International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 384-392, 2017

# Watermelon Waste: A Potential Source of Omega-6 Fatty Acid and Proteins

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**Abstract :** This study presents the extraction of oil and proteins from watermelon Fruit waste. In our present work we used watermelon seed of Indian origin for extraction of oil and proteins. Watermelon Seeds are waste product of Watermelon fruit. White seeds of watermelon contain 40% crude oil. Crude oil contains maximum amount of polyunsaturated omega-6 fatty acids (PUFA) especially Linoleic acid. Extraction of Watermelon seed oil was carried out using Soxhlet apparatus. Three solvents were used for the extraction of Watermelon seed oil namely; n-Hexane, Acetone and petroleum ether. From that hexane gives good results as compared to Pet. Ether and Acetone. The fatty acids profile of n-hexane solvent extracted watermelon seed oil showed polyunsaturated fatty acid content 65 %. Defatted seed cake is rich and concentrated source of protein. In this work protein was extracted from defatted seed meal (after extraction of oil from watermelon seeds). Alkali method was used for protein extraction from defatted seed cake. Amino acids were analyzed using high performance liquid chromatography method. Defatted watermelon seed cake contains mainly 39.68% Histidine, 31.43%Glycine, 8.34%Serine and 5.8%Alanine.
**Keywords :** Soxhlet, PUFA,Proteins,Amino acids.

## Introduction

Watermelon (*Citrullus lanatus*), comes from family cucurbitaceae[1].China is the world leader in watermelon production with 70.3% of total production. Other leading countries are Turkey (4.7%), Iran (2.3%), United States (2.2%) and Egypt (1.7%). India (290,485 mt) occupies 26th position in watermelon production[2].Watermelon found in the India's south and central regions. These areas are most suitable to growing commercial varieties. Many Indian states grow watermelon. These regions vary in their climate, but the adaptability of watermelon allows the fruit to grow in various soils.Hot weather is suitable condition for watermelon. In India, watermelon is cultivated in Himachal Pradesh, Uttar Pradesh, Rajasthan, Tamil Nadu, Gujarat, Punjab, Assam, West Bengal, Karnataka, Orissa, Andhra Pradesh, Maharashtra and Orissa. India grows approximately 26 commercial varieties. Watermelon comes into season during the summer months, generally from April to June. Watermelon is important from nutritional point of view[3]. It is an important source of lycopene and beta-carotene[4].Watermelon seeds contain high levels of proteins and lipids[5]. As India produces 290,485 mt of watermelon, a big quantity of watermelon wastes and byproduct from fruit processing industry are available. A large proportion of these waste including watermelon seeds are discarded either as a cheap animal feed or simply thrown away. Alternatives to such disposal methods could be processing to extract value added products such as oil and proteins. In this study we used Watermelon seeds for extracting value added products such as oil and proteins.

Watermelon seed oil also known as Ootanga oil or Kalahari oil mostly suitable for cosmeceutical applications. High amounts of unsaturated fatty acids,linoleic and oleic acids are present in seed of watermelon fruit.Watermelon seed oil contains considerable amount of PUFAs which are very receptive to oxidation and other side reactions that causes deterioration of oil[6].The present study was carried out to investigate the suitable solvent for extraction of omega-6 fatty acid rich oil from watermelon seed oil of Indian origin and its physical and chemical characteristics and proteins extraction from defatted seed cake.

## Experimental:

### Materials

Watermelon seeds were purchased from Mumbai market. Starch soluble,SodiumThiosulphate anhydrous, Potassium iodide,Hexane, Acetic acid glacial, pet ether, Chloroformand Acetone. Sodium Hydroxide (NaOH), Hydrochloric acid (HCl), distilled water ($H_2O$), PH papers were provided from Hi-Media (Mumbai, India).Gum Acacia and Maltodextrin were purchased from Hi-media (Mumbai, India). All Chemicals used were of AR grade.

### Sample preparation

Dehulled watermelon seeds were brought from the local market. Seeds were dried for 6 hrs at $105^0$C. After drying, seeds were well grinded. Grinded seed were again dried for 1hr at 105 $^0$C.

### Soxhlet Extraction

The watermelon seed oil was extracted using Soxhlet extractor and n-Hexane, acetone and Pet ether were used as solvent. Weight of the sample taken and then sample placed in a thimble. Then thimble covered and put in the inner tube of the apparatus. Apparatus then fitted to a round bottom flask which contains the solvent. Heat was applied to solvent boiling point for 4-6 hours. After some time the solvent started boiling and the water begins to drop from the top to the sample. The solvent siphoned over into the flask when it reached the top of the tube. During the process of refluxing the portion of oil has been extracted which eliminates in siphon cycle. The extracted oil was evaporated under vacuum at 44ºC using an equitronrote varotary evaporator(Germany). And solvent used was also recovered in this process. Oil extracted was collected and measured followed by filtration[7].

### Analysis of extracted watermelon seed oil

### Physical analysis of extracted watermelon seed oil

Moisture content of the Watermelon seed

The Oven method was used for moisture content determination. The principle was that a test portion was heated at $105^0$C until moisture and volatile substances are completely eliminated, and the loss in mass determine.

### Determination of density and specific gravity of oil

Ten millimeters (10ml) of specific gravity bottle was cleaned and dried in anoven. The weight of the empty gravity bottle was obtained as W1, the weight of the gravity bottle with the water was taken as W2, and the weight of the bottle with the oil was taken W3. The specific gravity and the relative density of the oil are calculated using the formula below:

$$Specific\ Gravity = \frac{weight\ of\ oil}{weight\ of\ equal\ volume\ of\ water}$$

$$Density = \frac{weight\ of\ oil}{volume\ of\ oil}$$

**Determination of acid value**

The acid value is expressed as % free fatty acid. One gram (1gm) of oil sample was taken in conical flask. About 25 ml of neutral alcohol was added in conical flask along with few drop of phenolphthalein indicator and shake vigorously. Solution was titrate against 0.1 N alkali solution with constant shaking until faint pink color obtained. Take a burette reading.

$$Acid\ Value = \frac{0.5 * 56.1 * Burette\ Reading}{Weight\ of\ sample\ taken}$$

**Determination of Peroxide value**

About 3 gm of oil was taken in dry Erlenmeyer flask with glass stopper. 30 ml of the 3:2 acetic acid-chloroform solutions was added into the Erlenmeyer flask and swirl to dissolve the sample. Then 0.5 ml of saturated KI solution was added. Allowed the solution to stand for exactly 1 minwith occasional shaking and then immediately 30 ml of distilled water was added. The solution was titrate with 0.01 N sodium thiosulphate solution till yellow color of iodine was disappeared. And about 0.5 mL of starch indicator was added. Again titrate with constant shaking till the blue color disappeared.

$$peroxide\ value = \frac{(Sample - Blank\ ) \times Normality \times 1000}{Wt\ of\ sample, gm}$$

**Determination of Iodine value**

About 0.3 gm of oil was taken inadry flask. Then 10 ml of carbon tetrachloride solution was added followed by 25 ml of Wijs solution and was kept in the dark place for 30 min. Then 10% of 15 mL KI solution added in to the mixture and was shaken. Then 100 ml of distilled water was and was shaken vigorously. Then starch indicator was added to it andthedark blue color was obtained. The mixture was titrated with 0.1N sodium thiosulphate till blue to thecolorless point was obtained. The Iodine Value was calculated as follows:

$$Iodine\ Value = (blank - sample) \times Normality \times \frac{12.69}{weight\ of\ sample}$$

**Determination of Saponification value**

2 gm of sample was weighted and taken in an Erlenmeyer flask. Then 50 ml of alcoholic KOH solution added. The mixture was boiled gently under the reflux until the sample was completely saponified. Few drop of phenolphthalein was added and titrate with 0.5N HCl until the pink color just disappeared.

$$Saponification\ value = \frac{(Blank - Sample) \times N \times 56.11}{Wt\ of\ sample, gm}$$

**Gas chromatography Analysis**

Fatty Acid composition of the soxhlet extracted watermelon seed oil was determined using GC after converting high boiling point fatty acids in low boiling fatty acids methyl esters by method mentioned in Christie[8].Gas chromatographic analyses were performed by injecting 1µL of the oil solution in the split less mode into an Shimadzu gas chromatograph GC1000equipped with BPX 70 column. The column was operated with Nitrogen as carrier gas (2 µL min -1) at an initial temperature of 75° C which was then raised at 5 °C/ min to 230 °C. The injector was kept at 280° C, and the flame ionization detector was set at 320° C. Chromatograms were displayed and integrated [9].

**FTIR Analysis of extracted Watermelon seed oil**

To detect the functional groups present in the extracted oil, FTIR analysis was used. FTIR spectrophotometer (Shimadzu 8400 Japan) used for recording FTIR spectra of extracted oil. ATR sampling

technique used to detect functional group by recording 60 scan in % transmittance mode in the range of 4000-500 $cm^{-1}$.

**Protein Extraction**

About 40 gm dried defatted seed was weighed and suspended in distilled water at a ratio of 1:100. While adjusting the pH at 9.0 with NaOH solution with the use of a magnetic stirrer, the mixture was stirred for 1 h. Then the mixture again stirred using overhead stirrer for 3 hrs. The mixture was centrifuged at 4500 rpm for 20 min at room temperature. Then supernatant was collected into a beaker. The 0.5 N HCl was added to supernatant to adjust the PH to 4.5 stirred for another 30 min. The supernatant was kept undisturbed for cold precipitation overnight at 4 $^0$C in a freezer. Protein slurry was washed three times with distilled water by centrifuging at 3500rpm for 10 min after supernatant was carefully siphoned off and pH was adjusted at 7.0. The slurry was kept overnight at 80 $^0$C and then it was freeze dried [10]. The sample was dried in the freeze-dryer. Analytical balance used for weighing protein concentrates.

**Amino acid analysis**

Amino acids were analyzed according to the method of Rees, M.W. 1946 [11] with minor modifications. Extracted protein powder analyzed for free amino acid using a high performance liquid chromatograph (HPLC) system.

## Results and Discussion

**Solvent Extraction Of watermelon seed oil**

**Table 1 Solvent Extraction of Watermelon seed oil**

| Batch no. | Time (hr) | Temp. | Solvent | Yield (%) | Acid value | Peroxide value |
|---|---|---|---|---|---|---|
| 1 | 4 | 60 | Hexane | 32.69 | 0.9181 | 1.98 |
| 2 | 5 | | | 31.32 | 1.18 | 2.07 |
| 3 | 6 | | | 32.98 | 1.32 | 2.17 |
| 4 | 4 | 70 | | 33.17 | 2.212 | 1.966 |
| 5 | 5 | | | 34.48 | 1.41 | 1.87 |
| 6 | 6 | | | 33.63 | 1.32 | 2.32 |
| 7 | 4 | 80 | | 35.19 | 1.02 | 2.01 |
| 8 | 5 | | | 35.57 | 1.81 | 2.07 |
| 9 | 6 | | | 36.81 | 2.08 | 2.54 |
| 10 | 4 | 60 | Pet. Ether | 23.93 | 4.40 | 2.37 |
| 11 | 5 | | | 31.38 | 3.81 | 2.67 |
| 12 | 6 | | | 31.62 | 3.27 | 2.19 |
| 13 | 4 | 70 | | 32.65 | 4.25 | 5.14 |
| 14 | 5 | | | 32.19 | 2.31 | 4.34 |
| 15 | 6 | | | 32.88 | 2.12 | 4.13 |
| 16 | 4 | 80 | | 33.23 | 3.25 | 2.12 |
| 17 | 5 | | | 33.75 | 2.77 | 3.24 |
| 18 | 6 | | | 34.87 | 2.83 | 3.98 |
| 19 | 4 | 60 | Acetone | 24.35 | 7.34 | 3.12 |
| 20 | 5 | | | 25.01 | 4.43 | 3.98 |
| 21 | 6 | | | 25.66 | 4.66 | 4.17 |
| 22 | 4 | 70 | | 25.18 | 1.50 | 3.65 |
| 23 | 5 | | | 25.83 | 3.32 | 4.18 |
| 24 | 6 | | | 26.46 | 3.07 | 4.53 |
| 25 | 4 | 80 | | 25.81 | 5.19 | 4.31 |
| 26 | 5 | | | 26.27 | 4.72 | 4.05 |
| 27 | 6 | | | 26.74 | 4.18 | 4.78 |

**Effect of solvent on extraction**

The solvent used for the extraction were Hexane, petroleum ether and acetone. Hexane and petroleum ether are non-polar and Acetone shows both polar and non-polar characteristics. It was found that, as the polarity of the solvent goes on decreases, the extraction yield of oil increases as shown in Table 1. Non polar solvents are highly capable of extracting fatty matter than polar solvents. From Table 1, hexane gives higher yield as compared to other two solvents followed by Petroleum Ether.

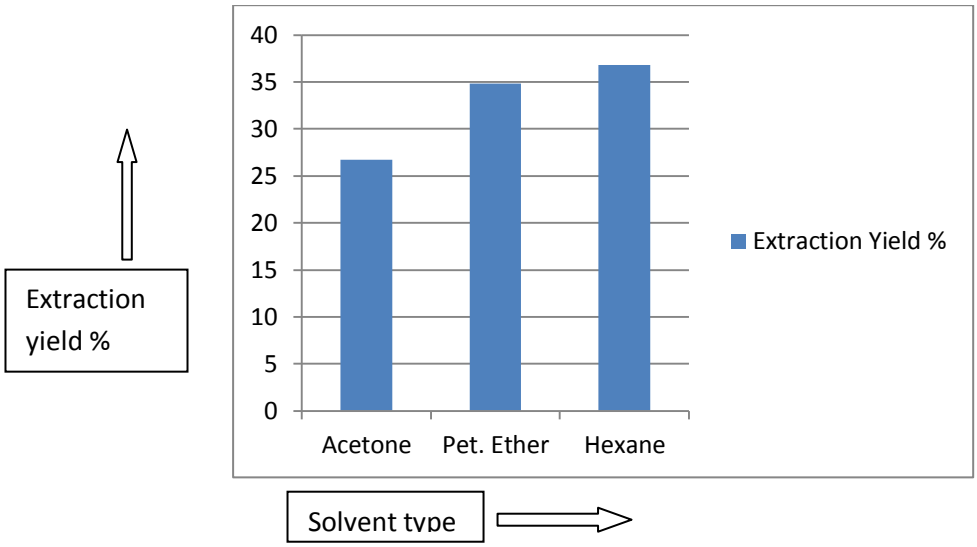


**Figure1: Effect of solvent type on the extraction yield @80$^0$C for extracted watermelon seed oil**

**Effect of temperature on extraction**

As shown in the table 1 the yield of watermelon seed oil increases with the increasing temperature in the solvent extraction. When temperature increases from 60-80$^o$C the extraction yield was also increased. Solubility and diffusivity increases with increase in temperature from 60$^o$C-80$^o$C, which results increase in mass transfer. Fig 2 shows the effect of temperature on extraction yield of watermelon seed oil for the hexane solvent.

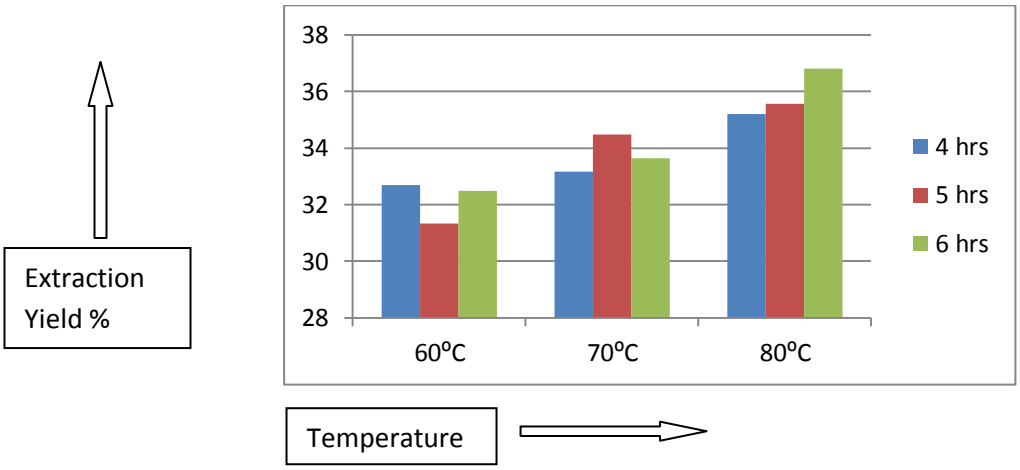


**Figure2: Effect of temperature and time period on extraction yield for Hexane extracted watermelon seed oil**

**Effect of time period:**

The table 1 shows that higher yield is obtained at 6 hours using n-hexane. The quality of that oil is also good. As increase in time above 6 hrs does not show that much increase in yield but it affects the quality of oil because watermelon seed contains ahigh amount of linoleic acid, which is very susceptible to oxidation.

**Physico-chemical Analysis of extracted Watermelon seed oil**

The Physico-chemical characteristic of the extracted watermelon seed oil was analyzed. Various `physical and chemical characteristics were calculated and noted in table 2.

**Table 2Physico –chemical Analysis of Watermelon seed oil**

| Analysis | Observation and results |
|---|---|
| Density (gm/ml) | 0.9073 ±0.014 |
| Specific Gravity | 0.831±0.012 |
| Moisture content (%) | 0.044±0.12 |
| FFA | 12.32±0.523 |
| Acid value (mgm of KOH/kg) | 1.02±0.836 |
| Sap. Value | 194.6±1.210 |
| Iodine value ($gl_2$/100gm) | 112.3±0.943 |
| Peroxide value (meq/kg) | 1.966±1.031 |

**Gas Chromatography Analysis of watermelon seed oil**

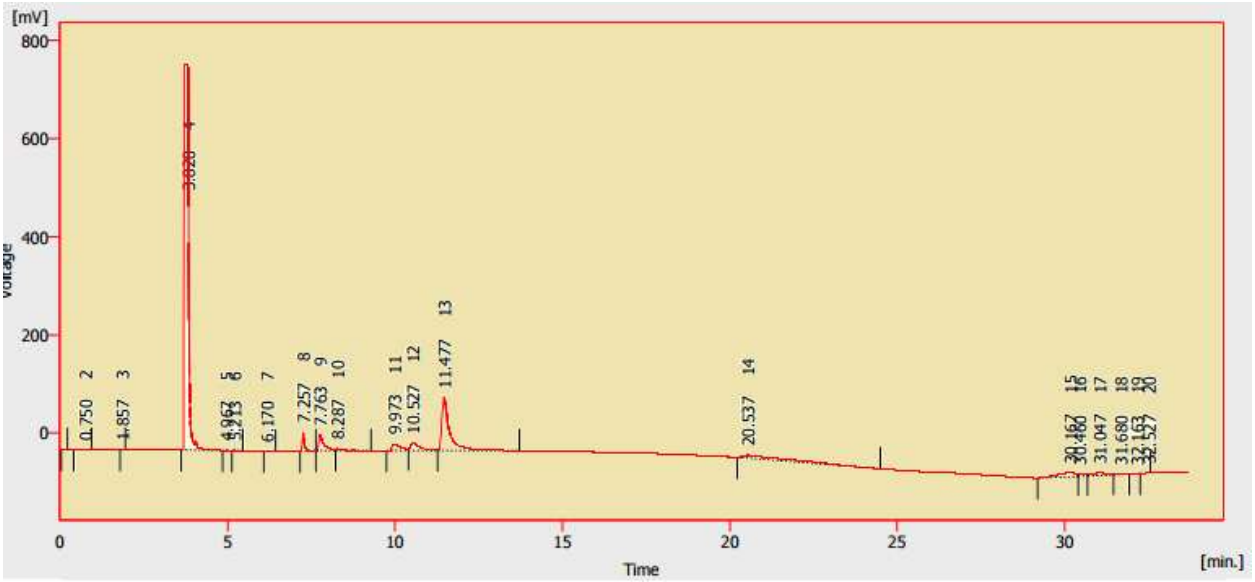


**Fig.3 Gas chromatograph of watermelon seed oil**

Fig.3 illustrates the chromatogram of Watermelon seed oil which shows the presence of various fatty acids such as Palmitic acid, oleic acid, stearic acid and mainly Linoleic acid (Table 3). As it can be remarked from Table 3 Extracted watermelon seed oil contains 43% Linoleic acids. Since extracted watermelon seed oil contains some amount of oleic acid, it may be useful in maintaining oxidative stability of oil [12].

**Table 3 Fatty acid profile of watermelon seed oil**

| Fatty Acids | Determined value % |
|---|---|
| Linoleic Acid (C18:2) | 43 |
| Oleic Acid (C18:1) | 8 |
| Stearic Acid (C18:0) | 6 |
| Palmitic Acid (C16:0) | 8 |

**FTIR Analysis of Watermelon seed oil**

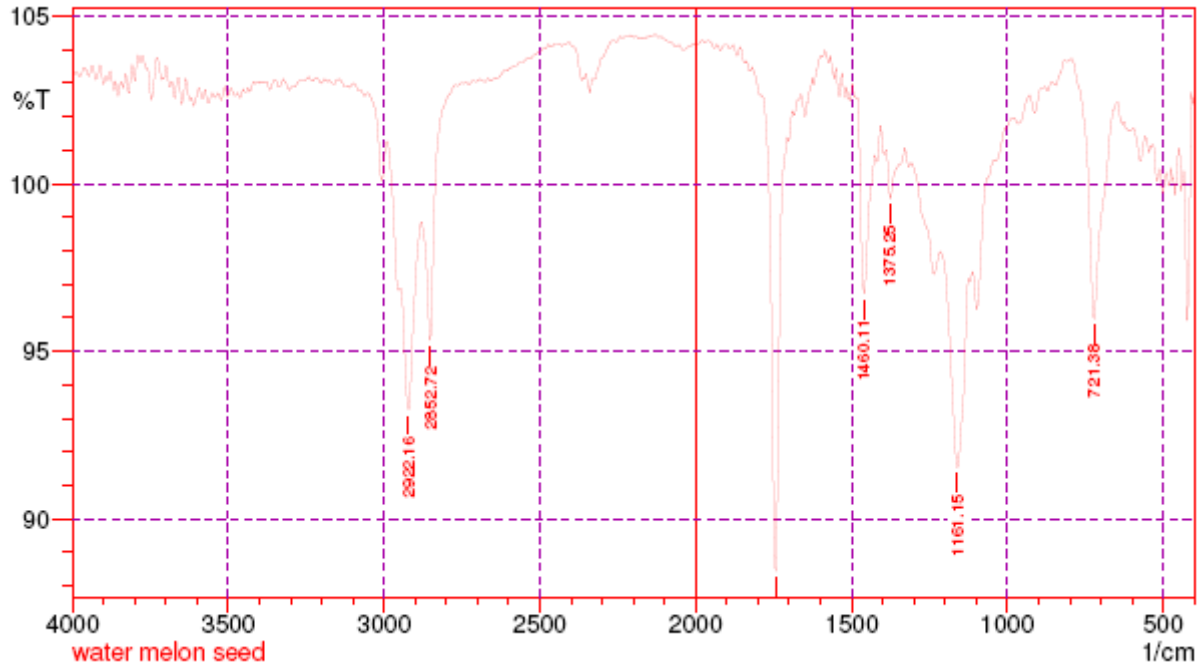


**Figure4: FTIR Spectra of Watermelon seed oil**

An analysis of the IR spectrum showed in figure 4, reveals the existence of ester functional group (C=O stretch) at a peak 1735 $cm^{-1}$ and at peak 1161 $cm^{-1}$ shows ether linkage. The peak at 2922 and 2852 $cm^{-1}$ corresponds to the asymmetric and symmetric vibrational modes of methylene groups, respectively. Stretching vibrations produce a strong band in the region 1200-900 $cm^{-1}$[13].

**Protein Extraction**

**Table 4: Protein Extraction from Defatted watermelon seed cake**

| Batch no. | Defatted seed powder taken (gm) | Yield % | Ash content % |
|---|---|---|---|
| 01 | 40 | 28.2 | 2.56 ±1.0 |
| 02 | 40 | 28.65 | 3.21±1.0 |

**Amino Acid Analysis by HPLC**

Figure 5 illustrates the high pressure liquid chromatograph of hexane extracted watermelon seed oil which shows the presence of various amino acids such as Histidine,glycine, serine, Alanine, Threonine, Aspartic acid, Tyrosine, Valine, Lysine, Leucine.

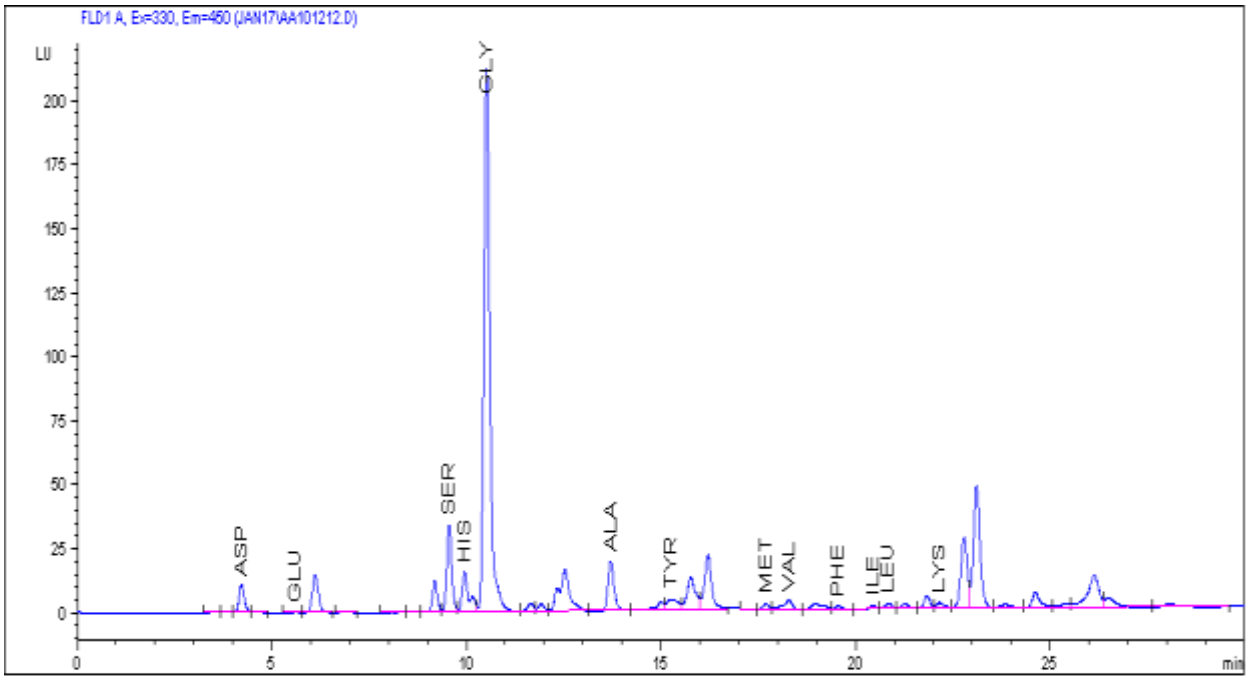


**Figure5: High pressure liquid chromatograph of hexane extracted watermelon seed oil**

**Table 5 Amino acid content of defatted watermelon seed cake proteins**

| **Amino Acids** | **Obtained value in %** |
|---|---|
| Histidine | 39.68 |
| Glycine | 31.43 |
| Serine | 8.34 |
| Alanine | 5.8 |
| Threonine | 3.86 |
| Aspartic Acid | 2.72 |
| Tyrosine | 2.1 |
| Valine | 1.59 |
| Lysine | 1.4 |
| Leucine | 0.8 |

Table 5 shows Amino acid content of defatted watermelon seed cake proteins. Defatted watermelon seed cake contains 39.68% Histidine, 31.43%Glycine, 8.34%Serine and 5.8%Alanine. Histidine is an essential amino acid and it helps in human growth and development [14]. Glycine is a key substance in metabolic reactions and also used as a sweetener or taste enhancer [15]. Serine is the precursor to several amino acids, including glycine and cysteine [16]. Alanine helps balance glucose and nitrogen in the body [17].

## Conclusion:

The solvent extraction method for extraction oil from Watermelon seeds was successfully developed after evaluating and comparing with three different solvents at various temperatures. The extracted oils were stable and liquid at room temperature. The oil extraction using n-hexane at 80$^{o}$C gave the highest yield with good quality. The extraction of oil using various solvent is less effective as compared to extraction of oil using n- hexane as solvents in terms of yield and quality of oil. Yield changes with the changing solvents, time and temperatures. The GC analysis showed the fatty acid composition of the extracted Watermelon seed oil. Watermelon seed oil is good source of omega-6 fatty acids. Watermelon seed has a great potential to use as an excellent source of edible protein. Amino Acid profile of watermelon seed proteins shows various amino acids, mainly Histidine and Glycine.

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