# Evaluation of the antioxidant activity of citrus flavedo extracts in food systems

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**Abstract**

The worldwide trend to avoid the use of synthetic antioxidants has motivated scientists to unveil and exploit new sources of natural antioxidants. In this study, the antioxidant potential of the flavedo extracts of two locally grown citrus fruits namely, mandarin *fizu* and pamplemousse *kaopan*, was investigated in mayonnaise prepared from unstripped soybean oil and in bulk unstripped soybean oil. The antioxidant activity of the mandarin extract at two concentrations (0.2% (m/m) and 0.4% (m/m)) was compared to 0.02% (m/m) BHT. Hydroperoxide formation was monitored during storage at 45°C by measuring peroxide value (PV) and conjugated diene value (CD). PV values showed that the mandarin extract was as effective as BHT ($p>0.05$) and more effective than pamplemousse *kaopan* extract ($p<0.05$) in retarding lipid oxidation in mayonnaise during storage. After 12 days of storage, the CD values of the mayonnaise samples increased in the following order: mandarin extract < BHT < control < pamplemousse extract. Increasing the concentration of the flavedo extract of mandarin *fizu* to 0.4% did not increase the effectiveness of the extract in controlling lipid autoxidation in mayonnaise ($p>0.05$). In unstripped soybean oil, mandarin extract at 0.4% (m/m) was found to be less effective than BHT but more effective than the extract at 0.2% (m/m) ($p<0.05$). A similar picture was noted for the conjugated diene data. The findings of this research point to the value of mandarin *fizu* flavedo extracts as potential sources of natural food additives.

***Keywords:*** *Flavedo, mandarin, pamplemousse, mayonnaise, soybean oil, peroxide value, conjugated diene value, antioxidants.*

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## 1. INTRODUCTION

Unsaturated triacylglycerols in lipid containing foods are susceptible to lipid autoxidation which takes place by via a free radical chain mechanism (Coultate, 2009). The propagation step of the reaction leads to accumulation of unsaturated triacylglycerol hydroperoxides which are unstable and cleave into small volatile molecules having rancid odours and flavours (Belitz *et al*., 2009). These reactions eventually lead to a decrease in the nutritional value of the food product and affect its sensory characteristics (Campbell-Platt, 2009). For many years, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and propyl gallate have been effectively used as food additives to control lipid autoxidation in food systems. However, toxicological concerns together with consumer preference for natural ingredients have caused a transition to the use of natural antioxidants (Lőliger, 1991). Considerable interest and efforts have been put in finding natural antioxidants which can impact positively on the management of food quality. Numerous natural antioxidants with various activities have been identified and a lot of attention has been drawn to the addition of polyphenols to foods and biological systems. Fruits are important sources of phenolic compounds such as flavonoids, phenolic acids, and anthocyanins and do also contain many vitamins, which express antioxidant activity, for example, vitamin C, vitamin E and β - carotene (Macheix *et al*., 1990). Several experiments have reported the antioxidant activity of fruit juice and fruit pulp from edible fruits (Mokbel *et al*., 2006; Luximon- Ramma *et al*., 2003; Macheix *et al*., 1990). However, recently the antioxidant activity of fruit peels has gained in popularity with scientific reports that the antioxidant activity of fruit peel fractions is higher than pulp fractions (Jayaprakasha *et al*., 2005; Li *et al*., 2006). In the local context, the crude flavedo extracts of citrus fruits grown in Mauritius have been found to exhibit good antioxidant activity in several *in vitro* systems (Ramful *et al*., 2010). Crude flavedo extracts thus represent an interesting cocktail of natural antioxidants for prospective use as a food additive. Thus, in this study crude extracts of pamplemousse *Kaopan* and mandarin *fizu* were tested in mayonnaise and bulk soybean oil in order to generate data on their antioxidant potential for food applications. Results from this study could form the basis for further characterization and isolation of the active antioxidant compounds from the crude flavedo extracts and their investigation for antioxidant activity in food systems.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials and reagents

Citrus fruits (Mandarin *fizu* and Pamplemousse *kaopan*) were obtained from La Compagnie Agricole de Labourdonnais situated at Mapou, in the north of Mauritius. Fruits were harvested at the mature stage when they were ready to be placed on the market or ready for processing. After harvest, the fruits were rapidly processed on the same day. They were carefully washed under running tap water and patted dry. The flavedo of at least 10 fruits of each variety was carefully removed with a manual peeler and cut into small pieces. Weighed portions of the peripheral peel of pooled samples of each variety were lyophilised for 48 hours and the freeze-dried weight was determined. Samples were ground into a fine powder in a coffee grinder and stored in airtight containers at -4°C until analysed.

All the chemicals and solvents were of analytical grade and were purchased from reputable suppliers. The ingredients used for the preparation of food systems were bought from a local supermarket.

### 2.2. Preparation of extracts

The extraction procedure was adapted from Franke *et al*. (2004) and Chun *et al*. (2003). The freeze dried powders were weighed and exhaustively extracted in 80% methanol at 4°C for three consecutive days. The samples were centrifuged at 3000 rpm for 15 minutes and supernatants from all samples were pooled together. The clear extracts were concentrated at 50°C to 5 mL using a rotary flash evaporator (Stuart RE300 DB, UK). After evaporation, the concentrated extracts were transferred to a clean glass vial and stored at 4°C until required for preparation of food systems.

### 2.3. Assessment of antioxidant activity in mayonnaise

The method used to prepare mayonnaise was according to Ramsaha *et al.* (2013) and Jacobsen *et al*. (1999). All the ingredients were weighed in a beaker on an electronic balance (Mettler Toledo B 303-S, Switzerland). A methanolic solution of butylated hydroxytoluene (BHT) or citrus flavedo extracts was added to soybean oil to obtain 0.02% (m/m) BHT or 0.2% (m/m) citrus flavedo extract in the prepared mayonnaise. Egg yolk was transferred to a ceramic bowl, 30 drops of oil were added at a time using a pasteur pipette and mixed using an electric hand mixer (Philips HR 1456, China) for 10 minutes at a low speed. After 10 minutes beating, all the other ingredients were added and mixed for a further two minutes at high speed. Table 1 presents the % composition of the mayonnaise in terms of ingredients. Each batch of mayonnaise was divided into three equal portions and transferred to glass pots which were covered with parafilm and stored at 45°C in an incubator (Gallenkamp Sanyo, UK). Sampling of mayonnaise was carried out before and during storage to monitor hydroperoxide formation. 5 g samples were taken, frozen at -40°C, thawed and centrifuged at 3000 rpm for three minutes at room temperature, to separate the oil from the mayonnaise emulsion. Isolated oil fractions from mayonnaise samples were used for peroxide value (PV) (AOCS 1997a) and conjugated diene value (CD) (AOCS, 1997b) determinations.

**Table 1.** Ingredients used in the preparation of mayonnaise

| **Ingredients** | **% (m/m)** |
|---|---|
| Soybean oil | 68.54 |
| Egg yolk | 19.88 |
| Distilled water | 6.44 |
| White vinegar | 3.12 |
| Lime juice | 0.90 |
| White refined sugar | 0.77 |
| White refined salt | 0.28 |
| Potassium sorbate | 0.07 |

### 2.4. Assessment of antioxidant activity in soybean oil

Unstripped soybean oil was weighed into glass pots. A methanolic solution of BHT or citrus flavedo extracts was added to obtain 0.02% (m/m) BHT or 0.2% (m/m) citrus flavedo extract in the oil. Soybean oil with or without BHT/citrus flavedo extract was prepared in triplicates, covered with parafilm and stored at 45°C in an incubator (Gallenkamp, UK). Sampling of soybean oil was carried out before and during storage to monitor hydroperoxide formation. 5 g samples were taken and analysed for PV (AOCS 1997a) and CD (AOCS, 1997b).

### 2.5. Statistical analysis

Results are expressed as mean value ± standard deviation (n=3). The time taken (mean number of days) to reach a given peroxide value was determined from exponential curves generated by plotting peroxide value against time using Microsoft Excel. Single factor ANOVA and the least significant difference (LSD) test at 5% level were then applied to determine significant differences between mean number of days.

## 3. RESULTS

### 3.1. Antioxidant activity of citrus flavedo extracts in mayonnaise

Peroxide value (PV) of all samples increased with storage time (Figure 1). A sharp rise in PV was noted for the control mayonnaise after day 6. However, the increase in PV was lower in mayonnaise samples containing BHT or mandarin extract compared to samples with pamplemousse extract and the control, during storage for 12 days at 45$^{O}$C. The conjugated diene (CD) data, depicted in Figure 2, illustrate an overall increase in levels of CD for the control and mayonnaise containing BHT or pamplemousse extract. After 12 days of storage, the CD values of the mayonnaise samples increased in the following order: mandarin extract < BHT < control < pamplemousse extract. Table 2 shows that the mean number of days to reach a PV of 10 was significantly higher for mayonnaise containing BHT or mandarin extract compared to the control and samples with pamplemousses extract ($p<0.05$).

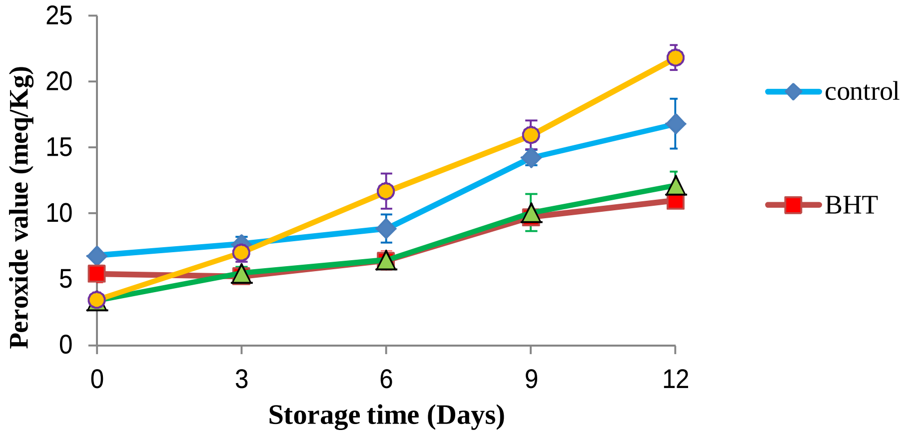


**Figure 1.** Effect of citrus flavedo extracts and BHT on the oxidation of mayonnaise prepared from unstripped soybean oil during storage at 45$^{O}$C, assessed by peroxide value.

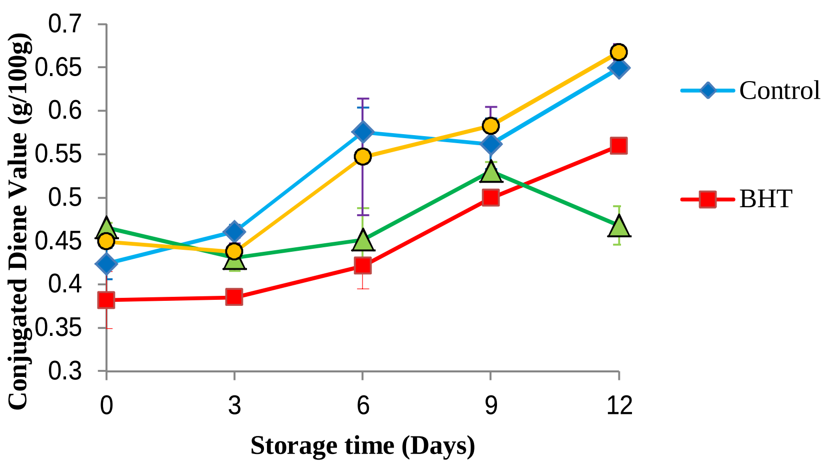


**Figure 2.** Effect of citrus flavedo extracts and BHT on the oxidation of mayonnaise prepared from unstripped soybean oil during storage at 45$^{O}$C, assessed by conjugated diene value

**Table 2.** Time taken for mayonnaise prepared with unstripped soybean oil to reach mean peroxide value of 10 meq/Kg during storage at 45ºC.

| Mayonnaise | Mean number of days |
|---|---|
| Control | $6.11 \pm 0.339^{b}$ |
| BHT – 0.02% (m/m) | $11.40 \pm 0.451^{a}$ |
| Mandarin *fizu* – 0.2 % (m/m) | $10.27 \pm 0.799^{a}$ |
| Pamplemousses *kaopan* - 0.2% (m/m) | $6.29 \pm 0.243^{b}$ |

Data represent mean ±: standard deviation (n=3). LSD = 1.645 at 5% level of significance. Different superscripts between rows represent significant differences between mean values. Control mayonnaise did not contain BHT nor citrus flavedo extract.

## 3.2. Antioxidant activity of two different concentrations of mandarin flavedo extract in mayonnaise

Mandarin flavedo extracts were tested at two different concentrations, 0.2% and 0.4% in mayonnaise. Figure 3 illustrates a slower increase in PV of mayonnaise samples containing BHT or mandarin extracts compared to the control mayonnaise, during storage for 19 days at 45$^{o}$C. A similar trend was recorded for conjugated diene values as seen in Figure 4. From Table 3, the mean number of days for PV to attain 7 meq/Kg was significantly higher for mayonnaise samples containing BHT or mandarin extracts than the control ($p<0.05$).

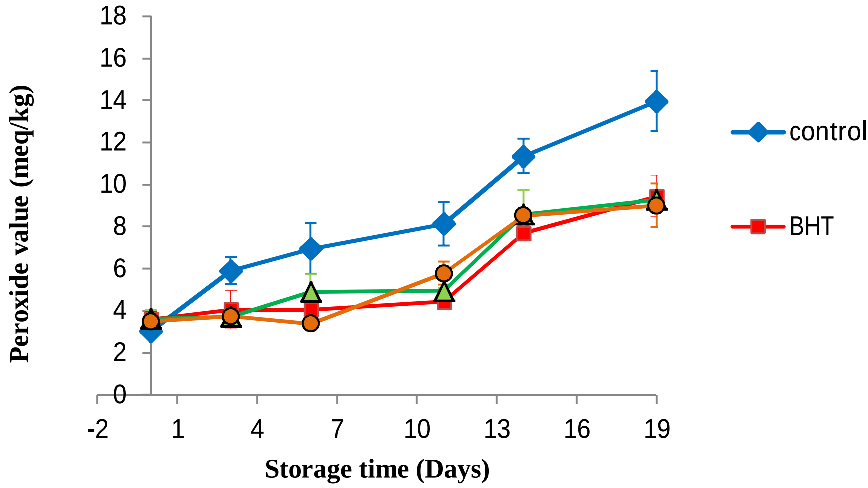


**Figure 3.** Effect of mandarin fizu flavedo extracts and BHT on the oxidation of mayonnaise prepared from unstripped soybean oil during storage at 45$^{O}$C, assessed by peroxide value.

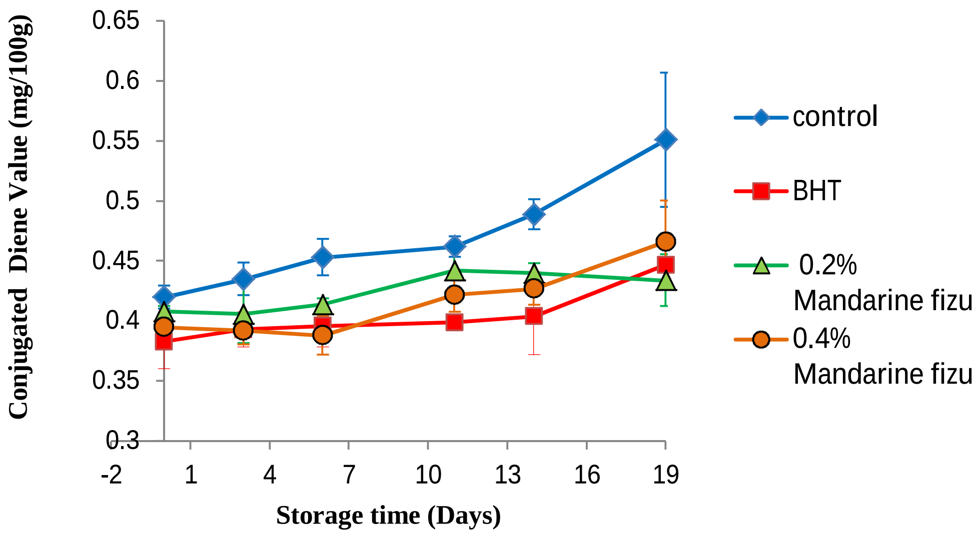


**Figure 4.** Effect of of mandarin fizu flavedo extracts and BHT on the oxidation of mayonnaise prepared from unstripped soybean oil during storage at 45$^{O}$C, assessed by peroxide value.

**Table 3.** Time taken for mayonnaise prepared with unstripped soybean oil to reach mean peroxide value of 7 meq/Kg during storage at 45ºC.

| **Mayonnaise** | **Mean number of days** |
|---|---|
| Control | 8.39 ± 0.228$^{b}$ |
| BHT – 0.02% | 14.70 ± 1.028$^{a}$ |
| Mandarin *fizu* – 0.2 % | 13.80 ± 1.110$^{a}$ |
| Mandarin *fizu*- 0.4 % | 14.35 ± 1.022$^{a}$ |

Data represent mean ±: standard deviation (n=3). LSD = 3.000 at 5% level of significance. Different superscripts between rows represent significant differences between mean values. Control mayonnaise did not contain BHT nor mandarin *fizu* flavedo extract.

### 3.3- Antioxidant activity of two different concentrations of mandarin flavedo extract in soybean oil

Figure 5 and Figure 6 present a slow increase in PV and CD of all soybean oil samples, up to 18 days storage at 45$^{O}$C. Between day 18 and day 25, the values increased more rapidly for the control compared to the other soybean oil samples containing BHT or mandarin extracts. From Table 3, the mean number of days for PV to attain 15 meq/Kg for soybean oil samples decreased in the following order: BHT > mandarin *fizu* (0.4%) > mandarin *fizu* (0.2%) > control (p<0.05).

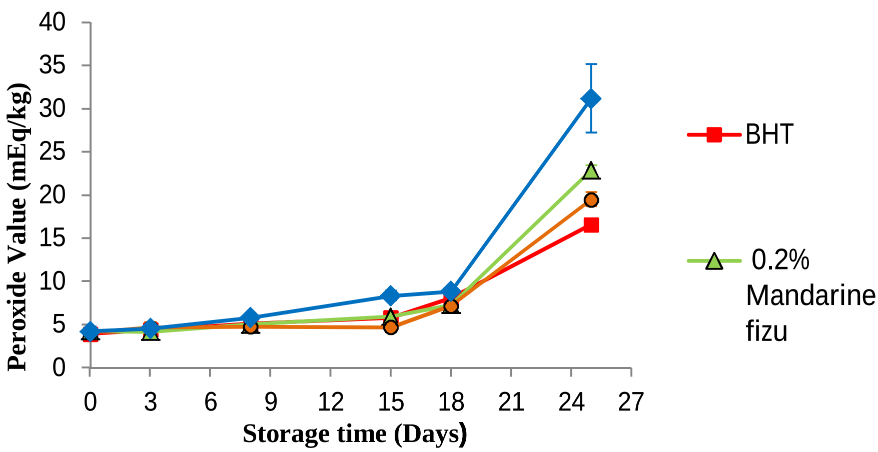


**Figure 5.** Effect of mandarin fizu flavedo extracts and BHT on the oxidation of unstripped soybean oil during storage at 45$^{O}$C, assessed by peroxide value.

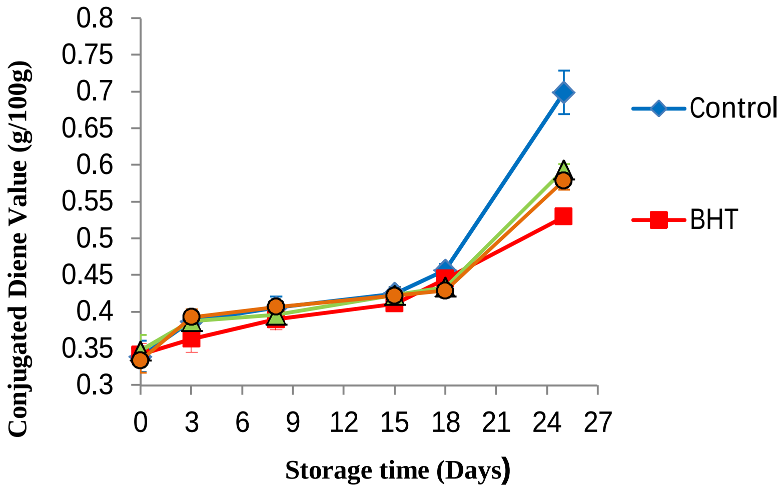


**Figure 6.** Effect of of mandarin *fizu* flavedo extracts and BHT on the oxidation of unstripped soybean oil during storage at 45$^{O}$C, assessed by conjugated diene value.

**Table 4.** Time taken for unstripped soybean oil to reach mean peroxide value of 15 meq/Kg during storage at 45ºC.

| **Soybean oil** | **Mean number of days** |
|---|---|
| Control | $19.30 \pm 1.191^{d}$ |
| BHT – 0.02% | $24.30 \pm 0.193^{a}$ |
| Mandarin *fizu* – 0.2 % | $21.60 \pm 0.346^{c}$ |
| Mandarin *fizu*- 0.4 % | $23.00 \pm 0.590^{b}$ |

Data represent mean ±: standard deviation (n=3). LSD = 1.306 at 5% level of significance. Different superscripts between rows represent significant differences between mean values. Control soybean oil did not contain BHT nor mandarin *fizu* flavedo extract.

## 4. DISCUSSION

Citrus peels, which are the primary waste fraction of citrus fruits, represent an important source of natural antioxidants as they contain numerous biologically active compounds such as phenolic acids and flavonoids (Bocco *et al.*, 1998). In fact, the peel, which constitutes roughly half of the fruit mass, contains the highest concentration of flavonoids in the citrus fruit (Manthey & Grohmann, 2001). Ramful *et al*. (2010) reported that the flavedo extracts of Mauritian citrus fruits represent good sources of bioactive phenolics with significant antioxidant propensities in various non-food systems. The present study aimed at investigating the antioxidant activity of the flavedo extracts of two Mauritian citrus fruits, mandarin *fizu* and pamplemousse *kaopan*, in two food systems namely, bulk soybean oil and mayonnaise prepared with soybean oil.

Flavedo extracts of mandarin *fizu*, at 0.2% and 0.4%, were effective in retarding lipid autoxidation in both mayonnaise and bulk soybean oil as monitored by the peroxide value and conjugated diene value. This protective effect to the oil systems could be attributed to the presence of flavonoid glycosides in the citrus extracts as reported by Ramful *et al*., (2010). Three types of flavonoids occur in citrus fruits: flavanones, flavones and flavonols. Several reports highlight the structure-antioxidant activity relationships of flavonoid subclasses in citrus extracts. Scientific evidence suggests that glycosylation, O-methylation and O-glycosylation influence greatly the antioxidant potency of citrus flavonoids (di Majo *et al*., 2005). It has been emphasized that antioxidant activity decreases with glycosylation and is enhanced with hydroxylation and the presence of C2-C3 double bond in conjugation with a 4-oxo function. (Rice-Evans *et al*., 1996). Apart from flavonoids in the flavedo extracts, other yet uncharacterised phytochemicals, might have also contributed to the overall antioxidant effect of the flavedo extracts in the two oil systems. Rehman (2006) reported that methanolic extracts of citrus peels were effective in stabilising corn oil stored at 25°C for a period of 6 months. A rapid decline in the PV (89.9-93.9%) of oil samples stabilised with citrus peel extracts at different concentrations was observed by the author.

Unlike flavedo extracts of mandarin *fizu,* extracts of pamplemousses *kaopan* were ineffective in inhibiting autoxidation in mayonnaise as evidenced by the peroxide and conjugated diene values which were not significantly different from control. This is quite an unexpected result as Ramful *et al*., (2010) reported that pamplemousse *kaopan* contain significant amounts of total phenolics and flavonoids comparable to mandarin *fizu*. One possible explanation for this weak observed antioxidant activity by the pamplemousse extract in the mayonnaise could be variations in extraction temperature used in the present study. Li and

Hossein (2006) reported that the main parameters that affected the yield of phenolics from citrus peels included the condition of the peels, temperature of the extraction, solvent concentration and species of citrus. A higher temperature of 80 °C was found to further increase the percentage extraction of total phenolics from citrus peels (Li and Hossein, 2006). Furthermore, a study on extraction of phenolic compounds from grape showed that a higher yield was obtained at 60°C than at 45°C (Giorgia *et al.*, 2007).

The observed trend in mean peroxide and conjugated diene values for unstripped soybean oil samples containing added mandarin *fizu* flavedo extract showed that antioxidative effect was concentration dependant. Mandarin flavedo extract at a concentration of 0.4% proved to be slightly better the extract at a concentration of 0.2 % in retarding oxidation in unstripped soybean oil ($p<0.05$). Recently, Bholah (2013) reported that methanolic leaf extracts (MLE) of *Moringa oleifera* Lam. at a concentration of 0.4% proved to be better than MLE at a concentration of 0.2% in retarding oxidation in both mayonnaise and unstripped sunflower oil. The work carried out by Siriwardhana and Jeon (2004) also indicated that an extract of cactus pear fruit exhibited higher antioxidant activity in oils and emulsions at a concentration of 0.1% than at 0.01%. Another study, testing citrus peel extracts in corn oil demonstrated that, as concentration of citrus peel extract increased, inhibitory effects on free fatty acid and peroxide value also increased considerably (Rehman, 2006). Hence, it can be inferred that, increased antioxidant effect with increasing concentration of extracts in the bulk oil test system could be attributed to the increased amount of antioxidant compounds added.

Apart from the concentration effect, polarity of antioxidant compounds is also influential in determining their antioxidant activity. In this study, extraction of polyphenols was performed on lyophilised citrus fruit flavedos using 80% aqueous methanol as the extracting solvent. Bronner and Beecher (1995) reported that citrus flavonoids were more soluble in methanol than in other solvents and that methanolic extraction yielded cloudy mixtures even following centrifugation, which could be corrected by addition of a small percentage of water to the extraction solvent. Use of 80 % aqueous methanol as solvent in the present study implies that the antioxidants extracted were a mixture of both polar and less polar compounds in nature, explaining the observed effectiveness of mandarin *fizu* extracts in protecting both mayonnaise and soybean oil against lipid oxidation. Citrus flavonoids have a wide range of polarity due to the presence of various functional groups According to the 'polar paradox' established by Porter (1993), polar antioxidants are more active in bulk oil systems than in emulsions since they have the tendency of orienting themselves at the oil-air interface, thereby conferring more protection against lipid oxidation. On the other hand, less polar antioxidants are more effective in oil-in-water emulsions as they are retained in oil droplets and/or may accumulate at the oil-water interface.

## 5. CONCLUSION

This study shows the potential antioxidant properties of flavedo extracts of locally grown mandarin *fizu* to retard autoxidation of food lipids in mayonnaise and soybean oil. However, further tests need to be carried out in other food systems to establish clearly their antioxidant potential and determine the active components.

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