

CHAPTER VI

PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED FERULATE ESTERS

6.1 Introduction

Today, there are ongoing attempts to use ferulic acid derivatives in several different areas of food (Manore et al. 2011), cosmetic (Di Domenico et al., 2009) and pharmaceutical (Suzuki et al., 2007; Shanthakumar et al., 2012) products due to their effective antioxidant properties by trapping and stabilizing radical species (Ergun et al., 2011). Transesterification with lipophilic molecules allows a better transportation of the compounds in different medium (Scapagnini et al., 2004) whereas selection of olive oil may improve the medicinal and cosmetic values (Viola & Viola, 2009) of the synthesized products.

Considering the promising results, this study was designed to delineate the profile for the synthesized ferulate esters. Mandloi et al. (2004) proposed that one of the most important stages to accomplish in the development of a new compound is the adequate physicochemical characterization, in order to determine the quality and help to describe the present conditions. Therefore, the physicochemical properties of synthesized ferulate esters were studied in term of sun protection factor (SPF) value, peroxide value, saponification value and iodine value.

6.2 Materials and Methods

6.2.1 Materials

Solvents and chemicals (ethanol, chloroform, potassium iodide, acetic acid, sodium thiosulfate, starch, potassium hydroxide, phenolphthalein, hydrochloric acid, Wij's solution) were purchased from Merck, Germany.

6.2.2 SPF Value

The procedure has been previously described by Dutra et al. (2004). 1.0 g of sample was weighed, transferred to a 100 mL volumetric flask, diluted to volume with ethanol, followed by ultrasonication for 5 min and then filtered through cotton, rejecting the ten first mL. A 5.0 mL aliquot was transferred to 50 mL volumetric flask and diluted to volume with ethanol. Then a 5.0 mL aliquot was transferred to a 25 mL volumetric flask and the volume completed with ethanol. The absorption spectra of sample in solution were obtained by using UV/Vis spectrometer (Perkin Elmer, Lambda 750) in the range of 290 to 450 nm using 1 cm quartz cell, and ethanol as blank. The absorption data were obtained in the range of 290 to 320, every 5 nm, and 3 determinations were made at each point, followed by the application of Mansur equation (Equation 6.1).

$$\text{SPF spectrophotometric value} = \text{CF} \times \sum_{290}^{320} \text{EE} \times \text{I} \times \text{Abs}$$

(Equation 6.1)

Where, CF = correction factor (=10)

EE = erythematous effect spectrum

I = solar intensity spectrum

Abs = absorbance of the sample

The values of EE x I are constants and showed in Appendix E.

6.2.3 Peroxide Value

About 5.0 g of sample was weighed into a 250 mL conical flask. 10 mL of chloroform was added and swirled to dissolve the sample. The mixture was then mixed with 15 mL acetic acid and 1 mL potassium iodide solution. The mixture was further leaved for 5 minutes in a dark place. Then, 30 mL of distilled water was added followed by 1 mL of starch indicator. The solution was titrated with 0.05 N of sodium thiosulfate until the blue color disappeared. The peroxide value is given by Equation 6.2.

$$\text{Peroxide value} = \frac{[(S - B) \times N \times 1000]}{W}$$

(Equation 6.2)

Where, S = volume of titrant (mL) for sample (ferulate esters)

B = volume of titrant (mL) for blank

N = normality of sodium thiosulfate solution (mmol/mL)

1000 = conversion of units (g/kg)

W = sample (ferulate esters) mass (g)

6.2.4 Saponification Value

2.0 g of sample was weighed into a 250 mL conical flask. About 25 mL of ethanolic potassium hydroxide solution was added. The mixture was then refluxed for 60 min. 1 mL of phenolphthalein solution was added and the mixture was titrated with the 0.5 N of hydrochloric acid until the pink color of the indicator just disappeared. The saponification value is given by Equation 6.3.

$$\text{Saponification value} = \frac{[(B - S) \times N \times 56.1]}{W}$$

(Equation 6.3)

Where, B = volume of titrant (mL) for blank

S = volume of titrant (mL) for sample (ferulate esters)

N = normality of hydrochloric acid (mmol/mL)

56.1 = molecular weight of potassium hydroxide (g/mol)

W = sample (ferulate esters) mass (g)

6.2.5 Iodine Value

The sample for about 2.0 g was weighed into a 500 mL stoppered flask and 10 mL of chloroform was added. After that, 25 mL of Wij's solution was pipetted into the flask, stoppered, and swirled to well-mix the mixture. The flask was then stored in the dark place for 30 minutes at room temperature and after that, 20 mL of potassium iodide (KI) was added followed with the addition of 100 mL freshly boiled and cooled distilled water. Then, the mixture was titrated with 0.1 N sodium thiosulphate solutions ($\text{Na}_2\text{S}_2\text{O}_3$) until the yellow colour almost disappeared. 1-2 mL of starch indicator was then added and the titration was continued until the blue colour just disappeared. A blank determination was carried out under the same condition. The iodine value is given by Equation 6.4.

$$\text{Iodine value} = \frac{[(B - S) \times N \times 126.9]}{W}$$

(Equation 6.4)

Where,

B = volume of titrant (mL) for blank

S = volume of titrant (mL) for sample (ferulate esters)

N = normality of sodium thiosulphate (mmol/mL)

126.9 = molecular weight of iodine (g/mol)

W = sample (ferulate esters) mass (g)

6.3 Results and Discussion

6.3.1 SPF Value

Level of sun protection on the skin has traditionally been estimated using the sun protection factor (SPF). Sunscreen products with high SPF value may provide more adequate protection against the sun (Latha et al., 2013). Gregoris et al. (2011) claimed that sunscreen products are classified in conformity with their SPF values as it follows: from 2 to less than 12 are defined as minimal sun protection products, from 12 to under 30 is moderate sun protection products while sunscreens with SPF values of 30 or above are defined as high sun protection products.

UV-Vis spectroscopy has been reported as a simple, rapid, and economic method in determining SPF values in many cosmetic formulations. This *in vitro* method is very useful as a screening test during product development before proceeding to the *in vivo* tests. In general, the method can be divided into two types: method that involve the measurement of absorption/transmission of UV radiation through sunscreen product films in quartz plates or biomembranes and method in which the absorption characteristics of the sunscreen agents are determined based on spectrophotometric analysis of dilute solutions (Dutra et al., 2004; Kaur & Saraf, 2010).

Khazaeli & Mehrabani (2008) have successfully applied this technique through dilute solutions in determining SPF values of sixteen plants dissolved in methanol;

Dracocephalum moldavica and *Viola tricolor* are found to have SPF values higher than 20 and suggested to be used in future topical sunscreens. Several nonvolatile and volatile herbal oils also being evaluated for their SPF values, where olive oil and coconut oil were revealed to have the best SPF values of 8 for nonvolatile herbal oil. On the other hand, peppermint oil and *tulsi* oil showed better SPF values of 7 than other volatile herbal oils which can be a potential candidate of perfumes during the formulation of sunscreens (Kaur & Saraf, 2010).

6.3.2 Peroxide Value

There are several methods for evaluation of antioxidative action of a sample (Becker et al., 2004). The present method was used for the assessment of early oxidative changes in the ester through formation of primary oxidation products (peroxides and hydroperoxides). Peroxide value is defined as the milliequivalents (mEq) of peroxide per kilogram of sample. It is a redox titrimetric determination. High quality of products will have a peroxide value of zero. Peroxide values >20 correspond to very poor quality which normally would have significant off flavours (Nielsen, 2003).

6.3.3 Saponification Value

Since the fatty acids are attached to the glycerol backbone with ester bonds, the saponification value reflects the number of ester bonds per gram sample. It is simply defined as the number of milligrams of potassium hydroxide required to saponify 1 g of

ester. Therefore, the saponification value is an important measurement to indicate the mean molecular weight of the sample's triacylglycerols (Wrolstad et al., 2005).

6.3.4 Iodine Value

The iodine value of an ester is used to measure the degree of unsaturation. The test measures the reaction of the double bonds with halogen, in this case iodine. Ekop et al. (2007) has reported that the higher the degree of unsaturation, the higher the iodine value. It expresses the concentration of the unsaturated fatty acids, together with the extent to which they are unsaturated, in a single number and therefore was a simple and very useful quality number parameter.

The properties of ferulate esters in terms of SPF value, peroxide value, saponification value and iodine value are displayed in Table 6.1.

TABLE 6.1: Properties of Ferulate Esters

No	Test	Unit
1	SPF value	16.0131
2	Peroxide value	17.52
3	Saponification value	191.3
4	Iodine value	88.02

6.4 Conclusion

Physicochemical profile of synthesized ferulate esters was well examined where medium SPF value and peroxide value were attained. This indicates the products synthesized have a good antioxidant value for further used.

