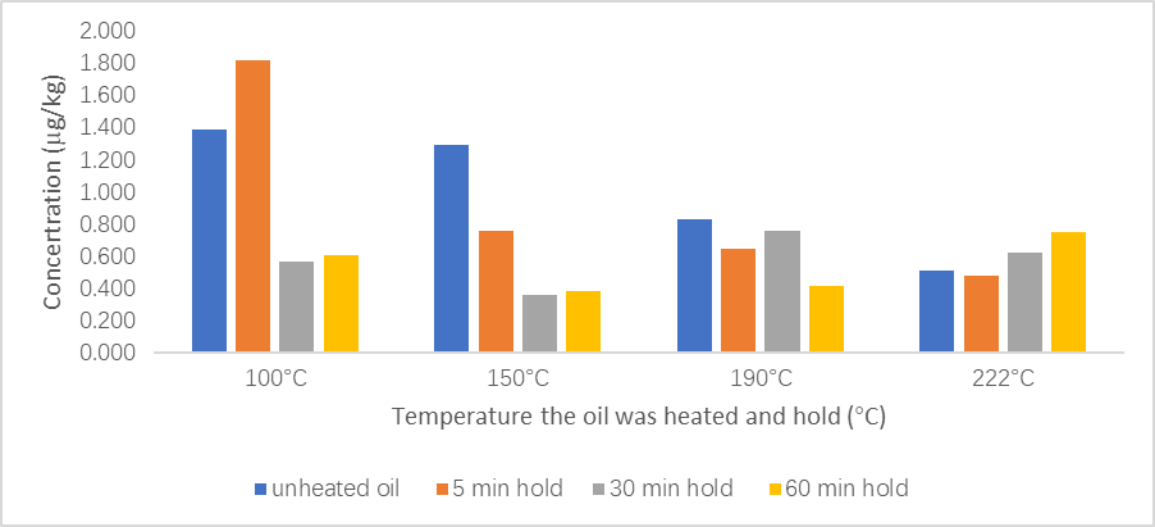


Figure 9: PYR level trends in Arawana Brand soybean oil after heated.

Table 11: B[a]A levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	0.982	1.070	1.111	1.237
150°C	0.586	0.803	0.930	0.952
190°C	1.229	1.219	1.407	1.043
222°C	1.075	1.137	1.313	1.309

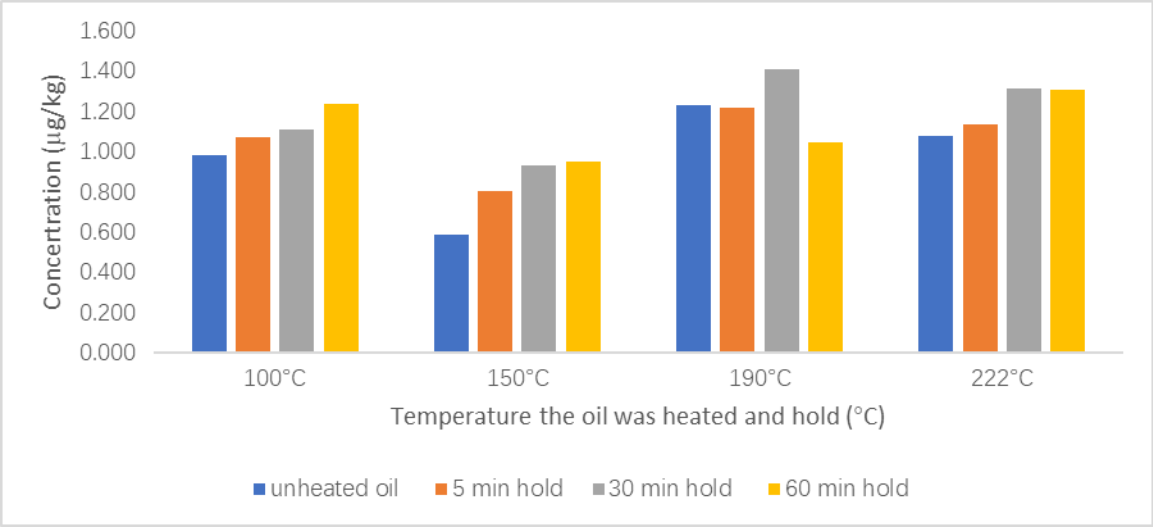


Figure 10: B[a]A level trends in Arawana Brand soybean oil after heated.

Table 12: CHRY levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	1.523	1.587	1.597	1.630
150°C	1.256	1.412	1.480	1.500
190°C	1.716	1.682	1.780	1.525
222°C	1.618	1.651	1.753	1.695

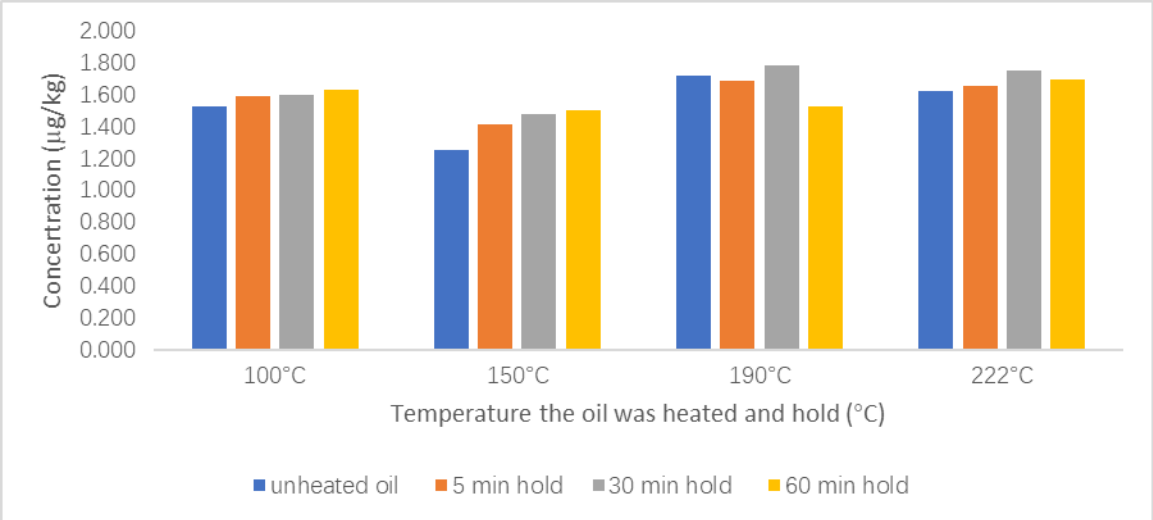


Figure 11: CHRY level trends in Arawana Brand soybean oil after heated.

Table 13: B[a]P levels in Arawana Brand soybean oil.

	Unheated oil (µg/kg)	5 min hold time (µg/kg)	30 min hold time (µg/kg)	60 min hold time (µg/kg)
100°C	0.856		0.982	1.859
150°C	0.743	0.805	0.863	0.874
190°C	0.937	0.972	1.092	0.901
222°C	0.885	0.921	1.019	1.167

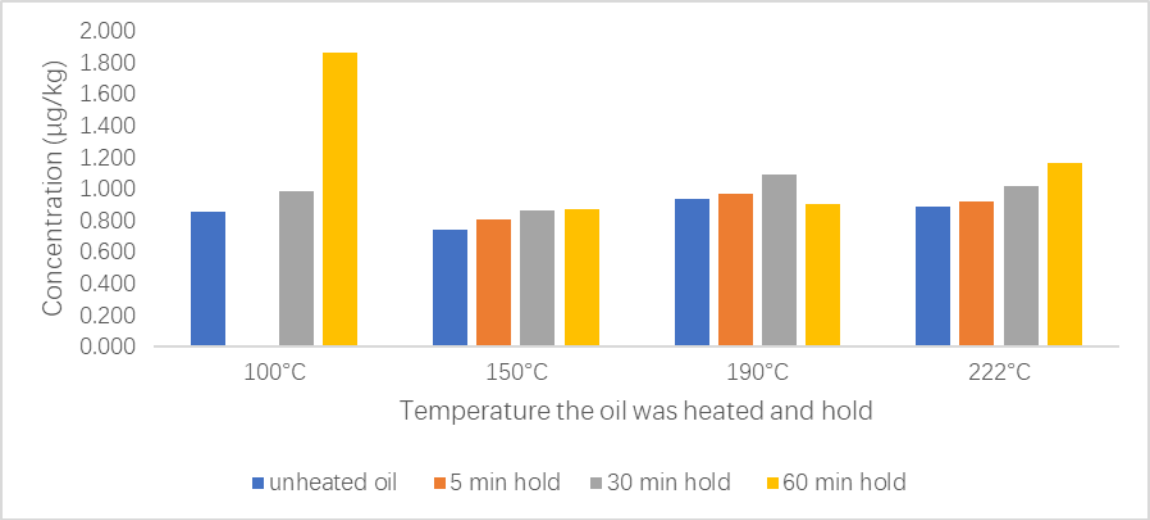


Figure 12: B[a]P level trends in Arawana Brand soybean oil after heated.

4. Discussion

4.1 Findings from the project and their explanation

Data of seven analytes, ACY, FLTH, B[b]F, B[k]F, IND, D[ah]A, and B[ghi]P, were considered not valid and were not used to find the law of their concentration change when the oil was heated. However, it does not mean that this null result is meaningless. These data were not valid due to their extremely low concentration. According to national standard in China, the maximum residue levels (MRL) for B[a]P are set at 10 μg/kg in edible oils (Wang & Guo, 2010). Extremely low concentrations of these PAHs in Arawana Brand soybean oil mean they are not reaching levels harmful to the human body when the oil is heated to any temperature between 100°C and 222°C within an hour.

NAP levels soar from unheated oil to the oil heated to 100°C and a hold time such as 60 minutes. This result is consistent with what Hao, Li & Yao (2016) had observed, which is a large increase in NAP concentration after the deep-frying process. This discovery does not match the statement that PAHs usually form at high temperatures (500–700 °C) while under lower temperatures (100-150°C), the formation of PAHs takes a "geological time-scale" period (Purcaro, Moret & Conte, 2013; Rose, 2010). If this statement is true, then cooking temperature and cooking time should not influence PAHs level too much because the highest recorded cooking temperature is

250°C, which is occasionally used to achieve effects such as a crispy crust. On the contrary, NAP formation turns out to be easy at temperatures as low as 100°C. Observing NAP data of oil heated to 150°C, concentrations maintain the rising trend but with a much gentle slope from unheated to reaching the target temperature and holding for 30 minutes. Regarding its boiling point, which is 217.97°C, vaporizing is a possible explanation for the gentle slope. NAP concentrations were kept at a low level when the oil was heated to 190°C, maintaining for 5 min and 30 min. So is the situation when oil is heated to 222°C. It is reasonable since most NAP should have vaporized at temperatures around 200°C.

There are four deviating members in this group of data. Explanation of two of them requires further analysis. The 150°C x 60 min data point, showing a sudden drop, is not in line with the former rising trend. The 190°C x 60 min one, which shows a sudden jump, differs from the former flat curve. Another two unusual numbers are from the unheated oil samples, which are supposed to be baselines for 190°C and 222°C samples to compare with. Data of these two points show extremely high NAP concentration. After recalling the operation process, the reason was the change of storage containers. The oil was weighed into plastic centrifuge tubes and stored in the 4°C refrigerator for a couple of days before started to extract. This explanation is supported by a supplementary experiment comparing the difference between two

samples of unheated oil stored in a glass bottle and a plastic centrifuge tube. Up to 100-fold NAP concentration was detected in the sample stored in a plastic centrifuge tube compared to the one stored in a glass bottle. Concentrations of the other 15 PAHs were found not to have a significant difference between samples stored in glass and plastic containers. Raw data of this supplementary experiment are provided in Appendix B.

For ACE, a moderate accumulation could be observed in the oil samples heated to 100°C with extended hold time. Specific reasons for the decrease in concentration from the 150°C x 30 min sample to 150°C x 60 min sample require further analysis. Data of 190°C and 222°C sample groups are not sufficient (data of 190°C x 30 min and 222°C x 30 min samples were not available) and not able to compare with each other. Considering their low concentrations, the difference between these samples may be caused by measurement error.

FLU could slowly accumulate in the oil samples heated to 100°C with extended hold time. The accumulation is not so obvious in oil samples heated to 150°C, but the concentrations are still higher than unheated oil. Concentrations fall in oil samples heated to 190°C and 222°C. The explanation for this phenomenon is that when the temperature is getting close to FLU's boiling point (295°C), the evaporation rate starts to exceed the generation rate.

PHEN and ANTH are isomers, which means they have the same molecular formula but with different structures. They both have a sharp concentration increase in their 222°C x 60 min samples compared to slightly fluctuating numbers of other samples. This phenomenon can probably be explained in terms of the increased formation of these compounds at this temperature and with a long heating time period. Confirmation of this explanation needs more supportive information.

The information provided by PYR data is complex, and a full explanation is not immediately obvious. Whether the PYR level in the oil has any significant change after heating requires further study.

B[a]A and CHRY are isomers. Their levels change similarly under the same experimental conditions. In oil samples heated to 100°C and 150°C, there is an increase in levels of both compounds compared to the non-heated oil sample. In oil samples heated to 190°C and 222°C, the concentration changes of both compounds are complex and slight. These changes, both ups and downs, are small. Whether these subtle changes are caused by measurement error or the joint action of formation and evaporation requires further investigation.

The pattern of increasing B[a]P levels in every temperature group mirrors the results of Hao, Li & Yao (2016) to some degree. According to their experiment, B[a]P content increased by 6.1- and 5.2-fold in deep-fried peanut and olive oils. This result is

much higher than the observation from this project which is less than double under all experimental conditions. The difference in the results between these two studies is probably caused by the potatoes and chicken nuggets Hao, Li & Yao (2016) used to fry.

B[a]P is a compound of great concern to scientists because it is classified as a Group A carcinogenic compound by the International Agency for Research of Cancer (IARC) for its established high carcinogenic character (IARC, 2002). It is used as a marker and a surrogate for PAH exposure in China (Shi, Zhang & Liu, 2016). B[a]P in the environment is considered to have high correlations with other carcinogenic PAHs and therefore is chosen to be representative (Bull & Collins, 2013). However, it turns out not so representative since different PAHs behave differently under identical experimental conditions according to the information obtained from this study. In other literature, a combination of several PAHs, such as PAH4 (including B[a]A, CHRY, B[b]F, and B[a]P) and PAH8 (which includes PAH4 along with B[k]F, D[ah]A, B[ghi]P, IND), were recommended as suitable marker substances of the presence and potential carcinogenic PAHs in foods (Shi, Zhang & Liu, 2016). However, the uneven distribution of different PAHs in heated soybean oil indicates that neither B[a]P alone nor any combination of several PAHs is representative enough to be the proper marker for the contamination level of PAHs in edible oils.

4.2 Implications for further research

Soybean oil is a member of the edible oil group. There are other edible oils widely used for various purposes. Different edible oils may produce different amounts of PAHs after being heated because of their different compositions and characteristics. The possible feature reflecting the oil structure and influencing PAH formation could be the degree of unsaturation. The higher unsaturation degree the oil has, the easier it is to produce benzene-like compounds (PAH precursors) due to the instability of its structure (Chen & Chen, 2001). Therefore, a suggestion for future research is to choose edible oils with a wide range of iodine value (indication of the degree of unsaturation) to provide comprehensive information on PAH levels in different oils under different degrees of heating.

This work showed that some PAHs, such as NAP, started to vaporize under medium cooking temperature such as 150°C and completely vaporize under temperatures around their boiling points. Therefore, future research needs to analyze gases produced by heated oil to better understand the amounts of PAHs produced when oil is heated.

This study showed that NAP accumulated rapidly when the oil was heated to 100°C. After the oil was heated to 100°C and held for 5 minutes, NAP level in the oil was more than 8-fold than that in unheated oil. After another 25 minutes, the NAP level

doubled. With another 30 minutes, it doubled again. In addition, NAP concentration also rises in 150°C data groups. Considering the result from Hao, Li & Yao (2016) that NAP was "especially prominent" in all the samples (all after the deep-frying process), it can be said that NAP is a compound that is very quickly formed and accumulated in the oil under normal cooking temperatures. When the oil is heated to higher temperatures, (190°C - 222°C), NAP vaporizes and enters the air and was not tested and added to the estimation of its total amount produced by heated oil. Therefore, it is safe to say that the amount of NAP determined in these two studies could still be underestimating the actual amount produced by cooking. In a real-world situation, some restaurants fry foods throughout the day. The amount of NAP produced during this process could be huge. However, with NAP being classified as Group 2B, possibly carcinogenic compound (IARC, 2002), it is rarely studied independently. Therefore, future research needs to pay more attention to the health effects of exposure to high NAP concentration.

4.3 Study strengths and limitations

This study focuses on PAH concentrations in heated soybean oil instead of fresh ones, reflecting the real-world situation and providing more real information on PAH levels in oil we might consume.

This study is not without limitation. With the influence of the pandemic, time to do the experiments was limited, so the project was not exquisite. For example, inter-day

precision was not good enough, so comparison between data from various days were avoided. Some of the calibration curves were not accurate enough, so data could be used only to compare relative relationship instead of accurate quantification. Lack of the use of an internal standard, which is a substance with a known concentration and is added to every sample analyzed, is also a reason for not accurately quantifying PAH concentration.

5. Conclusion

This study tested PAH concentrations in heated soybean oil. The results showed that concentrations of different PAHs in edible oil change differently when being heated. Therefore, B[a]P alone or a combination of several PAHs are not sufficiently representative of PAH exposure.

Adding up the valid PAH concentration data, with several of them increased at low cooking temperatures but decreased at higher ones, several increased sharply only at high cooking temperatures, and several increased slightly at all temperatures, the sum of PAH concentrations in heated oil did not show an obvious correlation with heating temperature and heating time. Taking evaporation, which caused several PAH concentrations to decrease at high cooking temperatures, into consideration, the overall amounts of PAHs generally increased at high temperatures with extended heating time. However, the exact increment was still unknown without quantifying the vaporized PAHs produced by the heated oil.

Appendix A

Table A.1: PAH concentrations in unheated soybean oil and in soybean oil heated to 100°C with 5 minutes hold time, 30 minutes hold time, and 60 minutes hold time.

	Unheated			Heated to 100°C, 5 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	29.1422	29.4155	31.0488	241.6209	249.4609	261.5701
ACY	8.7385	2.2951	7.871	13.1092	2.5033	7.871
ACE	6.9488	3.5638	7.0727	4.8748	5.1091	5.5216
FLU	15.2424	17.2341	15.8921	21.6938	22.9732	22.9272
PHEN	32.4623	41.0173	46.1217	20.7028	23.3392	30.6158
ANTH	9.5622	9.2409	10.2804	6.0012	34.8517	6.3566
FLTH	0	0	0	0	0	0
PYR	17.6359	15.8938	18.3957	21.5174	23.074	23.4521
B[a]A	12.2261	11.5538	13.0287	12.8905	13.5876	13.6372
CHRY	18.7923	18.6204	19.6961	19.7167	19.6929	20.1059
B[b]F	9.3569	17.5822	11.2541	15.5232	7.8905	12.64
B[k]F	12.9074	18.7755	13.3791	14.4932	11.7251	12.791
B[a]P	10.7029	10.67	10.7451	12.5875	9.4961	10.0094
IND	82.9943	78.2304	82.4799	87.8082	90.662	92.2502
D[ah]A	9.1323	9.1849				
B[ghi]P		9.3645	9.5343	9.5538	8.4759	
	Heated to 100°C, 30 min hold time			Heated to 100°C, 60 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	487.5288	495.6653	516.3745	978.2641	989.8884	1020.3974
ACY	4.0842	4.6404	26.7925	7.2101	23.5609	6.8263
ACE	6.5435	6.8224	6.794	8.2166	6.4879	7.0548
FLU	22.9381	23.5396	24.5764	24.9667	14.7136	17.655
PHEN	33.0291	33.1029	18.7749	55.5586	48.6884	21.935
ANTH	6.1587	6.278	6.286	6.3734	6.1553	6.2261
FLTH	0	0	0	0	0	0
PYR	6.7901	7.1034	7.3448	7.1649	7.8233	7.7329

B[a]A	13.7573	13.5991	14.3145	15.6908	15.0978	15.6028
CHRY	19.6842	19.9373	20.2685	20.6027	20.2457	20.2925
B[b]F	7.7709	12.079	12.62	9.8437	8.6498	12.502
B[k]F	12.2378	15.173	15.9766	14.3684	13.6885	15.0271
B[a]P	12.6243	12.0979	12.1004	22.7856	23.4799	23.4352
IND	70.2506	10.7018	10.7036	74.7224	77.9017	74.3052
D[ah]A				47.3295	46.4332	36.6691
B[ghi]P	9.5014	9.2739	9.7636		9.238	10.0521

Table A.2: PAH concentrations in unheated soybean oil and in soybean oil heated to 150°C with 5 minutes hold time, 30 minutes hold time, and 60 minutes hold time.

	Unheated			Heated to 150°C, 5 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	7.8716	8.0676	7.7759	33.7757	33.993	36.5832
ACY	5.5306	2.8967	1.0117	5.2207	8.7249	0.9468
ACE	0	0	0	4.6002	0.1496	3.6513
FLU	6.2005	6.0097	5.817	16.2983	17.2647	14.9743
PHEN	8.6963	18.0094	6.5484	16.3015	20.3821	10.9088
ANTH	13.9844	4.8555	4.914	23.6308	5.5084	5.8225
FLTH	0	0	0	0	0	0
PYR	16.7479	16.7242	14.9836	8.8065	9.8183	9.6754
B[a]A	7.4914	7.6342	6.8313	9.7695	12.2082	10.1494
CHRY	15.784	15.8677	15.4411	17.4379	17.7581	17.7517
B[b]F	6.4244	8.5628	8.3076	9.2871	9.3496	11.0458
B[k]F	12.4211	13.639	13.4936	12.4038	14.6041	13.6513
B[a]P	9.3316	9.1346	9.4093	10.0679	9.9538	10.1826
IND	6.9147	6.8981	6.7611	7.0733	6.6062	
D[ah]A		11.0953	10.5192			
B[ghi]P	8.9066	8.825	8.4524	9.0651	9.0176	9.0894
	Heated to 150°C, 30 min hold time			Heated to 150°C, 60 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	118.6101	121.1682	126.6797	6.7564	7.0092	7.0371
ACY	7.5227	3.127	6.8834	2.2855	13.0374	2.61
ACE	7.8951	5.619	7.982	5.7534	1.2276	1.1177
FLU	5.6445	18.5042	6.264	19.485	15.8888	16.0055
PHEN	9.2346	23.1431	9.1408	35.2315	18.5994	21.2528
ANTH	5.994	6.1291	6.0439	6.5856	6.2636	6.0981
FLTH	0	0	0	0	0	0
PYR	4.5529	4.4736	4.356	5.166	4.4235	4.8015

B[a]A	12.2609	10.9636	11.6449	12.1437	11.4769	12.0629
CHRY	18.1889	18.2541	19.0732	18.7915	18.5046	18.936
B[b]F	10.0297	8.8771	7.119	7.5925	11.4538	10.2216
B[k]F	14.2469	11.9425	11.9582	11.9435	14.8622	13.6012
B[a]P	10.399	11.0487	10.9178	10.3567	11.716	10.7014
IND	7.046	6.857	7.1067	6.9913	7.9467	6.8248
D[ah]A						
B[ghi]P	9.1333	9.1351	9.4436	9.2603	8.8883	8.9358

Table A.3: PAH concentrations in unheated soybean oil and in soybean oil heated to 190°C with 5 minutes hold time, 30 minutes hold time, and 60 minutes hold time.

	Unheated			Heated to 190°C, 5 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	161.466	129.6	164.3002	9.6405	4.1899	4.3476
ACY	20.68	14.8745	11.3279	0.8307	0.2338	0.9173
ACE	1.6788	0.1631	10.7197	0	4.5451	5.5535
FLU	29.0604	21.9543	30.4144	22.9104	19.6338	23.0448
PHEN	22.264	16.1667	24.2384	25.8881	36.6663	34.5503
ANTH	6.1427	5.5635	6.202	7.1367	7.2946	6.6641
FLTH	0	0	0	0	0	0
PYR	11.3944	6.8424	12.8553	7.8058	8.3639	7.93
B[a]A	16.0748	12.6525	17.3715	14.9218	15.1942	15.6111
CHRY	2.3682	19.3776	22.6077	20.815	21.168	21.0972
B[b]F	12.4826	8.0736	14.1264	8.1719	12.8767	7.6234
B[k]F	18.3977	11.8867	14.7841	13.4163	16.0957	13.104
B[a]P	11.9893	10.6953	12.4626	12.1881	12.1902	12.0535
IND	174.4911	130.1257	174.7523	126.576	136.7521	138.8443
D[ah]A						
B[ghi]P	9.5866	9.0059	9.8696	9.8372	9.5114	9.5987
	Heated to 190°C, 30 min hold time			Heated to 190°C, 60 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	3.8776	4.0437	3.9576	235.0165	268.875	258.5234
ACY	0.5895	0.0829	0.404	2.4426	2.4806	15.6699
ACE	0.7338	0.7624	0	3.0709	2.9446	2.595
FLU	15.6606	15.4911	14.4837	13.6518	13.1768	15.509
PHEN	24.8987	15.5547	27.9889	38.0757	17.8877	15.509
ANTH	6.388	6.229	7.0202	5.9847	6.1036	6.0604
FLTH	0	0	0	0	0	0
PYR	10.5337	9.1815	8.7875	5.1563	5.3653	4.9728

B[a]A	18.3026	17.7284	16.7207	12.8539	13.5684	12.7052
CHRY	22.8522	21.8603	22.0497	18.862	19.0953	19.2413
B[b]F	8.9646	10.1389	14.6082	10.741	7.5726	7.801
B[k]F	13.8678	14.5366	17.0818	21.4755	16.9024	13.2051
B[a]P	13.8861	13.7357	13.3124	11.0484	12.0525	10.6985
IND	205.7336	199.7441	192.2143	7.8475	6.8986	10.1913
D[ah]A	10.8584	11.2379				
B[ghi]P	10.4177	10.1996	10.4172	9.3333	9.2515	9.1433

Table A.4: PAH concentrations in unheated soybean oil and in soybean oil heated to 222°C with 5 minutes hold time, 30 minutes hold time, and 60 minutes hold time.

	Unheated			Heated to 222°C, 5 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	481.2627	477.7478	505.2182	17.3224	13.482	14.5809
ACY	18.7918	0.9742	27.3049	9.2866	2.6795	10.4975
ACE	4.9832	5.0032	5.3074	3.9757	0.7114	4.7602
FLU	22.8892	19.6752	19.827	10.8159	14.2928	16.618
PHEN	16.0126	22.7294	22.5295	21.6896	19.5698	23.8205
ANTH	5.9697	5.9904	6.0404	7.3376	6.2964	6.667
FLTH	0	0	0	0	0	0
PYR	6.2048	6.4886	6.4662	6.0185	4.9257	7.0613
B[a]A	12.883	13.6882	13.7366	14.1389	13.3336	15.147
CHRY	20.0833	20.3399	20.2434	20.9174	20.0255	20.988
B[b]F	11.4746	7.7468	11.4739	11.1377	7.5489	12.8675
B[k]F	15.1349	11.9244	16.2412	15.1053	13.0616	16.0905
B[a]P	10.8497	11.1368	11.2094	11.2683	11.1814	12.0849
IND	11.7412	106.1881	106.2377	102.5081	96.6444	110.086
D[ah]A	10.6353			9.1336	9.1222	10.7418
B[ghi]P	8.969	9.1327	8.8542	9.4195	9.2643	9.6132
	Heated to 222°C, 30 min hold time			Heated to 222°C, 60 min hold time		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	3.445	3.6876	3.5928	15.3988	16.1041	16.2378
ACY	17.6319	1.3201	0.8892	12.5114	9.3269	10.1545
ACE	0	0	0	4.2873	2.4785	2.227
FLU	11.9569	13.8375	14.1628	454.5376	465.7021	483.5729
PHEN	29.0161	40.1043	40.1802	48.4679	79.8194	26.2449
ANTH	7.4316	8.8697	7.3591	21.1248	22.5693	22.9818
FLTH	0	0	0	0	0	0
PYR	7.5215	7.9465	7.7956	9.7071	9.7532	8.5958

B[a]A	16.3985	16.4036	16.4494	16.1677	16.7075	16.2103
CHRY	21.8042	21.9546	21.9927	20.901	22.5456	21.4795
B[b]F	8.413	9.3653	9.9158	10.8308	8.7781	8.6775
B[k]F	13.5536	14.096	14.4095	12.9947	13.7615	12.9582
B[a]P	12.4978	13.6856	12.0372	15.1	13.6945	14.9692
IND	158.5641	169.8267	17.8252	110.6279	12.1186	106.8256
D[ah]A				9.5472	12.229	9.8471
B[ghi]P	10.7346	11.3102	9.6847	10.3164	9.5674	10.2478

Appendix B

Table B.1: PAH concentrations in two unheated soybean oil samples which are stored in a glass bottle and a plastic centrifuge tube.

	Stored in a glass bottle			Stored in a plastic centrifuge tube		
PAH	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)	First analyses (ng/ml)	Second analyses (ng/ml)	Third analyses (ng/ml)
NAP	3.4352	3.7635	3.8802	403.1878	355.7299	378.7211
ACY						
ACE	0	0	0	4.8606	5.7711	4.8766
FLU	6.6054	8.1784	8.1364	17.7321	17.1922	19.2907
PHEN	19.127	15.8573	11.0124	6.9878	30.9154	19.8553
ANTH	5.1165	5.221	5.0837	5.496	5.4869	5.2413
FLTH						
PYR	1.7428	0.8072	2.4301	2.0317	1.5763	2.141
B[a]A	14.6301	15.0866	15.6059	17.7724	18.0572	18.5915
CHRY	23.1967	21.6441	22.1227	24.9778	24.1471	24.4794
B[b]F						
B[k]F						
B[a]P	11.9896	12.7073	13.2791	12.4736	13.2258	13.5157
IND						
D[ah]A						
B[ghi]P	9.7036	9.7214	9.8648	10.145	9.9322	10.3218

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