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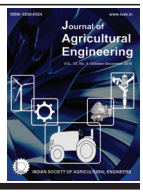

Effect of Enzymatic Aqueous Extraction on Quality Characteristics of Maize Germ Oil During Storage

Deepika Shende[1] and Gagandeep Kaur Sidhu[2]*

[1]Research Scholar, [2]Senior Research Engineer, Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana.*Corresponding author email address: gagandeep@pau.edu

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ABSTRACT

Enzyme-assisted aqueous extraction (EAAE) method is among the emerging technologies and eco-friendly process for oil extraction. The aim of this study was to evaluate the effect of oil extraction methods and packaging material on shelf life of maize germ oil. Maize germ oil extraction was done by EAAE and solvent extraction method. HDPE and glass bottles were used as packaging materials. Quality characteristics evaluated were acid number, pH value, total phenolics content, colour change, unsaponifiable matter, saponification value, peroxide value and density at 15-day intervals for 90 days of storage period. The quality characteristics of oil changed with the passage of storage periods, and maximum change upto 190 % increase in peroxide value occurred when oil was solvent extracted and stored in HDPE bottle. The quality parameters of EAAE extracted oil were superior to solvent extracted oil. During storage acid number, pH, total phenolics, unsaponifiable matter and peroxide value of oil were significantly affected ($P< 0.05$) by packaging material, storage period and method of oil extraction.

Fats and oils from any origin of animal, vegetable or marine represent the highest source of energy per unit weight that man can consume (Rauken *et al.,* 1997). Domestic production of edible oils was estimated at 9.98 MT, and 13.97 MT was imported to meet local demand making the total available oil during 2017-18 to 23.95 MT (Anon, 2018).Here is a need of an efficient oilseed processing industry as well as exploitation of non-conventional vegetable oils to bridge the demand-supply gap (Jha *et al.*, 2012). Increase in demand of edible oil was likely due to enhancement in income level, improvement of living standards and population in India (Narayan, 2016). Diversified use of maize adds higher value to its cultivation and production.

Maize is the third important cereal crop (after rice and wheat) in India (Shende and Sidhu, 2015a), and yields several useful products (Rajendran *et al.,* 2012). Wet milling process increases the nutritional and economic value of maize kernel by separating it into homogenous fractions, each having its specific identity and end use (Shende and Sidhu, 2016). Maize germ is a valuable by-product being rich in oil (Shende and Sidhu, 2015b).

Oil extraction from maize germ is conventionally done by solvent extraction (Abdulkarim *et al.,* 2005; Shende and Sidhu, 2015b). In 2001, the U.S. Environmental Protection Agency issued strict guidelines for hexane emissions by vegetable oil extraction facilities (Anon., 2001), providing new incentives to develop alternative methods of edible oil extraction. A number of aqueous (Tabtabaei and Diosady, 2013), aqueous enzymatic (Latif, 2009), and enzyme-assisted solvent extraction (Owusu-Ansah ,1997) methods have been developed; but the current consensus is that hexane extraction is still much less expensive than any of these alternative approaches (Shende and Sidhu, 2014). Enzyme-assisted aqueous oil extraction has emerged as an eco-friendly process for oil extraction (Mcglone *et al.,* 1986). The addition of specific enzymes during extraction enhances the oil recovery by breaking the cell wall and lipid bodies (Shende and Sidhu, 2016).

Oxidation constitutes a major factor for quality deterioration of oil (Pristouri *et al*., 2010). The rate of oxidation depends on a number of factors; including the availability of oxygen, presence of light and temperature

(Kanavouras and Coutelieris, 2006). Auto-oxidation, that is oxidation in the absence of light, follows a free radical mechanism where initially absorption of oxygen results in the formation of hydro-peroxides (Yousif and Haddad, 2013). Oil oxidation is considered a principal means of deterioration in the quality of foodstuffs. It imparts rancid and undesirable flavours to fat products. Oil oxidation also generates reactive oxygen species that are linked to carcinogenesis, inflammation, aging and cardiovascular disorders (Siddhuraju and Becker, 2003). Lipid oxidation also influences the chemical, sensory, and nutritional properties of edible oils and fatty foods, and thus plays an important role in determining their use and shelf-life (Anwar *et al.,* 2003).

This study was planned to evaluate the shelf life of maize germ oil extracted by different oil extraction methods, and to determine the effect of different packaging materials on acid number, pH value, total phenolics content, colour, unsaponifiable matter, saponification value, peroxide value and density.

MATERIALS AND METHODS

Plant Material and Chemicals

Fifty kg maize kernel of PMH-1 variety was procured from Punjab Agricultural University farm, Ludhiana, Punjab, India. The kernels were cleaned and graded to remove undesirable matters, and healthy kernels were selected. Maize germ was extracted batch-wise using manual wet milling method (Eckhoff *et al.,* 1993; Shende and Sidhu, 2016). Five kg of cleaned maize was batch steeped at 55 ºC for 18 h in steeping solution of 0.2 % sulphur dioxide (SO_2) and 0.55 % lactic acid (1,900 ml total solution) for germ separation (Eckhoff *et al.,* 1993). The sample was ground using a grinder (Make-Sujata 750 W, 1000 ml capacity jar) for 15 s, followed by batch-wise skimming process in 2000 ml capacity open steel container that allowed the germ component and other kernel component to be separated by density differences.

Skimmed germ was dried in a hot air tray dryer (Model-012B, Kilburn Oven, Macneill & Magor Ltd., India) upto 5 % moisture content (w.b.). About 450 g dried germ was obtained from 5 kg maize kernel, which was stored in a refrigerator at temperature of 4-5 ºC for further use. Petroleum ether (60-80 ºC) solvent was procured from LOBA CHEMIE Pvt. Ltd., Ludhiana, India, and enzymes (Cellulase and Protease) were obtained from Himedia Pvt. Ltd., Ludhiana, India.

The experiment was conducted in the Department of Processing and Food Engineering, PAU, Ludhiana in the year 2016.

Extraction of Maize Germ Oil

Solvent extraction method

Germ oil was traditionally extracted with soxhlet apparatus with electric heater (JSGW combine jheldhal dig and distill unit with glass parts, 3*500 W, attached with Allihn reflux condenser)as shown in Fig. 1, using petroleum ether as solvent. About 12 g of dried and ground maize germ sample was weighed and filled into the empty thimble. The soxhlet apparatus electro-thermal heating mantle was set at temperature of 40 ºC for 3 hours (Fig. 2). On completion of oil extraction, the

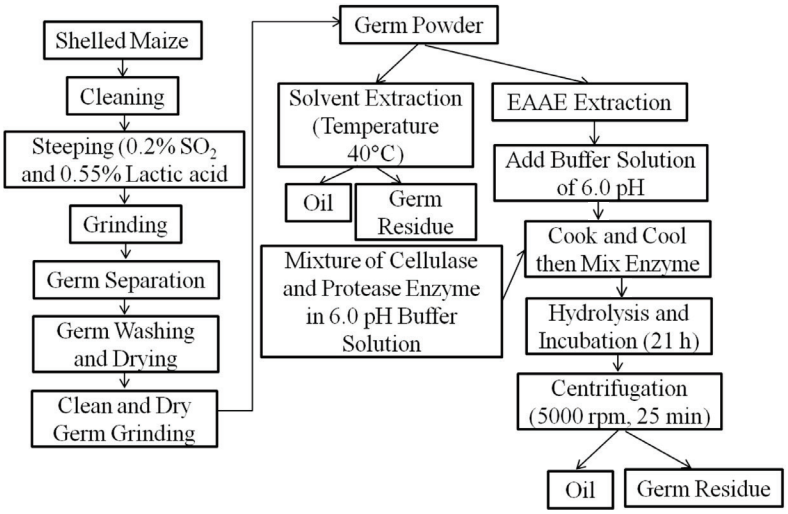

Fig. 1: Process flow diagram for extraction of maize germ

residue was removed from the thimble, and the oil was heated for 20 min at 50 ºC to enable residual solvent to escape (Abdulkadir and Abubakar, 2011; Shende and Sidhu, 2015b).

Fig. 2: Maize germ oil extraction using solvent extraction process

Enzyme assisted aqueous extraction (EAAE) method

Maize germ oil was extracted using enzyme assisted aqueous extraction (EAAE) method as shown in Fig. 3. The optimized results for EAAE maize germ oil extraction to yield maximum oil from slurry were 6.0 pH of slurry, 1:7 seed to water ratio, 45 ºC temperature of incubation and 1 h time of hydrolysis (Shende and Sidhu, 2016).

About 40 g of ground germ was mixed with distilled water at seed-to-water ratio(s/w) ratio of 1:7. The mixture was cooked [Fig. 3(a)], and allowed to cool down to room temperature [Fig. 3(b)] (Abdulkarim *et al.*, 2005). The pH of cooked slurry was adjusted at 6.0 with the help of 0.5 N NaOH and 0.5 N HCl. After the slurry was cooled, enzyme buffer concentration (0.50 % seed weight basis) was mixed with it. The mixture was stirred or hydrolyzed for 1 h and incubated for additional time of 20 h at temperature of 45 ºC in an incubator (Orbital shaking incubator-cum-BOD incubator, Model-256, Narang Scientific Work Pvt. Ltd., India, range = 5 ºC to 80 ºC), Fig. 3(c). After incubation, the mixture was centrifuged for 20 min using a hot centrifuge machine (R8C, Model- BHLO 878, Remi Motors, India), Fig. 3(d). The oil phase floating on the top was collected. The EAAE method extraction results were compared with traditional solvent extraction method.

Fig. 3: EAAE maize germ oil extraction (a) cooking of ground germ in distilled water, (b)cooling of cooked slurry, (c) hydrolysis of enzyme buffer solution with cooked slurry, and (d) centrifugation of processed slurry

Determination of Physico-chemical Properties of Maize Germ Oil

Different physico-chemical characteristics of conventional and EAAE extracted germ oil viz. acid number, pH value, total phenolics content, colour change, unsaponifiable matter, saponification value, peroxide value and density were determined using standard methods.

The extracted oil was packed in HDPE and glass bottles of 30 ml capacity to study the shelf life of oil. Oil was packed in ten bottles of HDPE and glass material for each treatment as shown in Fig. 4. The dimension and permeability values of bottles are given in Table 1. About 20 ml of oil was filled in each bottle. The average temperature and humidity during the months of storage (January to March) were 12.8-19.4 ºC and 74-62 %, respectively. The different quality characteristics of samples stored in different stored bottles were evaluated at regular interval of 15 days for a total period of 90 days under ambient condition.

Acid number

Oil sample of 4.0 ml was pipetted into a 250 ml conical flask, and 25 ml of 95 % alcohol was added to it. The flask with the content was boiled, and then titrated with N/10 potassium hydroxide to a faint pink colour using phenolphthalein as indicator (FSSAI, 2012).

$$\text{Acid number (mg KOH.g}^{-1}) = \frac{5.6 \times (V_1 - V_2)}{W_1} \quad \ldots (1)$$

Where,

V_1= Volume of potassium hydroxide solution required for sample, ml,

V_2= Volume of potassium hydroxide solution required for blank, ml, and

W_1= Weight of sample, g.

pH value

The pH of an oil sample was measured using a pH meter (pH Analyser, Remi R8C, Elico Limited Li – 614, range: 0-14, readability: 0.001, accuracy: ±0.001). The pH meter was calibrated with 7 pH buffer using manual setting at 20 ºC, and the electrode was kept dipped in buffer solution during further pH measuring process (Shende and Sidhu, 2015b).

Total phenolics content

Total phenolic content was determined using the modified Folin- Ciocalteu procedure (Chaovanalikit and Wrolstad, 2004; Tuan, 2011). Oil (0.5 ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent (Loba Chemie Pvt Ltd, Mumbai, India) and 7.5 ml deionized water.The mixture was held at room temperature for 10 min and then 1.5 ml of 20 % sodium carbonate (w/v) was added. The mixture was measured in a spectrophotometer (Rayleigh UV – 2601) by the absorbance at 755 nm for total phenolics (Shende and

Fig. 4: Storage of maize germ oil

Table 1. Dimension and permeability values of storage bottle

Sl. No.	Parameter	Bottle	
		HDPE	Glass
1.	Height, mm	95	50
2.	Diameter, mm	35	23
3.	Thicknenss, mm	1.0	1.5
4.	Cap height/dimeter, mm/mm	15/25	15/24
5.	O_2 Permeability, $cm^2.m^{-2}$-24h-bar	1100	0.03
6.	Water permeability, $g.m^{-2}$-24h	0.3	0.01
7.	Light transmission (400-700 nm wave length), %	10-40	78-86

(References: Coltro and Borghetti, 2007; SABIC, 2013; Shimadzu, 2015)

Sidhu, 2016). The results were expressed as mg of gallic acid equivalent (GAE) per kg of fresh weight.

Colour

The colour of oil was determined using a Colour Reader CR-10 (Konica Minolta Sensing Inc.) colourimeter (Hunter, 1975). 'L', 'a' and 'b' values were recorded at D 65/10º. The change in colour during storage was measured by the equation given by Gananasekharan *et al.* (1992). The chroma (saturation or vividness) value determined the colour chromaticity, and hue (tint of colour) value determined an angular measurement in which 0º equal to red and 90º equal to yellow (Itle and Kabelka, 2009).

Unsaponifiable matter

Unsaponifiable matter was determined by the method prescribed by FSSAI (2012). It was defined as all the substance after saponification and later by diethyl either followed by potassium hydroxide extraction present in oil, which are not volatile under the specified operating conditions (Abdulkadir and Abubakar, 2011; Shende and Sidhu, 2016). This method uses diethyl ether, extraction. Two grams of the sample was placed into a 500 ml conical flask containing 50 ml of 1M solution of ethanolic potassium hydroxide. This was refluxed for 1 h using an electric heater (JSGW combine jheldhal dig and distill unit with glass parts, 3*500W) attached with Allihn reflux condenser. One hundred ml of water was added through the top of the condenser with continuous swirling of the flask. The flask was then allowed to cool and the contents transferred into a 500 ml separating funnel. The conical flask was rinsed by using 100 ml of diethyl ether. Stopper was inserted into the separating funnel and then inverted after vigorous shaking.

The content was allowed to completely separate into two phases. The lower layer was then completely run-off into a second separating funnel, and washed until the solution no longer gave pink colour on addition of a drop of the phenolphthalein indicator. The ethereal solution was transferred through the top of a separating funnel into a 250 ml conical flask previously dried and weighed. The solvent was added and the content completely evaporated in a gentle current of air. The residue was dried in an oven maintained at 103 ºC for 15 minutes. This was allowed to cool in a desiccator and then weighed to the nearest 0.1 mg. The same procedure was used for the other samples and the blank.

$$\text{Unsaponifiable matter (\%)} = \frac{a}{m} \text{ X } 100 \qquad \ldots (2)$$

Where,

a = Weight of residue after oven drying, g, and

m = Weight of sample, g.

Saponification value

Saponification value was determined by the method prescribed by FSSAI (2012). It was calculated as the weight of potassium hydroxide (KOH) expressed in milligrams required to saponify 1 g of oil (Abdulkadir and Abubakar, 2011; Shende and Sidhu, 2016).

Peroxide value

Peroxide value is among idiocratic physicochemical index (Ni *et al*., 2016). It indicates the extent of oxidation suffered by oil.

Half gram of oil sample was taken in a 250 ml conical flask and 30 ml of acetic acid: chloroform solution was added to it and swirled to dissolve the sample. Half ml of saturated potassium iodide solution was added and then allowed to stand with occasional shaking for 1 min, and subsequently 30 ml of water was added to it. The solution was slowly titrated with 0.01 N sodium thiosulfate and vigorous shaken until yellow colour almost disappeared. Half ml of 1 % starch solution was then added. The titration was continued, shaken vigorously to release all iodine from the chloroform layer, until blue colour disappeared (FSSAI, 2012).

$$\text{Peroxide value (milliequiv. } \frac{\text{Peroxide}}{\text{kg}} \text{ sample)} = \frac{(S - B) \times N \times 1000}{\text{sample weight (g)}} \qquad \ldots (3)$$

Where,

S = 0.01 N $Na_2S_2O_3$ required by sample, ml,

B = 0.01 N $Na_2S_2O_3$ required by blank, ml, and

N = Normality of $Na_2S_2O_3$ solution.

Density

Density of oil sample was expressed in unit of $kg.m^{-3}$. Density of oil is temperature-dependent property, and was determined at ambient atmospheric temperature (Noureddini *et al.,* 1992).

Twenty ml oil was pipetted out using a pipette, and then weighed by means of a digital balance (AND electronic balance FX-320).

$$\text{Density (kg.m}^{-3}) = \frac{\text{mass (g)}}{\text{volume (m}^3)} \quad \ldots (4)$$

Statistical Analysis

Experiments were performed in triplicate, and the results were expressed as mean± standard deviation (SD).

All experimental data were analysed using spreadsheet program of Microsoft Excel (2007). One-way analysis of variance (ANOVA) was performed to test the effects for significance of various parameters at ($P< 0.05$) by using SPSS (Version 19.0). Critical difference (CD) values were calculated from standard error of the mean (SEM) obtained from ANOVA table for each quality characteristic of maize germ oil.

RESULTS AND DISCUSSION

Comparison of EAAE and Solvent Extracted Oil

Maize germ oil was extracted using enzyme assisted aqueous extraction (EAAE) method, and the oil recovery and quality of oil were compared with the traditional solvent extraction method.

Maximum recovery of maize germ oil using solvent extraction method was 24.86±0.70 % of maize kernel, whereas the maximum recovery of maize germ oil from optimized EAAE method process parameters (Shende and Sidhu, 2016) was 19.31±0.27 % of maize kernel. Similar results were obtained for yield (56 % to 93 %) of maize germ oil using aqueous enzymatic and steam explosion method by Ni *et al*. (2016).

Mean values of various quality characteristics of oil extracted by using EAAE and solvent extraction method are reported in Table 2. It was observed that the EAAE extracted oil was light yellow in colour and had a pleasant nutty maize aroma, while solvent extracted oil was reddish brown in colour and had unpleasant stink of solvent. EAAE extracted oil had lower acid number, pH, unsaponifiable matter, saponification value, peroxide value and density; and higher total phenolics content when compared with the traditionally solvent extracted oil quality characteristics. The quality parameters of EAAE extracted oil were thus superior to solvent extracted oil as with higher acid value unsaponifiable matter, saponification value and peroxide value of oil would have higher degree of its degradation (Li *et al*., 2014) and the protein extraction from the aqueous phase was strongly affected by the pH of oil (Hagenmaier *et al*., 1972).

Influence of Extraction Method on Physico-chemical Characteristics of Germ Oil During Storage

Acid number

The food industry considers acid value as an indicator of the quality of oil, and the degree of its degradation during heating.

The initial acid number of EAAE extracted oil and solvent extracted oil was 3.33±0.14 and 4.77±0.26, respectively. After 90 days of storage in HDPL and glass bottle, corresponding values of acid number decreased by 11.41 % and 9.60 %, respectively, for EAAE extracted oil; while by 15.93 % and 13.84 %, respectively, for solvent extracted oil, Fig. 5. The change in acid number of EAAE extracted oil was less

Table 2. Comparison of quality of maize germ oil extracted by solvent and EAAE methods

Sl. No.	Oil property	Solvent extraction	EAAE extraction
1.	Oil yield, %	24.86±0.70	19.31±0.27
2.	Colour	Red, Hue-52.9YR, Chroma-1.61	Yellow, Hue-49.05Y, Chroma-6.01
3.	pH	6.49±0.07	5.99±0.12
4.	Unsaponifiable matter, %	3.63±0.15	1.38±0.08
5.	Saponification value, mgKOH.g^{-1}	223.05±6.05	214.02±6.85
6.	Total phenolics, mg GAE.kg^{-1}	76.54±2.01	79.08±2.51
7.	Acid number, mg KOH.g^{-1}	3.73±0.03	3.35±0.05
8.	Peroxide value, meq.kg^{-1}	3.44±0.09	3.51±0.19
9.	Density, kg.m^{-3}	883.73±1.10	882.33±0.76

Note: Values are mean±SD

as compared to solvent extracted oil because enzymatic extraction offered the advantages of requiring a lower temperature for oil extraction (Li *et al*., 2014), thus ensuring higher storage stability of EAAE extracted oil. It can be observed from the Fig. 5 that after 60-day of storage, there was a sudden drop in acid number of solvent extracted oil stored in HDPE bottles. Higher acid numbers of oil result in elevated oxidation of oil causing higher deterioration of oil quality with development of unpleasant taste and odour in oils because both oxidation and oxidative polymerization occur with progressive heating (Li *et al*., 2014). The oxidation process was hindered by named antioxidants as tocopherols, phenolic compounds, carotenoids naturally appearing in plant tissues (Gromadzka and Wardencki, 2011). However, during the storage of oil, activities of natural antioxidants are decreased. It can be concluded that the decrease in acid number and antioxidant occur in the presence of light and air, called oxidation process. Similar results were determined by Abdulkarim *et al*. (2005) for moringa oleifera seed oil. The packaging material and method of oil extraction had significant effect (CD> 0.002) on acid number of stored oils, but storage period had a non-significant effect (CD< 0.002), Table 3.

pH value

Initial pH value of EAAE extracted and solvent extracted oil was 5.99±0.12 and 6.49±0.07, respectively. After storage for 90 days in HDPL and glass bottle, corresponding pH values decreased by 5.60 % and 4.40 %, respectively, for EAAE extracted oil, and 14

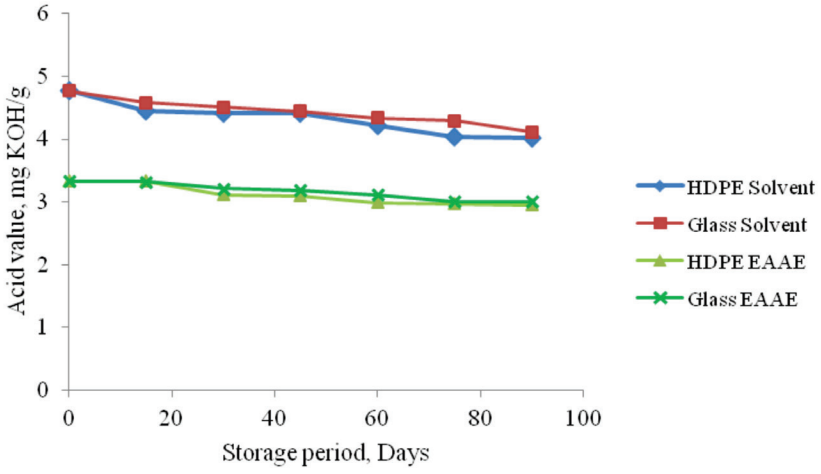

Fig. 5: Effect of process conditions on acid value of maize germ oil

Table 3. Analysis of variance for different quality characteristics of maize germ oil

	Acid number	pH	Total phenolics	Colour change	Unsaponifiable matter	Saponification value	Peroxide value	Density
	CD (5%)							
Packaging (P)	0.1202E-01	NS	0.2283E-01	0.4744	0.5750E-01	0.2136E-01	0.4049E-01	0.4832E-01
Storage period (S)	NS	NS	0.7658E-02	0.4481 E-01	0.4232	NS	0.4046	0.3728
Extraction method (E)	0.4792E-02	0.1495E-01	0.6006E-02	0.4429E-01	0.7571E-03	0.3485E-02	0.1706E-02	0.3904
P*S	0.7576E-03	NS	0.2287E-01	0. 3421E-03	0.7704E-03	0.2607E-12	0.9118E-03	0.3604E-06
P*E	0.3023E-02	0.5331E-01	0.8588E-04	0.3853	0.3733E-08	0.1996E-04	0.1169E-02	0.4360
S*E	0.6426E-03	0.1139E-02	0.9759E-02	0.3369E-03	0.5686E-03	0.1059E-11	0.7907E-03	0.3566E-04
P*S*E	0.9955E-05	0.2684E-04	0.8372E-03	0.3067E-05	0.6964E-05	0.8306E-20	0.1208E-04	0.1940E-09
SEM	0.5934E-02	0.1396E-01	0.1194E-01	0.2008E-01	0.1257	0.6584E-02	0.2063E-02	0.8715E-02

% and 11.26 %, respectively, for solvent extracted oil (Fig. 6). The increase in acidity of oil can be explained by triglyceride hydrolysis with the formation of free fatty acids (Gutierrez and Fernandez, 2002). Glass bottle seemed to retain initial quality characteristics of oil for longer time as glass does not allow penetration of oxidative gases (Gargouri *et al.,* 2015). Glass bottle was sensitive to the action of light on the fatty acids, which increased acidity of oil (Cuellar-Bermudez *et al*., 2015). HDPE bottle appeared to be the container in which the hydrolytic processes of the glycerides was more intense (Gargouri *et al.,* 2015), which could be related to the permeability of the container to oxygen and light. The EAAE extracted oil became less acidic as compared to solvent extracted oil during storage, similarly oil stored in glass bottles became less acidic than HDPE bottles during storage. The solvent extracted oil which was stored in HDPE bottle environment becomes more acidic than EAAE extracted oil, because EAAE extracted oil was stable in terms of storability. It can be observed from the Fig. 6 that the pH of solvent extracted oil stored in HDPE bottle dropped more, followed by solvent extracted oil stored in glass bottle. Change in pH of EAAE extracted oil was lower as compared to solvent extracted oil. Similar results were determined by Mendez and Falque (2007) for olive oil and Barku *et al.* (2012) for almond nut oil. Significant reduction in pH value of oil was due to method of oil extraction (CD> 0.005), but the packaging material and storage period had non-significant effect (CD<0.005) on it as reported in Table 3.

Total phenolics

Shelf-life was longer for oil samples containing higher amount of phenolics content (Silva *et al.,* 2010). These natural antioxidants provide the oil with certain characteristics at a chemical, organoleptic, and health level (Seppanne, 2010).

Initially high content of total phenolics was found in maize germ oil. Initial total phenolics content of EAAE extracted oil and solvent extracted oil was 79.08±3.61 and 76.54±2.53, respectively. Oil stored for 90 days in HDPE and glass bottle had corresponding values decreased by 1.15 % and 0.20 %, respectively, for EAAE extracted oil; and 18.08 % and 13.90 %, respectively, for solvent extracted oil, Fig. 7). The oil when stored in HDPE bottles had under gone upto 18.08 % decrease in total phenolics content due to oxidation of oil in presence of light and air. Oil stored in glass bottle showed upto 13.08 % decrease in total phenolics content, because glass did not allow penetration of gases for oxidation or deterioration of quality of oil (Gulla and Waghray, 2011). It can be observed from the Fig. 7 that after 45-day of storage, there was a change in trend of total phenolics content of solvent extracted oil stored in both HDPE and glass bottle as compared to EAAE extracted oil. The extent of decrease in total phenolics content of EAAE extracted oil was far less than solvent extracted oil because of storage stability (Latif, 2009). Similar results were reported by Silva *et al.* (2010) for olive oil. The decrease of total phenolics content in oil was significantly (CD> 0.0043) different and affected by packaging material, storage period and method of oil extraction as reported in Table 3.

Colour

Enzyme assisted aqueous extraction method results in superior quality of oil which was light yellowish

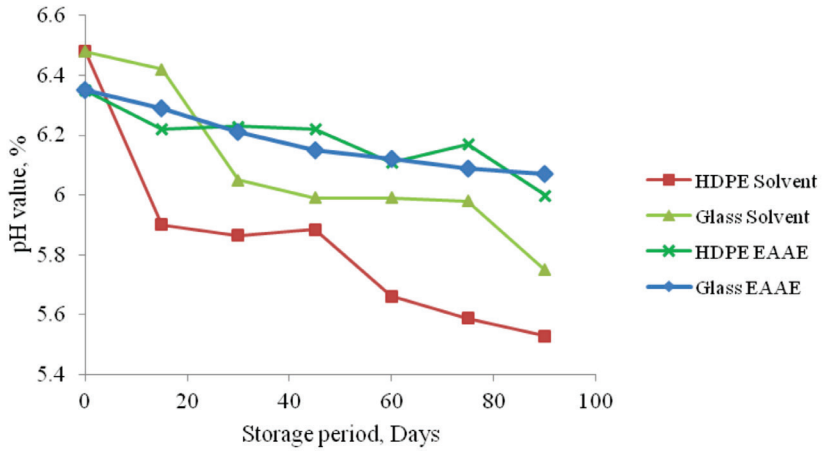

Fig. 6: Effect of process conditions on pH value of maize germ oil

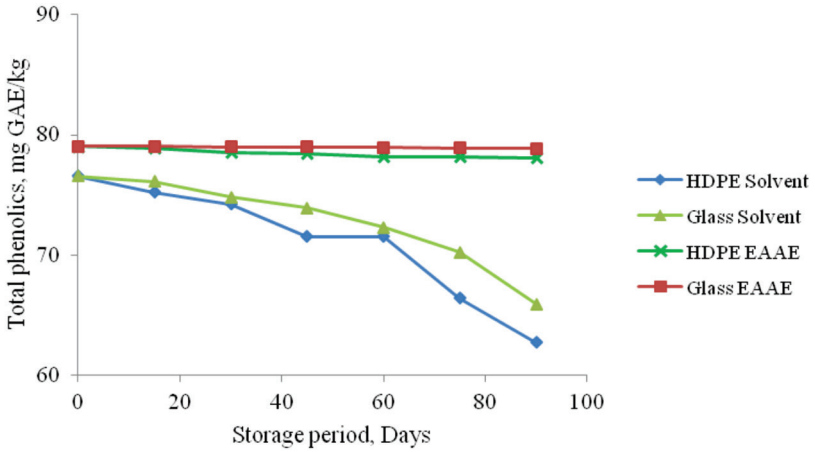

Fig. 7: Effect of process conditions on total phenolics content of maize germ oil

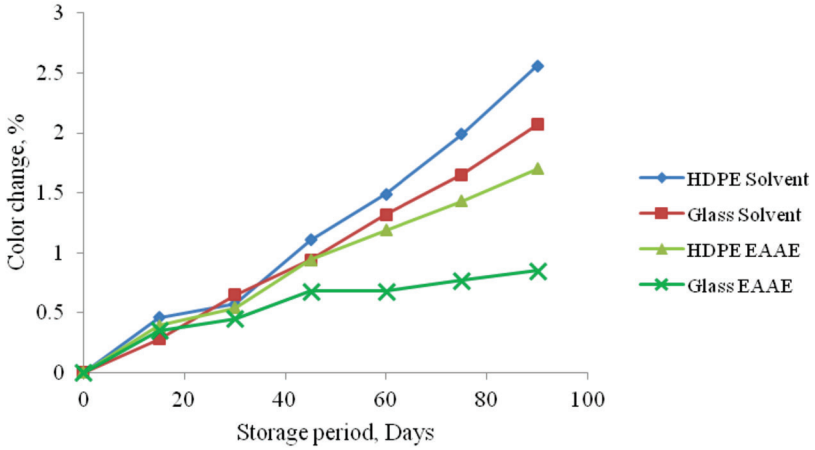

Fig. 8: Effect of process conditions on colour of maize germ oil

in colour as compared to solvent extracted oil which was brownish red in colour because of heat damage of oil due to repeated evaporation of solvent (Latif, 2009). The yellowish colour of oil was due to the presence of various pigments, such as chlorophylls, pheophytins and carotenoids (Cichelli and Pertesana, 2004). Such natural pigments can also considerably affect the preservation of the oil during storage. The L value among colour parameters of oil was of maximum significance as it indicated lightness, darkness and change in colour of oil.

The initial value of L of EAAE extracted oil and solvent extracted oil was 35.9±1.33 and 25.7±0.76, respectively. The higher the L value, the lighter the oil colour, which is more acceptable by consumers. The L value decreased with time indicating that the oil became dark in colour due to development of pigments during thermal decomposition and oxidation of fatty acids which diffused into the oil during storage (Li *et al*., 2014). The overall change in colour of EAAE extracted oil were 1.70 % and 0.85 %; whereas in solvent extracted oil were 2.56 % and 2.07 % when stored in HDPE and glass bottles after 90 days of storage. As it can be observed from Fig. 8 that there was maximum change in colour of solvent extracted oil stored in HDPE bottle, followed by solvent extracted oil stored in glass bottle and EAAE extracted oil stored in HDPE bottle. However, minimal change was observed in EAAE extracted oil stored in glass bottle. Glass bottle ensured better protection of oil from oxidation and colour change than HDPE bottles (Kiritsakis and Dugan, 1984).The quantity of chlorophyll and carotenes decreases in oil during storage, the extent depending upon the storage time and type of container (Dabbou *et al.,* 2011). Similar results for colour change

of oil during storage was reported by Kanavouras and Coutelieris (2006) for olive oil. The packaging material, storage period and method of oil extraction had significant (CD> 0.0067) effect on overall change in colour of germ oil (Table 3).

Unsaponifiable matter

The unsaponifiable fractions of vegetable oils consist of tocopherols as important functional constituent (Tuan, 2011).

The initial value of unsaponifiable matter of EAAE extracted oil and solvent extracted oil was 1.38±0.08 % and 3.63±0.15 %, respectively. The higher value of unsaponifiable matter in the solvent extracted oil was might be due to the ability of the solvent to extract other lipid-associated substances like sterols, fat soluble vitamins, hydrocarbons and pigments (Abdulkarim *et al.,* 2005). On storage in HDPL and glass bottle for 90 days, the corresponding values increased by 6.66 % and 3.33 %, respectively, for EAAE extracted oil; and by 12.57 % and 20.0 % for solvent extracted oil. It can be observed from Fig. 9 that after 75-day of storage, there was sudden increase in unsaponifiable matter solvent extracted oil stored in HDPE bottle. The increase in unsaponifiable matter might have occurred due to increase in non-volatile matter caused by auto-oxidation process triggered by light and air. The solvent extracted oil stored in glass bottle underwent negligible change in unsaponifiable matter as glass bottle did not allow the penetration of atmospheric gases. Solvent extracted oil when stored in HDPE bottle exhibited significant increase in unsaponifiable matter of oil after 90 days of storage period because of oxidation of oil in the presence light and air. The change in unsaponifiable matter of EAAE extracted oil was negligible during storage because oil extracted using enzyme assisted method had storage stability and longer shelf-life than solvent extracted oil. Similar results were reported by Latif (2009) for hemp seed oil. The increase in unsaponifiable matter of oil differed significantly and were affected by packaging material and storage period (CD> 0.042),but the method of oil extraction had non-significant effect on it (CD< 0.042) as shown in Table 3.

Saponification value

The saponification value of EAAE extracted oil was more stable during storage period when compared to solvent extracted oil. The initial saponification value of EAAE extracted oil and solvent extracted oil was 197.54±4.58 and 223.05±6.05, respectively. After 90 days of storage, saponification value decreased by 1.68 % and 0.67 % for EAAE extracted oil; and by 3.94 % and 1.10 % for solvent extracted oil when stored in HDPE and glass bottles, respectively (Fig. 10). A slight decrease saponification value was observed during storage because saponification or acid hydrolysis arose in the oil in the presence of light (Gargouri *et al.,* 2015) and air. The saponification value of solvent extracted oil stored in HDPE bottle decreased more after 45-day of storage than others as shown in Fig. 10. The solvent extracted oil when stored in glass bottle showed negligible change in saponification value because glass bottle did not allow the penetration of gases for oxidation or deterioration of quality of oil (Mendez and Falque, 2007). EAAE extracted oil showed stability during storage, and thus exhibited increased shelf life. Similar results were determined by Mendez and Falque

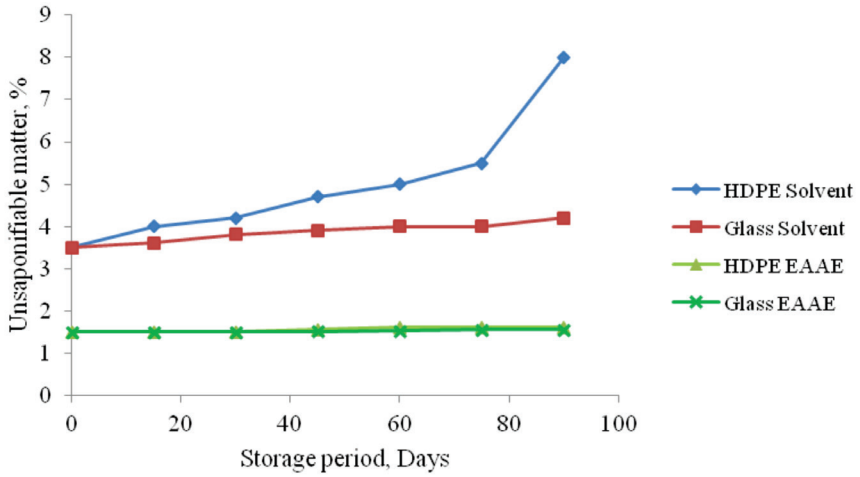

Fig. 9: Effect of process conditions on unsaponifiable matter of maize germ oil

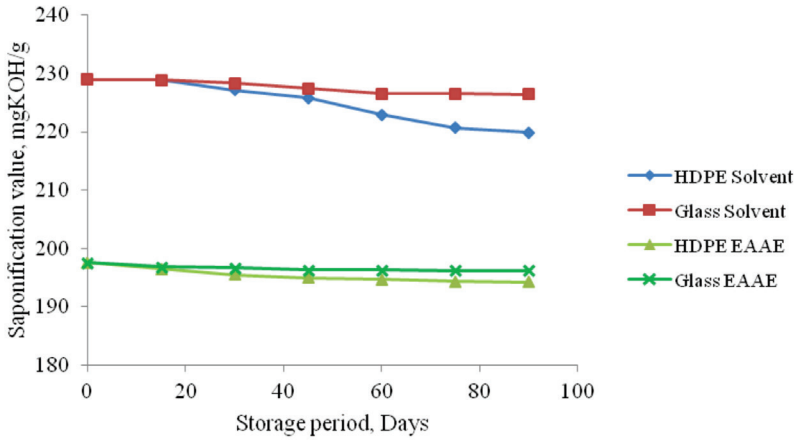

Fig. 10: Effect of process conditions on saponification value of maize germ oil

(2007) for olive oil. The decrease in saponification value of oil was significantly affected by packaging material and method of oil extraction (CD> 0.0022),but storage period had a non-significant effect on it (CD< 0.0022) as shown in Table 3.

Peroxide value

Peroxide value is most often used for indicating the oxidation level (Deiana *et al.,* 2002), and allows for the evaluation of the content of primary oxidation of products.

The initial peroxide value of EAAE extracted oil and solvent extracted oil was 3.51±0.19 and 3.44±0.09, respectively. Subsequent to storage for 90 days, the peroxide value increased by 125 % and 75 % for EAAE extracted oil; whereas it increased by 190.69 % and 76.47 % for solvent extracted oil when stored in HDPE and glass bottles, respectively (Fig. 11). Peroxide value increased due to the increased oxidation process (Gargouri *et al*., 2015). The degree of oxidation depended upon controlled reaction time, the intensity of the agitation and protection of the reaction components from light or atmospheric oxygen (Nouros *et al.,*1999). There was sharp increase in peroxide value of solvent extracted oil stored in HDPE bottles after 60-day of storage (Fig. 11), which might be due to penetration of gases from micro pores in it resulting in oxidation of oil. This sharp increase in peroxide value of oil was not exhibited by other samples than solvent extracted oil stored in HDPE bottle (Fig. 11). Similar results were determined by Gromadzka and Wardencki (2011) for vegetables oils. The increased peroxide value of oil differed significantly and was affected by packaging material, storage period and method of oil extraction (CD> 0.0007) as shown in Table 3.

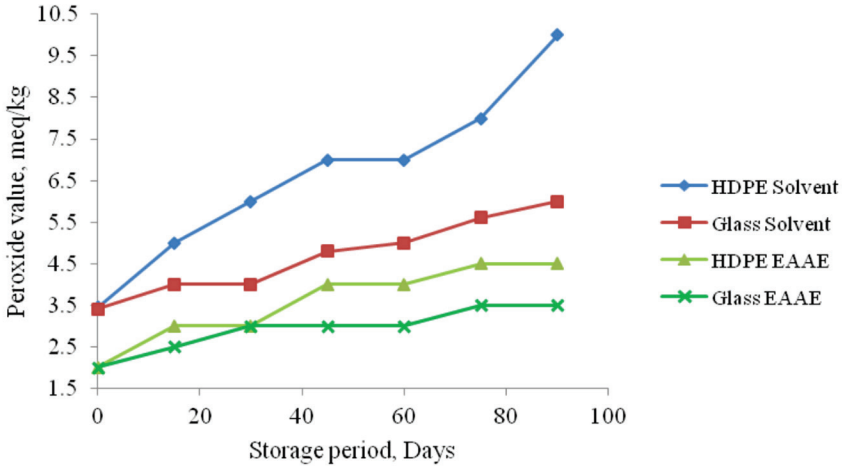

Fig. 11: Effect of process conditions on peroxide value of maize germ oil

Density of oil

Density of oil is necessary in the designing of unit operations such as distillation, heat exchangers, piping, and reactors (Rodenbush *et al.,*1999).

The initial density of EAAE extracted oil and solvent extracted oil was 883.73±1.10 and 882.33±0.76 $kg.m^{-3}$ respectively. On storage for 90 days, the corresponding values increased by 0.31 % and 0.15 % for EAAE extracted oil; and by 0.68 % and 0.37 % for solvent extracted oil when stored in HDPE and glass bottle, respectively, Fig. 12. The increase in density of oil might be attributed to increase in the level of unsaturation (Rodenbush *et al.,* 1999) and the formation of polymeric fractions of high molecular weight due to oxidative cleavage of fatty acids. Polyunsaturated acids could be a major factor for the increase in density and can be attributed to the higher content of linoleic acid in oil (Ackman and Eaton, 1977). The oil stored in HDPE bottles had increased deterioration than in glass bottle as glass bottle did not allow penetration of atmospheric gases and moisture for oxidation of oil (Kucuk and Caner, 2005). EAAE extracted oil when stored in glass bottle showed almost stable density of oil with only 0.15 % increase after 90 days of storage. There was a sharp increase in density of solvent extracted oil when stored in HDPE bottle after 45 days of storage (Fig. 12). There was 0.68 % increase in density of solvent extracted oil when stored in HDPE bottle after 90 days of storage, because HDPL bottle allowed penetration of gases from micro pores in it for oxidation of oil. Similar results were reported by Gulla and Waghray (2011) for sesame-rice bran blend oil. The density of oil increased significantly due to packaging material, storage period and method of oil extraction (CD> 0.003) as shown in Table 3.

CONCLUSIONS

EAAE oil extraction method enhanced oil recovery by using proper enzyme mixture adjuvant. Germ oil extracted by EAAE method had better colour and other quality attributes as compared to solvent extracted oil. Acid number, pH, total phenolics, unsaponifiable matter and peroxide value of oil stored for 90 days were significantly affected (P< 0.05) by packaging material, storage period and method of oil extraction. Colour change of oil was significantly affected (P< 0.05) by packaging material and method of oil extraction. Saponification value of oil was significantly affected (P< 0.05) by only packaging material. Density of oil was not significantly affected by packaging material, storage period and method of oil extraction at 5 % level of significance. Although the time required for extraction of EAAE method was more as compared to solvent extraction method, it gave superior colour, total phenolics and peroxide value of oil resulting in more stable oil during storage than solvent extracted oil. EAAE extracted maize germ oil can be stored in glass bottle at ambient storage conditions for more than 90 days without significant change in quality characteristics.

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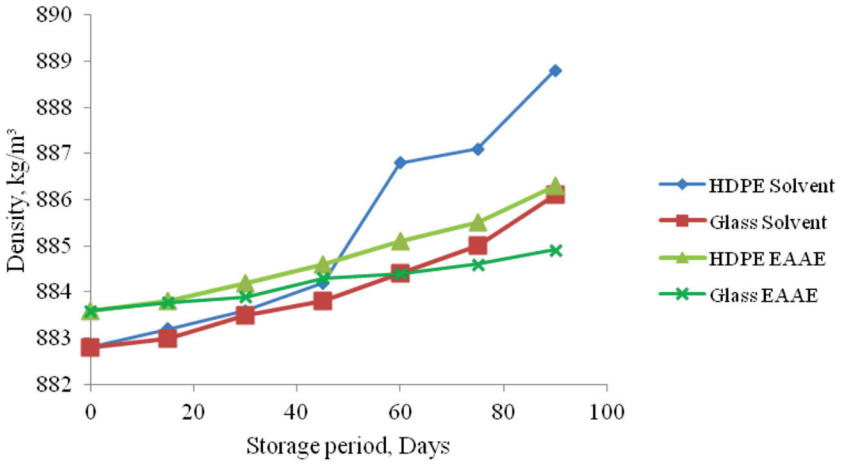

Fig. 12: Effect of process conditions on density of maize germ oil

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