Recycled palm oil is better than soy oil in maintaining bone properties in a menopausal syndrome model of ovariectomized rat

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Palm oil is shown to have antioxidant, anticancer and cholesterol lowering effects. It is resistant to oxidation when heated compared to other frying oils such as soy oil. When a frying oil is heated repeatedly, it forms toxic degradation products, such as aldehydes which when consumed, may be absorbed into the systemic circulation. We have studied the effects of taking soy or palm oil that were mixed with rat chow on the bone histomorphometric parameters of ovariectomised rats. Female Sprague-Dawley rats were divided into eight groups: (1) normal control group; (2) ovariectomised-control group; (3) ovariectomised and fresh soy oil; (4) ovariectomised and soy oil heated once; (5) ovariectomised and soy oil heated five times; (6) ovariectomised and fresh palm oil; (7) ovariectomised and palm oil heated once; (8) ovariectomised and palm oil heated five times. These oils were mixed with rat chow at weight ratio of 15:100 and were given to the rats daily for six months. Ovariectomy had caused negative effects on the bone histomorphometric parameters. Ingestion of both fresh and once-heated oils, were able to offer protections against the negative effects of ovariectomy, but these protections were lost when the oils were heated five times. Soy oil that was heated five times actually worsens the histomorphometric parameters of ovariectomised rats. Therefore, it may be better for postmenopausal who are at risk of osteoporosis to use palm oil as frying oil especially if they practice recycling of frying oils.

Key Words: palm oil, vitamin E, heated frying oils, ovariectomy, bone histomorphometry

INTRODUCTION
Palm oil is beneficial to health as it contains carotenoids, tocopherols and tocotrienols which have antioxidant, anticancer and cholesterol lowering effects.1,2,3 Studies have shown that palm vitamin E were able to prevent bone loss from FeNTA toxicity,2 hyperthyroidism,5 ovariectomy6 and orchidectomy.7 Palm oil is derived from the flesh of the fruit of the oil palm species Elaeis guineensis which is originated from West Africa. It has good resistance to oxidation and heat at prolonged elevated temperatures.8 Therefore, high percentages of palm oil are incorporated in frying oil blends for both performance and economic reasons. In many instances, palm oil has been used as replacement for the traditional hydrogenated seed oils such as soy oil.

Frying is the process of cooking food using heated oil as the heat transfer medium. Deep frying is a frying process where the food is completely immersed in the frying oil. It is a common practice in the household or in the commercial sector to use the same frying oil repeatedly to save cost. The oils would only be discarded if they produced smoke, foam and bad odour or if the colour had become darkened. These changes occur because during the process of frying, lipids especially polyunsaturated fatty acids (PUFA) undergo chemical reactions of oxidation, hydrolysis and polymerization. These complex series of chemical reactions lead to generation of degradation products, both volatile and non-volatile. The non-volatile products of degradation consisting of polymers and polar compounds remained in the oil.9,10 When frying oils are heated at 70°C, polar compounds like hydroperoxides and aldehydes are formed but when they are heated at 150 °C, aldehydes are mainly formed.11 Repeatedly heating of frying oils may also cause rancidity and destruction of vitamins and essential fatty acids.

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Fried foods may absorb some of the repeatedly heated frying oil along with their degradation products. When ingested, aldehydes may be absorbed by the gastrointestinal system into the systemic circulation. Aldehydes have been shown to be cytotoxic and are related to many degenerative illnesses. Increased cellular production of 4-hydroxynonenal and related aldehydes has been linked to pathophysiological effects associated with oxidative stress. Thus good frying oil would be able to withstand repeated heating with less formation of degradation products.

The use of repeatedly heated frying oils is an unhealthy practice. It has been linked to an increased risk of hypertension, disturbance of endothelial function and increased lipoprotein oxidation. There has been no report on the effect of using repeatedly heated frying oils on postmenopausal osteoporosis. Postmenopausal osteoporosis occurs in women with estrogen deficiency as a result of excessive bone resorption. The increase in bone resorption activities has been associated with an increase in oxidative stress. It is believed that the estrogen deficiency condition in postmenopausal women has lead to a decrease in thiol antioxidants, exposing the bone to oxidative damage. This was supported by a study which showed that women with postmenopausal osteoporosis have increased malondialdehyde levels and decreased superoxide dismutase levels.

This study concentrates on comparing the effects of repeatedly heated (recycled) palm and soy oil on bone histomorphometric parameters in an experimental model of postmenopausal osteoporosis (the ovariectomised rat).

**MATERIALS AND METHODS**

**Animals**

64 female Sprague-Dawley rats aged 3 months old (200-250g) were obtained from the University Animal House. The rats were divided randomly into 8 groups with 8 rats in each group. The first group was not ovariectomised and acted as the normal control group (NC). The second group is the ovariectomised control group (Ovx), while the rest of the groups were ovariectomised and given rat chow mixed with fresh soy oil (SOF), fresh palm oil (PO), oils heated once (SO1, PO1) or oils heated five times (SO5, PO5). The rats were treated daily for 6 months.

The rats were kept in cages in groups of three rats per cage at room temperature with 12 hour light and dark cycle. The rats were allowed to acclimatize for three days before they were given rat chow (Gold Coin, Selangor, Malaysia) mixed with fresh or heated soy or palm oil ad libitum. They have access to tap water ad libitum. All the rats except the first group were ovariectomised after anaesthesized with intraperitoneal injection of ketamine hydrochloride and xylazine at doses of 50 and 10 mg/kg body weight respectively. Bilateral ovariectomies were performed from a dorsal approach. There was no sham-operated group as the treatment period was long. The rat bones were fluorescence-labeled by injecting the rats twice with calcein at nine and two days before sacrifice. At the end of the study, the rats were anesthetized with ether and killed by cervical dislocation before the femurs were harvested. Success of ovariectomy was confirmed at necropsy by failure to detect ovarian tissue and by observation of marked atrophy of the uterine horns. This study was approved by the University Research and Animal Ethics Committee.

**Preparation of heated oils**

The soy oil or palm oil were not heated (SOF, POF) or heated according to modified methods of Owu et al., 1998. Briefly, the oils were used to fry fish crackers which were obtained from the same supplier, in a stainless steel pan for 10 minutes at 180°C. The oils were left to cool for five hours. This would be the once-heated soy oil or palm oil (SO1, PO1). To prepare oils that were heated five times, the procedure was repeated four more times. The oils were mixed with rat chow at weight ratio of 15:100 and were fed to the rats daily for six months. The oil: rat chow ratio represents the average amount of daily oil intake in human.

**Bone histomorphometry**

The distal portion of the femur samples were kept in 70% alcohol. For undecalcified samples, they were dehydrated and embedded in methyl methacrylate according to Difford, 1974. The block was sectioned at 10 μm with a microtome (Leica, Wetzlar, Germany) and stained with the Von Kossa stain. Bone samples were also decalcified with EDTA for 4 weeks and embedded in paraffin wax. The decalcified samples were sectioned at 8 μm with a microtome (Leica, Wetzlar, Germany) and stained with hematoxylin and eosin. The undecalcified samples were used to measure the structural parameters with an image analyzer (Nikon Eclipse 80i, Japan) while the decalcified samples were used to measure static parameters by using the Weibel Technique, a quantitative stereological technique for histological sections. The bones were decalcified for static parameter measurements to provide a clear background during staining so that the bone cells will be visualized easily. The bones labeled with the fluorescence marker calcein were examined with a fluorescence microscope and an image analyzer (Nikon Eclipse 80i, Japan) to measure dynamic parameters. All histomorphometric parameter measurements were performed at the metaphyseal region, which is located 3 to 7 mm from the lowest point of the growth plate and 1 mm from the lateral cortex. This secondary spongiosa area is rich in trabecular bone.

**Parameters**

Food intake and body weight were measured daily and weekly respectively. The structural parameters measured were bone volume per tissue volume (BV/TV %), trabecular thickness (TbTh, μm) and trabecular separation (TbSp, μm). The static parameter measured were osteoclast surface per bone surface (OcS/BS, %), osteoblast surface per bone surface (ObS/BS, %), eroded surface per bone surface (ES/BS, %), and bone formation rate per bone surface (BFR/BS, μm²/μm²/day). All the parameters were measured according to the American Society of Bone Mineral Research Histomorphometry Nomenclature Committee 1987.
**Statistical analysis**
The results were expressed as mean values ± SD. Data analysis was performed using SPSS for Windows software (SPSS Inc., version 12.0.1). Statistical test used was ANOVA followed by Tukey’s HSD (Honestly Significantly Different) for normally distributed data and Kruskal-Wallis and Mann-Whitney test for data that is not normally distributed. Changes were considered significance for *p* values less than 0.05.

**RESULTS**
There was no significant difference of food intake or body weight between the groups throughout the study (*p* > 0.05) (data not shown). In Figure 1 and 2, the density of bone trabecular at the secondary spongiosa which were stained black with Von Kossa for each group was

**Figure 1.** Bone trabeculae from rat femur were stained black with Von Kossa seen under the microscope (5X). A: normal control group; B: ovariectomised control group-reduction in the amount of bone trabeculae due to ovariectomy; C & D: the ovariectomised groups fed with fresh soy and palm oil – fresh oils restore the amount of bone trabeculae of the ovariectomised rats.

**Figure 2.** Bone trabeculae from rat femur were stained black with Von Kossa seen under the microscope (5X). E & F: ovariectomised groups fed with soy and palm oil heated once - partial restoration of the amount of bone trabeculae from the effects of ovariectomy; G & H: ovariectomised groups fed with soy and palm oil heated five times - the amount of bone trabeculae were similar or further reduced compared to ovariectomised control group (A).
compared. Grossly, trabecular density of ovariectomised group was reduced compared to the normal control group. Fresh palm and soy oil restored the trabecular density when they were heated once and five times, the trabecular density dwindles back to the pattern seen in ovariectomised group.

After six months, as expected, ovariectomy had caused negative effects on all the histomorphometric parameters (Table 1, Table 2 and Table 3). It reduced trabecular volume (BV/TV) (52.1%) and trabecular thickness (Tb.Th) (47.8%) but increased trabecular separation (Tb.Sp) (39.2%) for the structural parameters (Fig 3). It increased osteoclast surface (OcS/BS) (61.7%) and eroded surface (ES/BS) (52.3%) but reduced osteoblast surface (ObS/BS) (21.6%) for the static parameters (Fig 4). It increased single-labeled surface (sLS/BS) (43.1%) and reduced double-labeled surface (dLS/BS) (53.2%), mineralizing surface (MS/BS) (21.8%) and bone formation rate (BFR) (19.7%) for the dynamic parameters (Fig 5).

**DISCUSSION**

The term repeatedly heated frying or cooking oil has been used interchangeably with thermally oxidized oils or recycled oils. The heating process has changed the properties of the frying oil in terms of quality, colour, smell and taste due to presence of polymers and polar compounds. The repeated heating has oxidized the lipid content to potentially toxic lipid peroxidation products. Lipid hydroperoxides decomposed to highly cytotoxic products which are partly absorbed into the systemic circulation. Peroxyl radicals and aldehydes caused severe damage to membrane proteins, inactivating...
A great deal of attention on polar compounds has been focused on malondialdehyde, but there are other more noxious polar compounds such as 4-hydroxynonenal. Ideally, frying oils for human consumption must be discarded when the polar compounds concentration reaches more than 25

Figure 3. Effects of heated oils on structural parameters. a: significantly different to NC. b: significantly different to OVX. (p<0.05). NC: Normal control; Ovx: Ovariectomised control; SOF: Ovariectomised + fresh soy oil; SO1: Ovariectomised + soy oil heated once; SO5: Ovariectomised + soy oil heated 5 times; POF: Ovariectomised + fresh palm oil; PO1: Ovariectomised + palm oil heated once; PO5: Ovariectomised + palm oil heated 5 times. Value is expressed as mean ± SD. p<0.05 is considered to be significance.
In this study, we have compared the effects of ingestion of the two main vegetable oils used in the world, palm oil and soy oil, on bone histomorphometric parameters in ovariectomised rats. These oils were used to fry fish crackers, a well known delicacy in Malaysia. It was selected because fish is usually part of the daily meals of most households in Malaysia. Usually, the same oil was used several times to fry fish before it is discarded.

There may be questions raised on the probability that fish oil might get into the frying oil and affect the result of this study. Some studies have suggested the benefit of fish oil consumption on cardiovascular system and estrogen-deficiency bone loss. However, all the treated groups in our study had received frying oils that were used to fry fish cracker and therefore would be exposed to the same effects of fish oil, if there is any. Chicken or other food may be used instead, but fish was

Figure 4. Effects of heated oils on static parameters. a: significantly different to NC. b: significantly different to OVXC. (p<0.05).
NC: Normal control; Ovx: Ovariectomised control; SOF: Ovariectomised + fresh soy oil; SO1: Ovariectomised + soy oil heated once; SO5: Ovariectomised + soy oil heated 5 times; POF: Ovariectomised + fresh palm oil; PO1: Ovariectomised + palm oil heated once; PO5: Ovariectomised + palm oil heated 5 times. Value is expressed as mean ± SD. p<0.05 is considered to be significance.
selected as it was more representative of the local setting. There was no sham-operated group in this study. The purpose of sham is to expose the rat to the same surgical stress as the ovariectomised groups but since the duration of the study is long, the surgical stress that may influence the result would have subsided. Therefore the sham-operated groups were not included due to ethical reasons.

In this study, the ovariectomised rats, which are accepted models for postmenopausal bone loss,\textsuperscript{36,37} had shown deterioration in all the histomorphometric bone parameters. This was expected due to the increased bone resorption in estrogen deficiency. Estrogen deficiency has been shown to reduce the thiol antioxidants, exposing bone to free radical toxicity.\textsuperscript{19} Postmenopausal osteoporosis had also been associated with conditions of oxidative stress.\textsuperscript{20} In this study, we found that ingestion of heated oils further aggravate the negative effects of estrogen deficiency on bone.

Our findings have shown that soy or palm oil in the fresh form or heated once, when fed to ovariectomised rats, were able to prevent all the ovariectomy-induced changes on the histomorphometric parameters. Fresh and once-heated palm and soy oils had improved all the bone histomorphometric parameters except sLS/BS when compared to the normal control group. However when soy oil was heated five times, these protective effects were lost. In fact, it further deteriorates all the parameters in ovariectomised rats except for MS/BS by 24.6% to 63.7%.

Heating the soy oils five times had caused further increased in OcS and decreased in ObS in the ovariectomised rats. This could lead to higher bone resorption than bone formation and eventually the negative changes seen in the histomorphometric structural and dynamic parameters. However, this effect was not seen in the five times-heated palm oil group.

Palm oil seems to be able to withstand being heated five times better than soy oil. Although most of the protective effects on ovariectomy-induced changes were lost, it does not cause further deterioration of the histomorphometric parameters except for ES/BS. Some protective effects were still seen on dLS/BS and MS/BS. Therefore, palm oil may be a safer vegetable oil for postmenopausal women especially when the oil is repeatedly heated.

Previous studies have suggested that the unique composition of palm oil allows it to withstand heat better than soy oil. Firstly, it is rich in monounsaturated fatty acids (MUFA) but has low level of polyunsaturated fatty acids (PUFA) compared to soy oil.\textsuperscript{38} PUFA is more easily oxidised compared to MUFA.\textsuperscript{39} Repeated heating of vegetable oil high in PUFA results in formation of toxic compounds that increased the risk of hypertension.\textsuperscript{14} Whereas, oils that are rich in MUFA (oleic acid) such as palm oil and olive oil can better withstand oxidation and formed less degradation products when they are heated. Repeated

![Figure 5. Effects of heated oils on dynamic parameters. a: significantly different to NC. b: significantly different to O VX C. (p<0.05). NC: Normal control; Ovx: Ovariectomised control; SOF: Ovariectomised + fresh soy oil; SO1: Ovariectomised + soy oil heated once; SO5: Ovariectomised + soy oil heated 5 times; POF: Ovariectomised + fresh palm oil; PO1: Ovariectomised + palm oil heated once; PO5: Ovariectomised + palm oil heated 5 times. Value is expressed as mean ± SD. p<0.05 is considered to be significance.](image-url)
heating of olive oil generate less aldehyde compared to other oils.40,41

Secondly, vitamin E may play an important role in the oxidative changes. Inclusion of α-tocopherol to frying oil was found to render PUFA more resistant to oxidation.12 Rats fed on diet containing 15% oxidized frying oil (OFO) had significantly lower α-tocopherol concentrations in plasma and most tissues than rats fed on diet containing similar level of fresh soybean oil.42 These OFO-fed rats also had accelerated body α-tocopherol catabolism in a radioisotope study.53 Supplementation of vitamin E to olive oil was found to increase the stability of this oil under pro-oxidant conditions, and its intake was found to decrease the oxidative damage generated by adriamycin in rats.44 Vitamin E, which effectively protects fatty acids in the oil from oxidation deteriorate after each frying episode.45 Therefore, repeated heating of frying oils destroys the vitamin E content and exposes the fatty acids to oxidation. The vitamin E content of palm oil mainly consists of tocotrienols, while the main vitamin E in soy oil is tocopherols.46 Tocotrienols have better antioxidant capacity than tocopherols47,48 and this may contribute to the better resistance to oxidative changes due to repeated heating seen in this study.

In conclusion, repeatedly heated frying oils especially soy oil, may further deteriorate the ovariecotomy-induced bone changes in rats. Palm oil maybe more stable to heat than soy oil because of its unique composition of fatty acids and vitamin E. Therefore, it may be unhealthy for post-menopausal women to use repeatedly heated frying oils due to their negative effects on bone, but if this is not practical economically, then it may be better to use palm oil instead of soy oil.

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Effects of recycled oil on bone

Original Article

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回鍋棕櫚油在維持有更年期症狀卵巢切除的大鼠之骨質狀況較黃豆油佳

棕櫚油具抗氧化作用、抗癌及降低膽固醇之效力。它比起其他如黃豆油等炸油，在加熱時抗氧化作用。當炸油重複地加熱，會生成毒性降解產物像是醛類，在被攝取後可能被吸收進入系統循環中。我們研究卵巢切除的大鼠攝取混合黃豆油或是棕櫚油的大鼠飼料，對骨質組織型態學參數之影響。將母的Sprague-Dawley大鼠分成八組：(1)正常控制組；(2)卵巢切除控制組；(3)卵巢切除及新鮮黃豆油；(4)卵巢切除及加熱一次黃豆油；(5)卵巢切除及加熱五次黃豆油；(6)卵巢切除及新鮮棕櫚油；(7)卵巢切除及加熱一次棕櫚油；(8)卵巢切除及加熱五次棕櫚油。這些油脂與大鼠飼料以大鼠體重比為15:100的比例混合，每天給予此食物共六個月。卵巢切除對骨質組織型態學參數有負面影響。攝取新鮮及加熱一次的油可以提供保護作用，對抗卵巢切除術的負面影響，但是這些保護作用在加熱五次的油則消失。大豆油加熱五次確實使卵巢切除大鼠的組織型態學參數變得較差。因此，對於有骨質疏鬆危険性的已停經者而言，最好使用棕櫚油當做炸油，尤其是習慣使用回鍋油者。

關鍵字：棕櫚油、維生素E、加熱炸油、卵巢切除、骨質組織型態學。