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Volatile profiling of seaweed subjected to various drying & milling technique by e-nose using multivariate analysis: *Kappaphycus alvarezii*

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Abstract
Seaweed is a nutritious food source that has gained importance in recent times due to its health potential and landless cultivation. However, it has a high moisture content of 75-80%, which must be reduced to avoid spoilage and increase its shelf life before further processing. Drying and milling are common practices to reduce the moisture content to 10% or less, but they impact seaweeds volatile composition. This study examined the impact of drying and milling techniques includes sun drying, solar drying, hot air oven drying, freeze drying and the milling by rotary mill (pulveriser) and mechanical grinder. Around 60-80 compounds were identified based the drying and milling technique used. Principal Compound Analysis (PCA) and Discriminant Functional Analysis (DFA) revealed that both drying and size reduction influence seaweeds volatile profile. The Freeze dried *Kappaphycus alvarezii* had the least volatile change compared to other drying techniques, and rotary milling caused less volatile composition loss compared to mechanical grinding.

Keywords: Volatiles, E-nose, *Kappaphycus alvarezii*, PCA, DFA, drying, milling

Introduction
Global scenario of growing population, leads to societal challenges namely food supply, feed and fuel demand in global sustainability. But owing to climate change and current agricultural production and consumption pattern the food supply is not sufficient and it requires more than two planet earth to feed the population by 2050. Finding alternative food sources, thus brings into focus the untapped potential of marine and ocean resources. The most neglected food source, seaweed, offers many health advantages, does not need space to be grown, and are cost-effective, which has attracted many researchers and the government to feed the people in 2050. In addition to being utilised as food, seaweed also serves as a significant feedstock to produce biomass, biofuels, and animal feed. It is utilized in the pharmaceutical and nutraceutical industries as an antibacterial agent, antioxidant, lipid peroxidation inhibitor, antiproliferation agent, antidiabetic, and anti-inflammatory chemical [1]. Seaweeds were classified into three groups namely green algae (chlorophyte), brown algae (Phaeophyta) and red algae (Rhodophyta) [2].

The prospective red seaweed Kappaphycus alvarezii (KA) has a long history of producing carrageenan. It is the fifth-most grown macroalga in the world, and its chemical makeup and concentrations are very varied depending on the environment in which it grows, including temperature, salinity, sunlight, weather, light intensity, wave strength, depth, and others [2]. The bio stimulant properties of KA are also known for improving crop quality and plant development in a wide range of crop varieties. It has been examined primarily for its primary metabolites, such as polysaccharides, proteins, and minerals, and it also has beneficial nutraceutical and medicinal qualities. The secondary metabolites of the KA were produced by various stress conditions namely due to exposure to UV radiation, temperature change, salinity, or environmental pollutants. KA mainly used for the production of carrageenan, gel forming and polysaccharides [3].

Seaweed degrades faster within 10-15 days after harvest [4]; thus, steps have to be made to prevent seaweed from destruction. Drying is the oldest method of preservation employed to safeguard safety and increase the shelf life of the produces. it's easier transportation, storage and convenient for economical purpose [5]. A study on drying of red seaweed (*Pyropia orbicularis*) using Freeze drying, vacuum drying, solar drying and convective drying showed

that antioxidant properties and pigments of seaweed degrades significantly irrespective of drying [6]. Another study by [7] on three seaweed variety from Europe namely *Ulva rigida, Gracilaria sp and Fucus vesiculosus* was studied with respect to oven drying and freeze drying, the results suggest that oven drying had negative impact on phenolic and antioxidant properties as well based on temperature of drying. Another study by [8] on edible Irish brown seaweed *Alaria esculenta* on blanching and drying methods also found that blanching and drying has influence on volatile profile of the seaweed. Thus, during drying process and size reduction process, the bioactive compound quality changes, were well known and its characteristics changes must be studied in detail.

Flavor has a significant impact on consumer acceptance of food products, thus being a related factor. Volatiles, that are molecules with a moderate hydrophilic nature, higher vapor pressure, and low molecular weight, also have a relationship to food quality. The extensive variety of volatile metabolites included hydrocarbons, alcohols, aldehydes, carboxylic acids, esters, furans, pyrazines, pyridines, sulphur compounds, amines, and halogenated compounds, all of which were affected by the species, region of origin, and processing techniques [9]. The major secondary metabolites were phenolic compounds, halogenated compounds, sterols, terpenes, and small peptides. It also contains wide range of antioxidants, antibacterial, antifungal activities and only few studies have wide range of insight into it [10]. *Alaria esculenta* seaweed cultivated in two regions namely Scotland and Ireland of United Kingdom was evaluated for identifying the microbial quality of seaweed using volatile variation, stored at various condition over a period of time using e-nose reported by [4]. Seaweed volatile identification is a crucial aspect that has been effectively accomplished using GC-MS, Solid Phase Microextraction GC-MS, and Headspace. Only a small number of investigations have been done on e-nose in relation to GC-MS throughout the past few decades. The biological machine olfaction known as E-nose imitates the human nose. E-nose was used to analyse flavour components quickly and accurately in food products, without the need for sample preparation. It uses the volatile gases heated and collected in the head space to detect and identify the volatiles present in the sample [11]. E-nose with chemometrics was considered as an effective tool for non-destructive food quality monitoring. Thus, this study attempts the notable volatile changes during drying of seaweed using e-nose.

2. Materials and Methods

Seaweed (KA) was collected from Gulf of Mannar region, Ramanathapuram, Tamil Nadu, India. The samples were dried for 3 days by sun drying and then the moisture was brought down to 20% followed by that three different drying was performed namely tray drying, solar drying and hot air oven drying for 1 hour at 50 °C to bring down the moisture content to below 10%. Then the samples were grinded using three different size reducing machines namely mechanical grinder and rotary mill pulveriser for 30 seconds each. The dried samples were taken as control which then compared with two size reduction technique for notable change in volatile composition using e-nose. The samples were stored in air tight containers at -20 °C till further analysis. The samples were named as Kappaphycus Sun Dried Control (KSDC), Kappaphycus Solar dried (KSOD), Kappaphycus Hot air oven dried (KHAOD) and Kappaphycus Freeze dried (KFD) in case of drying. In case of milled samples mechanical grinded samples were named as Kappaphycus Solar dried Grinded (KSODG), Kappahycus Hot air oven dried grinded (KHAOD), Kappaphycus Freeze dried Grinded (KFDG) and the pulverised samples as Kappaphycus Solar dried Pulverised (KSDP), Kappaphycus Hot air oven dried Pulverised (KHAODP) and Kappaphycus Freeze Dried Pulverised (KFDP) respectively.

2.1 E-nose

The volatile profiling of seaweed samples dried and pulverized were obtained with the help of Ultrafast gas chromatography- Flame Ionization Detector (UGC-FID) also known as E-nose. Volatile profiling of dried and milled KA samples was performed by Heracles II electronic nose (Alpha MOS, Toulouse, France). To calibrate the GC columns the standard used for reference is n-alkanes of C6-C16 (Sigma-Aldrich) that converts retention time into kovats indices. This method was adopted from Roy, *et al.*, 2022 [12] with slight modification based on the sample and treatment condition. The equipment contains an autosampler with sample holder, an injection needle, an agitator system, a detector system, and a computer system connected to it. The sample holder or sample port contains a provision of keeping 45 samples at a time. An autosampler (PAL-RSI, CTC analytics, Switzerland), trap composed of Tenax TA polymer, two metallic columns (10m Length x 0.18 mm diameter) and flame ionization detector (FID's). the column made up of two different polarities, a non-polar column (MXT-5) made of cross bond 5% diphenyl/95% dimethyl polysiloxane), medium polar (MXT-1701) of cross bond 14% cyanopropyl phenol/86% dimethyl siloxane). Volatile identification was performed by using alkane standard (Restek, Bellefonte, USA), along with kovats retention index were calculated (RI). Two grams of samples were placed in 20 mL vials which were capped with PTFE/Silicone seals in order to prevent penetration of environmental odours. Then the samples were placed in the agitator with the help of magnetic cap holder and then agitation along with heating takes place. It helps in trapping of volatiles at the headspace of the vails at the required temperature. The trapped volatiles were subjected to detector using injection needle to the detector. Based on adsorption and partition principle the volatiles will pass through the column and results in compound identification. Aro Chem Base (V6, Alpha MOS, Toulouse, France) library was used to identify the volatile compounds by three ways: by comparing the experimental Kovats RI with the RI in the aro Chem Base library data, by comparing the relevance indices given to each volatile compounds using the same software and by verifying the literature in its database provided with more than 99,000 different molecular information.

Table 1: The condition for volatile compound identification was given as below

Auto sampler condition	
Incubation time (min)	20 min
Incubation temperature (°C)	80
Agitation speed (rpm)	500
Agitation on/off (s)	5/2
Syringe temperature (°C)	90
Trap	
Trapping temperature (°C)	50
Injection volume (µL)	5000
Injection speed (µL/s)	125
Trap pre heating time (s)	35
Post injection transfer time (s)	10
Trapping duration (s)	50
Valve condition	
Valve temperature (°C)	250
Oven condition	
Initial oven temperature (°C)	50
Final temperature (°C)	250
FID 1 & 2 condition	260
FID 1 & 2 Detector temperature (°C)	

2.2 Data analysis

For classifying and pattern recognition of dried and milled samples, to discriminate the difference between the samples, the chemometric techniques were employed. Two techniques namely Principal component analysis (PCA) and discriminant function analysis (DFA) was performed. The PCA score plot helps in visualizing the difference among the drying and milling process, whereas DFA helps in estimation of possibility of separation among drying and milling treatment effect [13]. PCA is an unsupervised learning technique to reduce large size of dataset into smaller ones which retains original information in reduced form and identify the correlations and patterns in between the samples. PCA makes the data in clusters, to explain the difference among the sample's treatment conditions. In score plot, each PC represents the variance direction in the data, with the largest variance as the indication of most important data. A loading plot with PCA represents how strongly each variables impact the discrimination of the sample [11]. DFA is a multivariate statistical tool to predict the variations among treatments (drying and milling) into predefined groups or clusters. It is an efficient tool to classify among groups [12].

3. Results and Discussion

3.1 E-nose analysis

Volatile compound of food and its changes with respect to processing varies based on numerous factors. The volatile profile of KA samples implemented to drying and milling was done using e-nose (Alpha MOS). Volatile compounds were identified, based on e-nose retention time, kovats retention indices and aero-chem based library of about 60-85 compounds based on treatment conditions. The major contributing peak area compound identified in e-nose after KSDC was 1-heptadecene (29.64%), 2-propanol (9.91%), 2,3-pentanedione (8.55%), 3-methylpropanal (6.93%), butanoic acid (4.18%), 2-methylpropanoic acid (3.37%), 3-methylhexane (2.63%), benzene (2.08%), 1-propanol (1.52%), (E, Z)-2,4- Heptadienal (1.48%), 2-methylpropanal (1.23%), phenol (1.20%) and 2-octanol (1.06%) respectively. The KSOD possess 3-methylheptadecane (34.76%), 2-propanol (7.36%), (E)-4-octene (5.58), 2-tridecanone (4.82%), 2,3-pentanedione (4.60%), pentanoic acid (4.56%), n-nonanal (3.22%), 2-butyloctanol (3.22%), butan-2-one (2.88%), pyridine (2.62%), 2-methyl-octane (1.17%), 1-octen-3-one (1.08%), 2-octanol (1.21%), (E,Z)-2,4-heptadienal (1.48%), 5-methyloctadecane (1.38%), 2-nonenal, (E)- 1.14% respectively. The major components of KHAOD were namely 2,6,10, 14-tetramethylpentadecane (38.60%), 2-propanol (4.10%), pentanal (2.96%), pyridine (2.79%), E-4-octene (4.71%), pentanoic acid (2.53%), 2-octanol (1.85%), n-nonanal (3.16%), decanal (1.02%), 2-undecanol (1.51%), 2,4-decadienal, (E, E)- (1.93%), 2-methyltetradecane (1.91%), 2-tridecanone (6.56%), and 5-methyloctadecane (1.51%) respectively. KFD showed higher relative area of propenal (59.09%), 2-propanol (20.29%), butan-2-one (1.06%), (E)-4-octene (1.18%), 2, 6, 10, 14-tetramethylpentadecane (6.11%) and cis-9-hexadecenoic acid (1.30%) respectively.

The KA samples subjected to various drying were grinded using mixy, whereas the highest retained milled compound in KSODG sample was 2-propanol (5.35%), chloroform (2.24%), 2,3-pentanedione (6.44%), 3-methylheptane (1.20%), (E)-4-octene (6.27%), 3-methybutanoic acid (1.22%), pentanoic acid (1.68%), dimethyl trisulfide (1.06%), psi-cumene (1.33%), 2-methyldecane (1.15%), 3-nonen-2-one (1.45%), 2-butyloctanol (1.85%), 2-methyltetradecane (1.44%), 3-methyltetradecane (6.60%) and 3-methylhexadecane (41.52%) respectively. KHAODG contained compounds were 2-propanol (4.91%), 1-propanol (1.00%), 3-methylpentane (4.27%), 2-propanol (4.91%), 1-propanol (1.00%), 3-methylpentane (4.27%), 2,3-pentanedione (4.41%), 3-methylheptane (1.52%), (E)-4-octene (5.03%), pentanoic acid (1.72%), 3-octanone (2.36%), n-nonanal (1.90%), 2,4-decadienal (E,E)- (1.92%), 2-methyltetradecane (1.99%), 2-Tridecanone (6.62%), 3-Methyl hexadecane (45.23%), 2-octadecene (Z) (0.612%) and 5-methyloctadecane (0.624%) respectively. KFDG had highest intensity compound namely propenal (60.72%), 2-propanol (15.48%), 2-tridecanone (1.16%), 2,6,10,14-Tetramethylpentadecane (8.38%) and 4-methylnonadecane (1.79%) respectively. The KSODP had the highest relative area percentage in acetaldehyde (1.12%), 2-propanol (6.60%), 3-methylpentane (2.97%), 3-methylhexane (1.09%), pentanal (8.05%), propanoic acid (2.99%), 3-methylheptane (2.00%), (E)-4-octene (14.13%), 2-methylbutanoic acid (14.95%), m-xylene (1.28%), 1,2-dimethylbenzene (1.56%), nonane 3-methyl (1.06%), 3-octanone (1.27%), (E,Z)-2,4- Heptadienal (1.04%), hexanoic acid (1.65%), 2-acetylthiazole (1.10%), 1-octanol (1.20%), n-nonanal (1.98%), 5-methylundecane (1.70%), 2-tridecanone (2.45%) and 2-heptadecene (E) (14.49%) respectively. KHAODP had highest peak area % of 2-methylpropanal (3.94%), 3-methylpentane (2.84%), 3-methylfuran (2.75%), benzene (2.69%), 3-methylhexane (3.73%), 1-hexen-3-ol (1.87%), 2-methylthiophene (1.08%), 3-methylheptane (3.69%), 1-hexanol (12.21%), heptanal (1.04%), 4-pentanolide (1.47%), phenol (2.02%), 2-butyloctanol (1.44%), 2-tridecanone (4.75%) and 2-heptadecene (E) (35.19%) respectively. The volatiles identified in our study was compared with the results of [14] in which around seven dehydrated species of edible seaweed volatiles were examined and the results suggested that the most of the volatiles of seaweeds were similar in nature, which are the most potent seafood and seaweed odour

characteristics. The results of our study showed that there was a clear influence of drying and milling on the volatile profile of the compound and are displayed into radar graph (figure 1). Similar observation was made by [15] using GC/MS coupled MOS e-nose and flash GC e-nose for volatile characterization of Chinese jujubes as affected by various drying methods. The results suggested that the compounds identified were mostly similar with respect to drying, but there was significant difference in the aroma profile using various techniques. There was significant variation among the volatile profile of different drying techniques observed and found that freeze drying preserves most of the volatile compound similar to the results of this study. This may be due to the operating condition of the sample at freeze drying and low temperature ultimately prevent the volatile loss of the sample material. The influence of volatiles with respect to milling was observed similar to our result by [16] for rice milling and the results showed that degree of milling affects the volatile content of rice. The result also suggest that e-nose could be an effective tool in discrimination of volatile nature of compounds with respect to treatment.

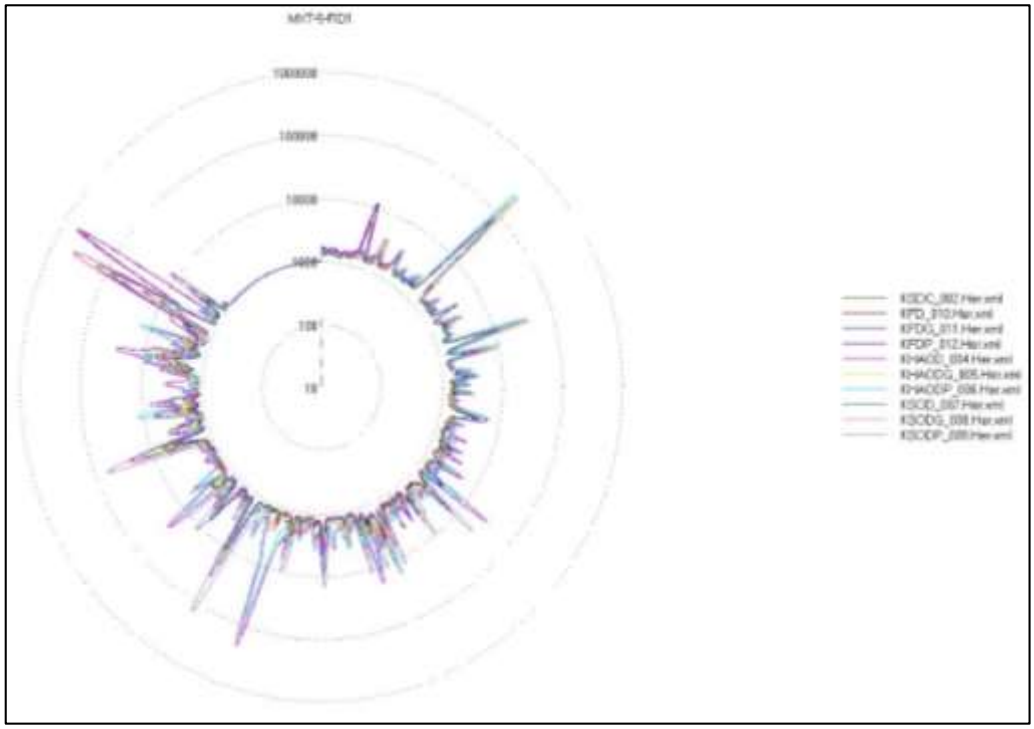

Fig 1: Radar Diagram and superimposed plot of dried samples

3.2 PCA for dried samples

The PCA a multivariate statistical tool was employed to reduce the dimensions of the obtained e-nose dataset and for the primary assessment of the relationship between the groups [11]. PCA helps in discriminating the sample before and after treatments. The 2D plot of PCA contains x and y axis representing the contribution rate of two major components obtained in the data set. In general, PC1 confronts to most variance in the sample, whereas PC2 represents the second most variance and it goes on [17]. All the variables were represented in different colour (groups) in the loading plot of PCA with positive loading and negative loading given below. The scattered diagram of PC1x PC2 was grouped based on the drying condition was observed on the figure 2. The scattered diagram reveals that, there was distinct difference between the control (KSDC), KHAOD, KSOD in comparison with KFD. The results represent that 100% discrimination was observed among the samples with PC1-56.458% variance and PC2-42.944% variance respectively. From PCA plot it was clear that KHAOD, KSOD, KSDC forms separate cluster, which denotes a close relation of volatile compounds concentration associated within these group, whereas the volatiles with KFD were different within the group. The KFD lies in the positive loading of the PCA and all other lies in negative loading side represents the KFD had the most valuable component in comparison with other dried samples. Thus, from figure 2, it could be concluded that KFD is best technique to be adopted to preserve the volatiles of KA seaweed in comparison with other different technique employed. When dried seaweed subjected to milling figure 3 and 4 represents the mechanical grinded and pulverised samples in comparison with control KSDC. The scattered plot of figure 2 (PC1xPC2) had variance of PC1 57.754% and PC2-35.577% were the KSODG lies in the positive loading and KSDC, KHOADG lies on the negative side. This represents for mechanical grinding the most volatile retained was by KSODG in comparison with other drying technique and it was not advisable to go for mechanical grinder when the seaweed samples were freeze dried since it lies in 3rd quadrant the most negative side of the PCA plot. The KSODG could be adopted in case of industrial application because of cost effectiveness, energy saving and could easily be adopted in large scale processing, but uniform particle size could be possible only after sieving. The figure 4 represents the pulverised sample PCA plot in this case, it was indeed opposite to the figure 3. It represents to KSDC, KHAODP had similar volatile compounds when subjected to pulveriser and lies in negative loading side, whereas KFDP lies in the positive loading side and KSODP lies on the most negative loading side. This represents that KFD along with pulverizing could save the most volatile compounds in

comparison with other grinding technique and drying technique. It could possibly be adopted in industry with high economic background and the industry which needs more volatile protection of seaweed for further processing of it. From the overall observation of PCA it was clear that KFD with pulverisation could be effective in comparison with KHAOD, KSOD, KSDC, KSODG, KSODG, KHAODG and KHAODP respectively. E-nose with PCA as chemometric approach found promising to distinguish the volatile distribution between different drying technique and milling technique for seaweed samples in our study.

3.3 Discriminant Function Analysis (DFA)

The DFA is basically another chemometric technique employed. This study was done for grouping the data set and separating among groups. DFA provides full discrimination of all the groups or cluster [12]. The method helps in understanding the differentiation between all the groups of dried and milled samples. The DFA of (figure 5) DF1xDF2 represents the variance of DF1 as 98.704% and DF2 as 0.864% like that of PCA figure 2. All the other drying technique employed lies in the negative side of the quadrant whereas the control lies in the most negative side of the quadrant and only positive side of the quadrant noted was KFD. This clearly represents the KFD could be an alternative drying technique adopted instead of Sun drying to preserve volatiles of seaweed. To confirm with grinding techniques figure 6 and 7 of DFA was employed with the grouping of dried and mechanical grinded samples as well as dried and pulverized samples. The results of figure 6 represents that KFD along with mechanical grinding could not be advisable and KSODG could be possibly adopted technique if the industry adopt mechanical grinding, whereas figure 7 represents that KSODP and KFDP could possibly the alternative technique for grinding and drying in comparison with conventionally adopted mechanical grinding with sun drying. The KSODP and KFDP preserves the high value volatile compounds of seaweed and this DFA provide more insight and knowledge over the PCA to distinguish the groups for better clarity in the results. Thus, from the chemometric techniques along with seaweed volatile profiling by drying and milling it was suggested that KSODP and KFDP could be adopted as new technique for better retention of volatiles in seaweed in comparison with conventional practices. Among the KSODP and KFDP according to PCA it was suggested that KFDP could be best suitable method for preserving flavour of seaweed.

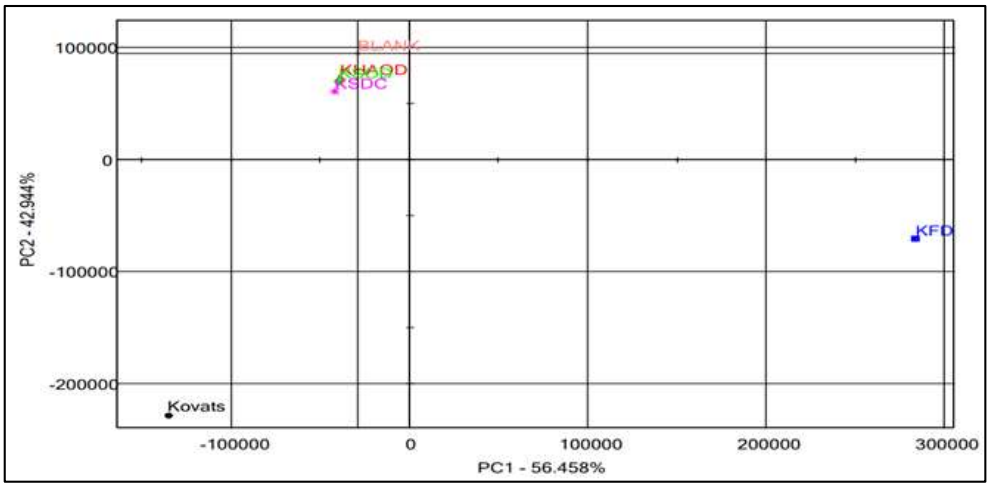

Fig 2: PCA plot of dried samples

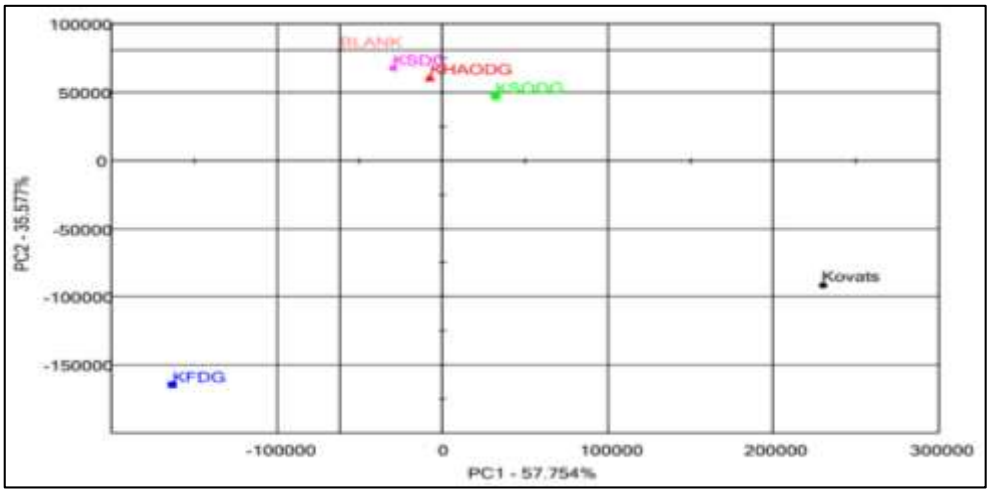

Fig 3: PCA plot of Dried-Mechanical Grinded samples

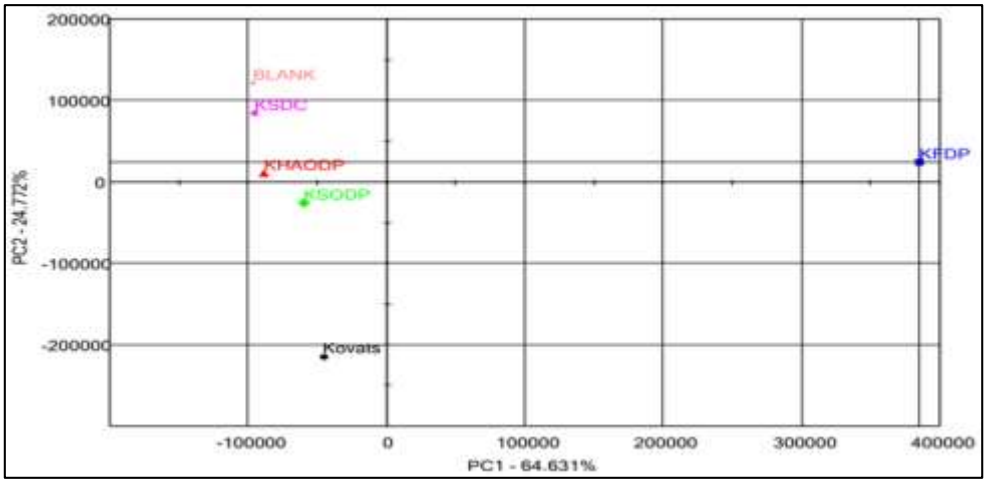

Fig 4: PCA plot of Dried-Pulverised samples

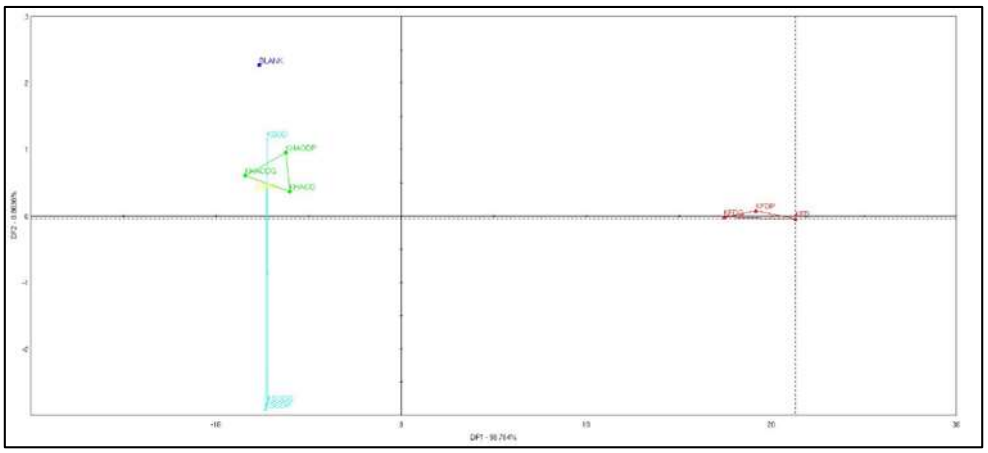

Fig 5: DFA plot of dried samples

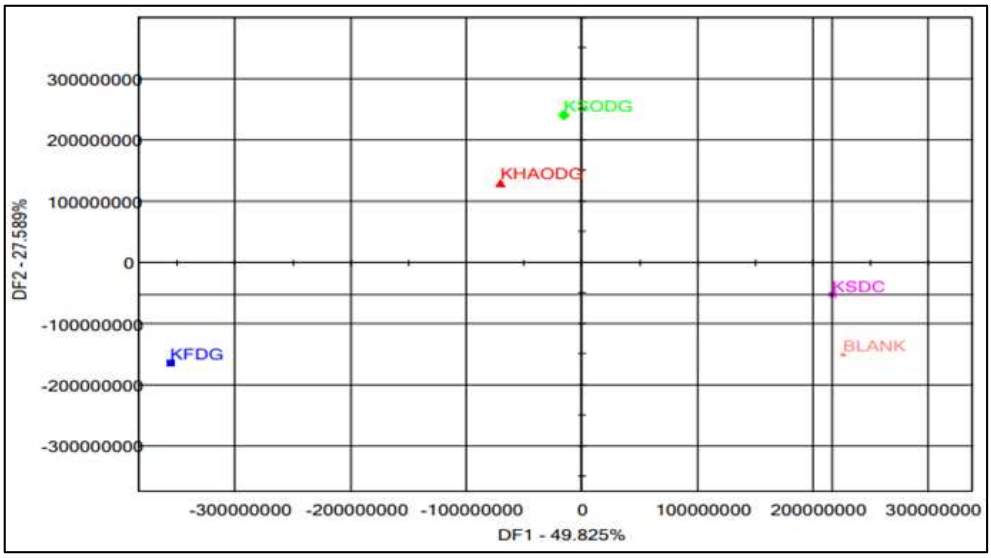

Fig 6: DFA plot of Dried-mechanical grinded samples

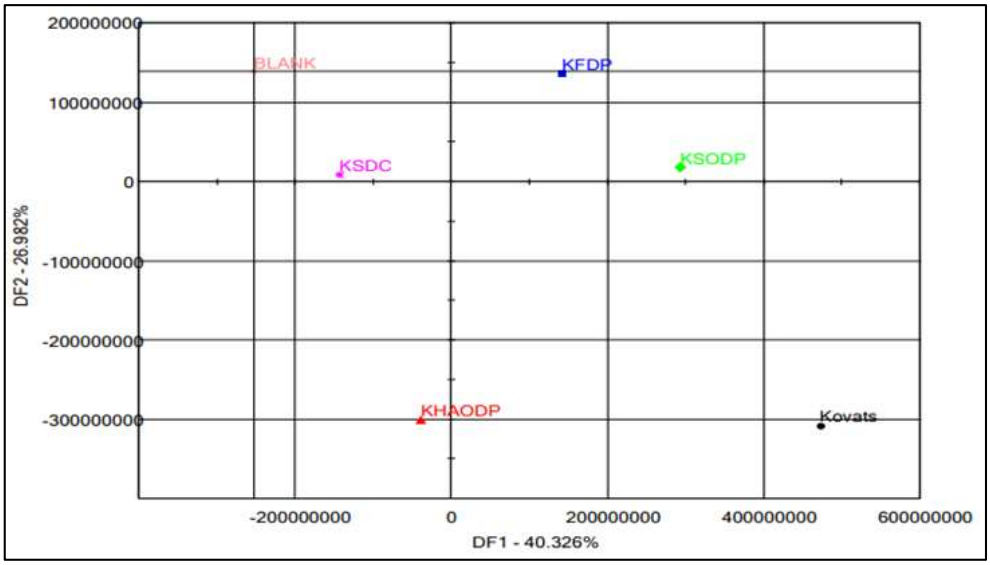

Fig 7: DFA plot of Dried-pulverised samples

4. Conclusion

The volatile profile of seaweed was less explored area of research and was carried out to know the effect of drying and milling on volatile profiling of seaweed. The obtained e-nose datasets were further used for two different classification models namely PCA and DFA. The predictive accuracy for calibration obtained by PCA and DFA method was 99.402% and 85.706% respectively. This study conclude that e-nose coupled with chemometric technique (i.e.., PCA and DFA) could be a promising tool to explore the difference between the treatment effect and the control. The PCA and DFA clearly distinguish the volatile difference in between the groups and found that KFDP could possibly be the technique adopted for preserving the volatile composition of seaweed in comparison with the conventional technique. Further volatile compound identification and fishy odour compound identification could be employed by e-nose coupled chemometric technique which could be used in flavour mimics of seafoods and elimination of fishy odour in use of seaweed as food ingredient.

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