

## CORRELATION BETWEEN PHYSICOCHEMICAL PROFILES OF BLACK PEPPER SEED OIL FROM SOXHLET AND COLD PRESSING

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### ABSTRACT

This present study deals with the physicochemical profiles of black pepper (*Piper nigrum* L.) seeds oil which was determined by two extraction methods i.e., Soxhlet apparatus and Cold pressing. The physicochemical characterization of black pepper seed oil was saponification value, peroxide value, free fatty acid, acid value, specific gravity, ester, pH, iodine and wax. The mean values of physicochemical parameters of black pepper seed oil between two extraction systems were saponification value (64.4 to 109.39 mg KOH/g  $\pm$  31.81), peroxide value (8 to 17 meq O<sub>2</sub>/g  $\pm$  6.36), free fatty acid (3 to 9 mg/g  $\pm$  4.78), acid value (6.17 to 19.63 mg/g  $\pm$  9.51), specific gravity (0.840 to 0.849 g/cm<sup>3</sup>  $\pm$  0.006), ester (58.23 to 89.76 mg/g  $\pm$  22.29), pH (8.06 to 8.07  $\pm$  0.007), iodine (1.27 to 2.54 mg/g  $\pm$  0.89) and wax (3.18 to 4.9 mg/g  $\pm$  1.21). The results showed that black pepper seeds oil extracted by both extraction methods are strong association with each other and both method can be used for extraction of oil. Cold pressing method is more suitable procedure for the oil extraction without expenditure of solvent (n-hexane used in Soxhlet extraction). It also indicates that vegetable oils are non toxic to environment and biodegradable. Apart from its nutritional importance, fats and oils have major role to play in food cookery. They are almost indispensable when it comes to cooking food.

**Key words:** Physicochemical profiles, black pepper seed oil, Correlation, Saponification, Rancidity, Flavor reversion, Food cookery and Smoking point.

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### INTRODUCTION

Black pepper is commonly known as flowering vine belonging to family Piperaceae (Abbasi *et al.*, 2010). *Piper nigrum* L. is very precious due to presence of piperine including its various isomers (Zaveri *et al.*, 2010, Fan *et al.*, 2018). *P. nigrum* is commonly utilized formation of medicine in India, Latin America and West-Indies for its numerous medicinal values (Scott *et al.*, 2008). Black pepper can be utilized as human dietaries, medicine, preservatives and biocontrol agent (Awen *et al.*, 2010; Hussain *et al.*, 2011).

Black pepper utilized as food for prevention of the chronic ailments due to their physiological effects which is addition to its nutrition values (Sruthi *et al.*, 2013). Black pepper is also consumed for its antimicrobial, antioxidant, anticancer, gastro protective effect and anti-inflammatory (Butt *et al.*, 2013). Piperaceae family is good source of medicine and food species (Scott *et al.*, 2008, Murshed *et al.*, 2017). Plants are very important in our daily life and fulfill our needs (Szallasi, 2005).

In the recent study, sodium caseinate and chitosan-based composite edible films were developed with the incorporation of black pepper essential oil in various concentrations for potential food packaging applications (Bhatia *et al.*, 2024). The free radicals produced in our bodies which are responsible for many diseases (Ahmad *et al.*, 2011a). Free radicals produced in the body which is responsible for causing oxidation of lipid, reduction of fluidity of membrane, loss of receptor activities, degradation of different enzyme actions and cell inactivation by degradation of proteins present in membranes (Ahmad *et al.*, 2011b). Old people and most of herbal practitioners have firm belief that uses of peppercorn powder in green tea prevent asthma (Abbasi *et al.*, 2010; Ahmad *et al.*, 2010). Local people, herbal practitioners and herbal industries used peppercorn for treating diarrhea in all ages (Singh and Duggal, 2009).

Fruits of black pepper which is known as kali mirchi commonly used as pungent condiment and on the bases of pharmacognostic, phytochemical and physicochemical properties of black pepper fruit used as aphrodisiac, antipyretic, diuretic, immune stimulant, antioxidant, digestive, counter irritant, rubefacient, antiseptic and antispasmodic agent (Kadam *et al.*, 2013).

Different methods are used to extract oil or fat from plant source. These are chopping or rendering, cold pressing or crushing, solvent Soxhlet extraction and refining (Ferdosiet *al.*, 2021). With increased demands of vegetables oil and spice in the country, the investigation has been done on black pepper seeds as following aims

- To determine the physicochemical parameters of black pepper seed oil by Soxhlet apparatus and cold pressing.

- To correlate the efficiency and significance of physicochemical properties of black pepper seeds oil as affected by two extraction system

## MATERIALS AND METHODS

### SAMPLE COLLECTION AND PREPARATION

Black pepper seeds were collected from local market, Rawalpindi. These seeds were washed through water and dried under shade. After that the sample was grounded in powdered form with the help of grinder. The sample was stored in well plastic bags and placed in refrigerator at 4 °C for further examination.

### EXTRACTION OF BLACK PEPPER OIL THROUGH SOXHLET APPARATUS

The black pepper grinded sample was weighted and 15g of the sample was set in the filter paper thimble. The thimble was located in the loading compartment and extracted for about 6 hours in Soxhlet with 120mL of n-hexane which was placed in the 250 mL conical flask. The oil was transferred into the solvent and it was observed by changing in its colour (Quan *et al.*,2004).

When extraction had done with the help of Soxhlet, the n-hexane (solvent) was evaporated by using vacuum evaporator at 45°C. The oil was permitted to stand at room temperature for the evaporation of remaining n-hexane from the sample for 24 hrs.

The sample was measured to find out the amount of black pepper oil from 15g of black pepper seeds. The process was done for three times in the sake of accuracy and black pepper oil was saved at 4°C for further analysis.

### EXTRACTION OF BLACK PEPPER SEED OIL THROUGH COLD PRESSING

Black pepper seeds were pressed at room temperature by cold pressing machine without any thermal treatment. The seeds oil was collected in glass bottle for further analysis.

### PHYSICOCHEMICAL PROFILES OF BLACK PEPPER SEED OIL

Black pepper powdered sample had analyzed for physical and chemical parameters such as saponification value, peroxide value, free fatty acids, acid value, specific gravity, ester value, pH value and iodine value with the help of AOAC methods (AOAC,2003)

Wax content of the oil was calculated by (Gohari *et al.*, 2012). 5 g of black pepper oil was put in a flask and 25 ml of acetone will be added. Then after, solution mixture was settling down and put in freezer for 24 hrs at 4°C to crystal the wax. The acetone represented the insoluble part of the solution which was filtered on pre-weighed filter paper Whatman No.1. Filter paper was dried out in vacuum chamber at 45 °C and weighed to obtain the wax contents.

## RESULT

The physicochemical characterization of black pepper seeds oil had been determined which included saponification value, peroxide value, free fatty acids, acid value, specific gravity, ester value, pH value, iodine value and wax value which were reported in Table 1.

**Table 1.** Physicochemical profiles of Black pepper seed oil by Soxhlet and Cold Pressing.

Physical & Chemical property	Extraction methods		Mean Value Ranges
	Soxhlet extraction	Cold pressing	
Saponification (mg KOH/g)	64.4 ± 0.26	109.39 ± 0.51	64.4 to 109.39 ± 31.81
Peroxide value (meq O <sub>2</sub> /g)	8 ± 0.57	17 ± 0.57	8 to 17 ± 6.36
Free fatty Acid (mg/g)	3.10 ± 0.23	9.87 ± 0.50	3 to 9 ± 4.78
Acid value (mg/g)	6.17 ± 0.21	19.63 ± 0.36	6.17 to 19.63 ± 9.51
Specific gravity (g/cm <sup>3</sup> )	0.840 ± 0.005	0.849 ± 0.003	0.840 to 0.849 ± 0.006
Ester value(mg/g)	58.23 ± 0.16	89.76 ± 0.35	58.23 to 89.76 ± 22.29
pH value	8.06± 0.26	8.07± 0.19	8.06 to 8.07 ± 0.007
Iodine value (mg/g)	1.27 ± 0.15	2.54 ± 0.18	1.27 to 2.54 ± 0.89
Wax value(mg/g)	3.18 ± 0.15	4.9 ± 0.15	3.18 to 4.9 ± 1.21

Each value represents Mean ± S.D

The mean values of physical and chemical parameters of black pepper seed oil between two extraction systems were reported as saponification value (64.4 to 109.39 mg KOH/g  $\pm$  31.81), peroxide value (8 to 17 meq O<sub>2</sub>/g  $\pm$  6.36), free fatty acid (3 to 9 mg/g  $\pm$  4.78), acid value (6.17 to 19.63 mg/g  $\pm$  9.51), specific gravity (0.840 to 0.849 g/cm<sup>3</sup>  $\pm$  0.006), ester (58.23 to 89.76 mg/g  $\pm$  22.29), pH (8.06 to 8.07  $\pm$  0.007), iodine (1.27 to 2.54 mg/g  $\pm$  0.89) and wax (3.18 to 4.9 mg/g  $\pm$  1.21). The correlation and significance between chemical parameters of two extraction systems was determined by One Way ANOVA test and  $\alpha$  significance at 0.05 ( $P < 0.05$ ) as reported in Table 2.

**Table 2.** Correlation and significance of various physicochemical parameters by two extraction systems.

Parameters	Physicochemical parameters Mean values	One Way ANOVA Test ( $P < 0.05$ )	Status	Correlation	Remarks
Saponification (mg KOH/g)	64.4 to 109.39 $\pm$ 31.81	0.000	More Significant	1	Strong Association
Peroxide value (meq O <sub>2</sub> /g)	8 to 17 $\pm$ 6.36	0.000	More Significant	1	Strong Association
Free fatty Acid (mg/g)	3 to 9 $\pm$ 4.78	0.000	More Significant	1	Strong Association
Acid value (mg/g)	6.17 to 19.63 $\pm$ 9.51	0.000	More Significant	1	Strong Association
Specific gravity (g/cm <sup>3</sup> )	0.840 to 0.849 $\pm$ 0.006	0.000	More Significant	1	Strong Association
Ester value (mg/g)	58.23 to 89.76 $\pm$ 22.29	0.000	More Significant	1	Strong Association
pH value	8.06 to 8.07 $\pm$ 0.007	0.000	More Significant	1	Strong Association
Iodine value (mg/g)	1.27 to 2.54 $\pm$ 0.89	0.000	More Significant	1	Strong Association
Wax value (mg/g)	3.18 to 4.9 $\pm$ 1.21	0.000	More Significant	1	Strong Association

Each value represents Mean  $\pm$  S.D

The comparison of physicochemical properties of black pepper seed oil with reported values of the physicochemical properties of black pepper present in Bangladesh and India was reported in Table 3.

**Table 3.** Comparison of physicochemical properties of black pepper seed oil.

Physical Properties		Present study	Reported values (Aziz et al., 2012)	
			Bangladeshi Black Pepper	Indian Black Pepper
Organoleptic	Taste	Pungent	Pungent	Pungent
	Odor	Spicy	Spicy	Spicy
	Color	Slight green	Colorless	Slight Greenish
	Appearance at Room Temperature (30 °C)	Homogenous, transparent liquid	Homogenous, transparent liquid	Homogenous, transparent liquid
Specific gravity		0.84	0.84	0.84
Solubility	60% Alcohol	Opaque	Opaque	Opaque
	70% Alcohol	Hazy	Hazy	Hazy
	80% Alcohol	Slightly Hazy	Slightly Hazy	Slightly Hazy
	90% Alcohol	Soluble in 5 volume	Soluble in 5 volume	Soluble in 5 volume
	100% Alcohol	Soluble	Soluble	Soluble
	Distilled water	Not Soluble	Not Soluble	Not Soluble
	Chloroform	Soluble	Soluble	Soluble
	CCl <sub>4</sub>	Soluble	Soluble	Soluble
Diethyl ether	Soluble	Soluble	Soluble	
n-Hexane	Soluble	Soluble	Soluble	
<b>Chemical Properties</b>				
Acid value (mg/g)		6.17	3.75	4.26
Ester value (mg/g)		58.23	30.00	25.25

## DISCUSSION

### CORRELATION BETWEEN PHYSICO-CHEMICAL PROPERTIES

The value of Probability between different parameters of physicochemical properties of black pepper seed oil by Soxhlet and cold pressing is 0.000 which is less than  $P < 0.05$  which indicates the significance, efficiency and

stability of black pepper seed oil extracted by Soxhlet and cold pressing. The two different extraction systems are showed the positive correlation with each other in table 2. The positive correlation represents that there are strong relation between chemical parameters by two different extraction methods and it indicated that the extraction systems can not affect the efficient, significance and stability of the chemical characterization of black pepper seed oil. Both methods can be used for the characterization of vegetable oils.

The saponification value, peroxide, acid, free fatty acids and iodine of black pepper seeds oil is associated with the saponification value, peroxide, acid and iodine of Bangladesh (Hossain *et al.*, 2014) with slight variation. The specific gravity of black pepper seeds oil is linked with specific gravity of Bangladeshi black pepper as well as Indian black pepper seeds oil (Aziz *et al.*, 2012). The ester value of the black pepper seeds oil is associated with the Bangladeshi black pepper seed oil (Aziz *et al.*, 2012).

### SAPONIFICATION

The pH of black pepper oil also indicated that it has nature of alkalinity. The **saponification** is defined as the number of milligrams of potassium hydrate which is required to saponify completely 1g of the substance. It can be used in soap making due to high saponification value (Mir *et al.*, 2014). Oils or fats are hydrolyzed by the action of alkali, a mixture of alkali salts of fatty acids and glycerol is formed. Such salts of fatty acids are used in the manufacturing of soap. This process is called **saponification**.

The term “**unsaponifiable matter**” in fats and oils includes all those substances which are not soluble in water but soluble in light petroleum spirit. Its determination may be conventionally carried out on the neutralized solution produced after determining the saponification value. The result so obtained point to the presence of the mineral salts, waxes, etc.

### PEROXIDE VALUE

Peroxide value is to determine the oxidation of food stuff for the period of preservation and freshness of the fat content present in oil and is a good indicator of lipid oxidation products. It determined the lipid oxidation which responsible for the presence of saturated fatty acids in it. The elevated level of peroxide oxidation is responsible for the more oxidization of oil. High peroxide value of extracted oil predicted that the oil contained lot of free active oxygen species which involved autoxidation of the oil (Mowla *et al.*, 1990; Jacobs, 2006). The high peroxide value of black pepper oil is responsible for oxidative rancidity.

**Rancidity** is the deterioration (spoilage) of fats and fat containing foods due to lipid oxidation. Rancid foods and oils develop highly reactive chemicals which produced unpleasant and obnoxious odors and flavors, and destroy nutrients in food. Rancidity is accelerated in highly unsaturated fats and storage conditions conducive to chemical changes. Rancidity is of two kinds, hydrolytic rancidity and oxidative rancidity.

**Hydrolytic rancidity** is caused by the hydrolysis of fats into the free fatty acids and glycerol by the action of the enzymes. The enzymes bring about the decomposition of fats. It can be prevented by heating of the fat to destroy the enzyme and prevent hydrolysis.

**Oxidative rancidity** is caused by oxidation and the changes that accompany the reactions. It is a problem with only unsaturated fats and foods that have lipoxigenase. The reaction causing oxidative rancidity is a chain reaction and is self perpetuating. It does not require any catalyst.

### ACID VALUE

The acid value of a fat or oil is a measure of the free fatty acidity and is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in 1g of fat or oil. Acid value is to determine the quality of vegetable oils. Acid value defined as 1 g of oil required the amount of KOH in mg to neutralize free fatty acids present in it. The free fatty acids content is a usually determined the amount of total fat present in percentage mass-fraction. The high acid value of black pepper seeds oil indicated the higher proportion of the free fatty acids content as compared to other edible oils such as rapeseed oil (0.02 %), soybean oil (0.015 %), sesame seed oil (0.02 %) and palm oil (0.012 %), respectively (Mowla, 1990; Jacobs, 2006). High level of free fatty acid is sign of unfit of oil for utilization purpose. Black pepper oil would not appropriate for edible purposes. It could be refined before utilized for consumption.

### IODINE VALUE

The iodine value is defined as the number of grams of iodine that combine with 100 g of oil. It gives a measure of unsaturation in oil. Iodine value is determined by the known excess of Wij's solution (iodine monochloride in glacial acetic acid) to a solution of known weight of the oil. We used Wij's solution because iodine as such does not react with the unsaturated oils. The black pepper oil had low iodine value which also indicated that oil mostly contained unsaturated fatty acids. It is also indicated that unsaturated fatty acids do not allow close packing of the

molecules, there will be weaker molecular interactions present in the oils thus responsible for lower melting points. Therefore triglycerides of unsaturated fatty acids are liquid at room temperature.

Oils and fats are liquid or solids have a greasy touch. The oils are lighter than water (specific gravity less than 1) thus floating on the surface of water. The high specific gravity of black pepper indicated that it had fatty acids of high molecular weight.

### FLAVOR REVERSION

This refers to change in the flavor of fats or oils prior to rancidity. The reasons attributes to these changes which are as exposure to ultra violet light and visible light, heating of the fats or oils, the presence of oxygen for the reaction to occurs, triglycerides containing linolenic acid are precursor for flavor reversion reaction and traces of iron or copper which catalyze the reaction and acts as pro-oxidants. Metal in-activators or sequestering agents stabilize the flavor stabilize the flavor reversion.

### SMOKING POINT

Smoking point is that temperature of which a fat give off smoke from the surface of the fat or oil. The point at which one can see smoke coming out from the fat or oil surface is smoking point. Fats or oils are different in their smoking points and therefore different fats or oils are used for various purposes depending upon the highest temperature they have to be heated at. For frying purposes such fats or oils are used that do not smoke at the temperature used.

### FOOD COOKERY

Fats or oils are used as medium of cooking in a variety of preparations. The advantage of using fat or oil lies in the ease with which it heats up and its quality to transfer heat. It is better than water or air as it has a higher operating temperature and greater specific heat. Fat or oils used both for shallow and deep frying. Heating oil reacts with the food being cooked and either causes it leaching into the oil. Food cooked in oil, imparts the medium a color and cause darkening. Butter (fat) is an exceptional case which is not suitable for frying. The reason is the presence of water which restricts the cooking temperature and short chain esters which causes rapid breakdown and discoloration on heating.

Shallow frying uses moderate temperatures and so prevent smoking. On the hands, deep frying causing smoke and there is direct transfer of heat to food till food cooks. This causes loss of moisture from food surface and cooking of entire food by heat transfer to its interior. Therefore the outer surface is crisp and brown due to evaporation of water from the surface. Apart from its nutritional importance, fats and oils have major role to play in food cookery. They are almost indispensable when it comes to cooking food.

It was observed that comparison of physicochemical parameters of compressed black pepper seed oil is relatively higher than the Soxhlet apparatus oil. Compressed oil has high acid value which indicates that it is not fit for cooking purpose. It has less oxidative stability due to high peroxide value. That's why cold pressed black pepper oil was more susceptible for oxidation as compared to solvent extracted oil.

### CONCLUSION

The physicochemical parameters such as saponification value, peroxide value, free fatty acids, acid value, specific gravity, ester value, pH value and iodine value are used to control the quality of oil for the production of various products on commercial scale. It revealed that black pepper seed oil has used to make soaps, skin products, candles and other cosmetics. It also indicates that vegetable oils are non toxic to environment and biodegradable. Fats and oils have major role to play in food cookery. Further investigations may also be required to determine the essential oils (pleasant smelling liquids) in black pepper seed oil.

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