STUDIES ON IMPROVEMENT OF FRIED AND BAKED FOOD PRODUCT QUALITY AND PREFERENCE USING ELECTRONIC NOSE TECHNOLOGY

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PROFORMA-1

I**, Anupama Bose** registered on **18.05.2016** do hereby declare that this thesis entitled "**Studies on improvement of fried and baked food product quality and preference using electronic nose technology"** contains literature survey and original research work done by undersigned candidate as part of Doctorial studies.

All informations in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct. I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019" , and the level of similarity as checked by iThenticate software is 10%.

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CHAPTER 1:

Introduction and literature review

1. Introduction

Frying and baking are very important unit operations associated with the manufacture of daily consumable most popular food products. The current investigation encompasses these two important thermal processing operations in relation to fried potato crisps and bread, respectively. Globally consumed fried food products, prepared by conventional frying process are potato crisp and chip, tortilla chip and breaded chicken; and those by baking are bread, cake, cookie and biscuit. Fried and baked food products have unique texture, flavor and color and thus have very high sensory appeal and these organoleptic qualities are commonly assessed by sensory evaluation techniques.

Sensory evaluation plays a vital role in taking decisions of acceptance or rejection of fried and baked products which are performed using several discrimination testing and measurement techniques (Stone and Sidel, 2012). Fundamentally, sensory evaluation can be divided into two categories: subjective i.e., linguistic expression about the product,and objective (Das et al., 2011).Just-about-right scale (subjective evaluation of food products) isused for quantification of responses to stimuli for the purpose of utilizing descriptive and inferential statistics (Stone and Sidel, 2012). This scale is frequently encountered in large scale consumer testing and anchored with the comments such as “too much”, “too little” or “about right for each product’s quality attribute”. The outcome of such evaluations are associated with numerous problems since the same do not follow standard rules for determining how significant the differences are among the responses corresponding to each sensory attribute of a food product is to be considered significant (Stone and Sidel, 2012).

The hedonic scale on the other hand is an objective evaluation of food products and is kost commonly used in sensory science. This 9-point (1-9) scale is unique and useful for its general applicability and easy-to-use nature (Stone and Sidel, 2012), where “1” and “9” imply “dislike extremely” and “like extremely”, respectively(Lawless and Heymann, 2013).Althoughhedonic rating is widely used in research and industries alike, it delivers a good assessment of a food product, provided the panellists are trained. Otherwise, there is a probability to receive a biased and fuzzy opinion about the food products especially for the scores 5 and 6 which signify “neither like nor dislike” and “slightly dislike”, respectively. Hiring trained sensory professionals is difficult for laboratory investigations. Therefore, it is more realistic to use linguistic assessments instead of numerical values (Lin, 1999) in

laboratory investigation as well as in consumers' survey thereby mitigating chances of incorrect assessments by untrained mixed populations.

In the present work, sensory evaluation of fried and baked products were performed by a 30-member of semi-trained panellists(15 men and 15 women)comprising of Jadavpur University faculty members and research scholars (in the age group of 20-45 years). Although the panellists willingly performed subjective sensory evaluation, sensory-perceptual alterations and biasness in human perceptions in the mixed population semi-trained panel obscured objective sensory analysis of the food products. Fuzzy logic analysis of sensory outcomes can successfully overcome these shortcomings of sensory evaluation and was therefore used in this work for sensory evaluation of both fried and baked products.

1.1. Basic concept and applicability of fuzzy logic

Fuzzy logic (a state-of-art technique) is an important tool in the domain of artificial intelligence (AI) by which imprecise, vague data can be analysed and important conclusions regarding rejection or acceptance of the product can be drawn (Das, 2005). Fuzzy logic uses its unique subjective linguistic expression scale for sensory evaluation. The outcomes of the subjective sensory evaluation are considered as input variables in fuzzy modelling. Based upon this fact, sensory scale factors are designed for sensory attributes in the following way: "not satisfactory," "fair," "medium," "good," and "excellent". Panelists are asked to provide tick (√) mark to an appropriate scale factor for apposite quality attributesof a food product (Das, 2005). They are also asked to rank the quality attributes of the food products in general by putting a tick (√) mark to an appropriate scale factor, *viz*. "not at all important," "somewhat important," "important," "highly important," and "extremely important" (Das, 2005). All these subjective evaluation data are fed to fuzzy modeling to develop a relation between the above-mentioned input variables and the dependent variables ("acceptance, rejection, ranking, strong and weak attribute") with the help of membership function and set theory (Chakraborty et al., 2011). The stages involved in fuzzy modeling are elaborated in **Chapter 2**. The general steps involved in fuzzy logic are presented in Figure 1.

Applications of fuzzy logic in food science and technology:

Fuzzy logic has been applied for different purposes in different areas of food technology as shown in Figure 2 (Perrot et al., 2006) as a 3-D pie chart. The key areas of application of fuzzy logic in development of popular food products are presented in Table 1.

1.2. Application of fuzzy logic in the present work

Virgin coconut oil (VCO), although a very popular cooking oil of south-India has limited sensorial acceptability nationally and globally since it imparts a typical obnoxious odor (owing to presence of octanoic acid) to fried products jeopardizing the organoleptic acceptability of the same (Ghosh et al., 2016). Previous investigations conducted in our 'Green Technology Laboratories I and II', were successful in completely removing the obnoxious odor of VCO by gamma-irradiation of the oil at 4.2 kGy followed by 28 day-storage at ambient conditions (23 ± 2 °C, 80% RH). Absence of this typical disagreeable odor note was judged sensorially by a semi-trained panel (of similar composition as has been described in section 1) (Ghosh et al., 2016). This deodorized virgin coconut oil (DVCO) has been successfully used in formulating a green-tea based beverage providing body to the drink (Ghosh et al., 2016) and cookies as a partial substitute of hydrogenated fat (Mondal et al., 2022).

To the best of my knowledge, application of deodorized virgin coconut oil (DVCO) as a frying oil has not been reported yet. Since gamma-irradiation did not alter the phytochemical and antioxidant properties of VCO (Ghosh et al., 2016), DVCO could possibly be used as a healthy frying medium, as a substitute of the most commonly used polyunsaturated fatty acid (PUFA)-rich soybean oil. There remains a possibility that if an unpleasant odor persists in DVCO or is regenerated by flavor-reversion during frying, it would impart unacceptable flavors to fried products; whereas in the above-mentioned food products, masking of unpleasant odor of DVCO (if any) by green-tea flavors in the beverage, and by baked flavors in cookies did not compromise their sensory acceptabilities. Thus it is necessary to re-validate complete removal of obnoxious odor notes in DVCO prior to its application as a frying oil, lest it imparts disagreeable flavor to the fried product and further obscures detection of onset of rancidity in the same. Therefore, re-validation was conducted by application of fuzzy logic to ensure complete absence of obnoxious odor in DVCO. Themethodologies developed (importance for development of new equation under special condition, elaborated in Appendices 2A) to fulfill this aim has been elaborated in Chapter-2and the findings validated that DVCO was completely devoid of its obnoxious odor and thus could be a used as a new frying oil.

2. Frying

Frying is a very complex phenomenon including simultaneous heat, mass and momentum transfers accompanied by a series of physical and chemical reactions (Eichenlaub et al., 2015). During frying, heat transfer occurs from oil to the product being fried which leads to mass transfer i.e., water evaporation and oil uptake (Xu et al., 2020). Momentum transfer is accompanied by convection (i.e., vapor leaving the fried materials) or by molecular forces (i.e., viscous stress or pressure) (Farid et al., 2009). In addition to this, chemical constituents (e.g., sugars, amino-acids and water) of food materials react with each other at high frying temperatures, and physical reactions (e.g., water evaporation and oil uptake) too occur in tandem, rendering changes in the structures of fried products (Bouchonet al., 2001).

There are three types of frying as under:

(a) *Deep or immersion frying*: Deep frying is defined as a process where food is fried at temperatures between 170 °C to 190 °C for 3 min by submerging it completely in hot oil. The surface to volume ratio (S/V) of food matrix in oil for this type of frying is around 1.0 cm^{-1} (USDA, 2020). It always imparts better color, texture, mouthfeel and overall appearance to the products vis-à-vis other modes of frying. It is most used by food industries.

(b) *Shallow frying*: Shallow frying is performed at a relatively low temperature of 130 °C for a longer period of time i.e., more than 5 min in accordance with Pedreschi et al. (2004). Therefore, shallow-frying is considered as a less intense cooking technique. The S/V ratio of food matrix in hot oil for this type of frying is around 0.5 cm^{-1}.

(c) *Par frying*: Par frying refers to the technique of partially cooked foods so that they can be finished later. Par frying is performed in the temperature range of 160 °C to 200 °C for 10 s to 150 s. The S/V ratio of food product in hot oil for this type of frying is approximately 0.75 cm^{-1} (USDA, 2020).

2.1. Attributes induced by frying:

Frying imparts several attributes to the fried products which are compiled in Table 2.

Apart from these attributes, few undesirable compounds also form as a result of degradation of the fatty acids in the oil and in the food product which are retained in both the frying oil and in the fried products. These compounds undergo reactions with our body proteins,

hormones and enzymes post consumption and are therefore detrimental to human health. They are therefore classified as toxins.

2.2. Toxin(s) formation in fried potato crisps during frying in DVCO

A potent toxin namely acrylamide (AA) is formed in fried potato crisps at high temperature by Maillard reaction and lipid peroxidation of PUFA via formation of acrolein (Stevens and Maier, 2008). Since DVCO is a PUFA-lean oil, the AA formation in fried potato crisps via acrolein would most likely be negligible. Therefore, the responsible factor for formation of AA in DVCO-fried potato crisps is solely Maillard reaction (Gökmen et al., 2007).

2.2.1. Dietary exposure to AA

The presence of AA in heat-processed foods is a world-wide health concern, since this compound has been classified as a probable human carcinogen by the International Agency for Research on Cancer, Lyon, France (Rifai and Saleh, 2020). Different categories of foods contain different levels of AA as shown in Table 3. Among different food products, fried potato products such as French fries contain the highestamount of AA followed by potato crisps owing to their large surface areas provided by their thin top and bottom surfaces. The food sources providing ~38% of caloric uptake (Petersen and Tran, 2005) and havingdark colors(Rifai and Saleh, 2020) are assumed to be the major sources of AA. The average intake of AA by consumers may vary according to origin and dietary habits. Tolerable daily intake for averting the carcinogenic effect has been estimated to be 2.6 $mg/kg.day^{-1}$ (WHO, 2006; Tardiff et al., 2010).

2.2.2. Mechanism of AA Formation in fried potato crisps

It has been reported that AA formation during heating involves two main precursors, namely, reducing sugars and the amino-acid asparagine (Zyzak et al., 2003). This reaction follows first order kinetics and is a surface phenomenon. High temperature (> 120 ℃) and high moisture content (> 40%) of foods favor formation of AA (Zyzak et al., 2003). During Maillard reaction, reducing sugars (glucose and fructose) condense with amino-acids, mainly asparagine, to produce a Schiff base (explained in section 2.3.1) which forms AA via decarboxylation (Figure 3). Research has shown that reducing sugar is the limiting factor for AA formation in potatoes (Muttucumaru et al., 2017) during frying.

Formation of AA via acrolein:

Acrolein is formed during pyrolysis of PUFA (in oil) during high temperature frying. Formation of acrolein mainly depends on the composition of the frying oil and the temperature (> 150 ℃) of frying (Ubaoji and Orji, 2016). Acrolein further undergoes oxidation to form acrylic acid which reacts with ammonia (generated by amino-acid degradation by heat) to produce AA (Figure 4).

2.2.3.Toxicities induced by AA

Neurotoxicity, genotoxicity and carcinogenicity are reportedly the major consequences of AA consumption by human (Rifai and Saleh, 2020). AA is also known to disrupt the central nervous system by inhibiting human neuroblastoma and glioblastoma cellular differentiations (Chen and Chou, 2015).

2.2.4. Mitigation strategies for AA

Since 2002, scientists from all over the world in collaboration with agencies such as FAO and WHO have been working on different ways to reduce the levels of AA in the most widely consumed products, namely potato and cereal-based products. On the day this thesis was compiled, 276 research articles were cited on Scopus web engine on mitigation strategies for AA. It is evident from the literature review that treatments for AA attenuation in different food products including fried potato products were either single-stage which comprised of addition of different additives (such as enzymes, inorganic salts, fiber and plant extract);or modification of blanching, fermentation and/or processing conditions. A comprehensive and updated review on different mitigation strategies for reduction of AA in different food products is presented in Table 4.

2.2.5. Mitigation strategy of AA formation in fried potato crisps in the present work

It is evident from the above literature survey that treatments used to mitigate AA in fried potato crispscouldachieve a maximum of 90% reduction and with no improvement of sensorial parameters of the fried crisps. The current work envisages mitigating ~99% AA in fried potato crisps by adopting hurdle technology accompanied by improved sensorial acceptability of the products. These aspects have been critically enumerated in **Chapter 3**. Frying industries also face an additional challenge from another toxin, mediated by oxidative degeneration of linoleic acid present in commonly used frying oils.

3. Industrially used frying oils and mechanism of formation of 2,4-decadienal

Among different aldehydes (formed during frying), *z,z*-2,4-decadienal (*tt*-DDE or 2,4-De) is the major fatty acid decomposition product in the linoleic acid-rich oils (Boskou *et al*., 2006; Wu et al., 2001). Palmolein and soybean oilare commercially used common frying oils which contain significant amounts of linoleic acid i.e., ~10 % (Chenyan et al., 2018) and ~49 %, respectively. During high temperature frying, i.e., ≥ 150 ℃, 2,4-De forms (Figure 5) by homolytic cleavage of linoleic acid (Grootveld et al., 2020) in such common frying oils which subsequently accumulates in the fried products (Boskou et al., 2006).

3.1. Toxicity induced by 2,4-Deadienal

Ingestion of 2,4-Deadienal (2,4-De) has been reported to induce cellular toxicity in liver and kidney of human (Hageman *et al*., 1991). There is also a potential link between 2,4-De and development of cancer in humans (Hageman *et al*., 1991). In view of above, 2,4-De is regarded as one of the potent toxicant whose formation during frying needs to be prevented. It is one of the important total polar compounds (TPC) labelled as harmful toxins in frying oils (Li et al., 2017).

3.2. Mitigation strategies for 2,4-De

Few methods have been reported to mitigate 2,4-De formation in soybean and corn oil. Hydrogenation, genetic modification and blending of oils are the main processes to decrease the UFAs of frying oil and thus enhance frying stability of the oil (Warner and Knowlton 1997).

- Hydrogenation produces trans-fatty acids and/or a metallic flavor, and it reduces the level of linoleic acid (Warner and Mounts 1993). There are reports on formation of amount of TPC in hydrogenated soybean oil with 0.1% linolenic acid (Tompkins and Perkins 2000). However, hydrogenated oil (contains higher amount of saturated fat) always has a negative impact on human cardiovascular health.
- Genetic modification of corn germ produces low linolenic acid-content corn oil which was suggested to be a potential alternative to hydrogenated frying oil (Mounts and others 1994) and had improved frying stability over normal corn oil (Warner and Knowlton 1997). However, this method is highly cost-intensive and applicability of

genetically modified crops is limited globally for several reasons (Qaim and Kouser, 2013).

- Blending of oils alter the fatty acid composition of oils (Shiota and others 1999; Mamat and others 2005) and can limit their oxidation during deep-fat frying. The blending process also causes variation in flash point of cooking medium which results in inadequate frying of the products with reduced sensorial acceptance with respect to color, texture and taste (Qaim and Kouser, 2013).

3.3. Mitigation strategy of 2,4-De in fried potato crisps in the present work

As 2,4-De formation is highly dependent on fatty acid composition of frying oil (explained in section 3.1), the current work selected a low linoleic acid frying oil viz. DVCO as an alternate frying medium under modified deep frying conditions (i.e., lower frying temperature:< 150 ℃). Thus, the aim of this study was to investigate whether this approach can be able to reduce the probability of formation of 2,4-De in fried potato crisps.

4. Rancidity in fried products

Mitigation of toxin production alone does not ensure complete safety of the fried products for consumption, rancidity development being a major predicament which occurs concomitantly with toxin production.

4.1 Mechanism of rancidity

Two major rancidification pathways are recognized in fried products (Robards et al., 1988).

(a) *Oxidative rancidity*: This occurs due to peroxidation of UFA(s) present in the frying oil and/or food product being fried (fat gets concentrated in the food product owing to oil uptake during frying). Oxidative deterioration of lipids occurs via free radical mechanism which follows three phases of initiation, propagation and termination (Feiner, 2006). UFAs (such as linoleic acid) are converted into hydroperoxides, which further break down into volatile aldehydes, esters, alcohols, ketones, and hydrocarbons; some of these compounds possess disagreeable odors. Thus unpleasant odor and taste in the fried products renders them not only sensorially unacceptable for consumption but also harmful for human health. Among all the degenerated compounds, hexanal is considered as the most important prominent rancidity marker compound (Alqahtani et al., 2018). The pathway of hexanal formation from linoleic acid (Cao et al., 2014) has been shown in Figure 6.

(b) Hydrolytic rancidity: Hydrolytic rancidity refers to development of off-flavors caused by release of short chain fatty acids from fat by the action of lipases of bacterial origin when microbial spoilage commences in fried products which have > 11% moisture content (Zeece, 2020). There are reports on deterioration of hydrogenated soybean oil with 0.1% linolenic acid by hydrolytic rancidity (*vide infra*) but with lower TPC formation (Tompkins and Perkins 2000).

4.2 Rancidity detection techniques

In order to examine the level of lipid oxidation i.e., rancidity in fried products, the content of primary and secondary oxidation productsare assessed. Hydroperoxides are formed by reactions between free radicals and unsaturated fatty acids which are tasteless and odorless and determined by peroxide value (PV). Hydroperoxides further decompose to secondary oxidation products by either peroxide scissions alone or simultaneous peroxide and fatty acid-chain scissions(Márquez-Ruiz et al., 2010). Short-chain volatiles are formed by chain scission such as aldehydes, ketones, alcohols and carboxylic acids, which are responsible for the characteristic off-flavors and odors of rancid (Zeece, 2020). Malonalaldehyde (MDA) is a potent secondary oxidation product determined by thiobarbituric acid reactive substances (TBARS) test.The conventional methods used for detection of rancidity in fried products (potato crisps)are titrimetric, spectrophotometric, chromatographic and instrumental methods (Table 5). However, these methods have inherent drawbacks (Table 6)since they can be applied only when rancidity has progressed sufficiently resulting in detectable changes in the fried products.

Well-established conventional methods (Table 5) to assess rancidity in fried potato crisps are not robust (reasons discussed in Table 6). At commercial level (industrial scale) for high sample throughput,a fast and accurate detection technique for predicting rancidity status (onset of rancidity and progression of rancidity) of fried products is required which should be simple, user-friendly and can be operated by semi-trained personnel. Therefore,there is clearly a need for development of new techniques having capabilities for rapid and accurate assessment of rancidity status of fried products, more so because these products are health-debilitating. The current work has endeavored to develop an alternative method based on electronic nose (e-nose) technologyfor rapid and accurate assessment of rancidity status offried potato crispsovercoming the above-mentioned shortcomings (Table 6) of conventional methods. It is opined that the e-nose based method would help fulfill fried food product

regulations by radio monitoring of their rancidity status during manufacturing, storage and transportation.

It was evident from our previous investigation (Ghosh et al., 2016) that linoleic acid (~2% w/w, lower than other common frying oils) in VCO did not undergo radiolysis by gamma-irradiation and therefore its content was unaltered in DVCO. Moreover, the oil uptake (~12 ml/100 g) of treated potato crisps during frying in DVCO (DFC: DVCO-fried crisps) was significantly lower vis-à-vis the soybean oil-fried products i.e., SFC (soybean oil-fried crisps). These aspects have been further elaborated in Chapter 3. During storage, oxidative rancidity of DFC produced comparatively low hexanal (owing to low linoleic acid content of DVCO and low frying temperature) vis-à-vis SFC (conventional deep frying temperature).Although rancidity progresses with storage time post frying, it initiates immediately when the oil is heated and the product is being fried. Detection of onset of rancidity in the same was not possible since the concentration of hexanal was below the LOD (0.89 ppm) of GC-FID analysis. However, this could be successfully accomplished by e-nose technology. Both onset and progression of rancidity w.r.t hexanal in DFC and SFC were rendered easy and accurate by e-nose analysis of hexanal, post-screening of sensors using linear support vector machine (SVM) learning tool.

5. E-nose system

An e-nose comprisesofgas sensors that simulate the human nose. E-nose has shown great promise and utility in fast and accurateassessments of quality characteristics of food samples compared with conventional detection methods (Tan and Xu, 2020). This state-of-art technology is highly sensitive and facilitates cost-effective, reliable, user friendly and time-saving analysis. On the day this thesis was compiled, 4 published research articles were cited on Scopus web engine on rancidity assessment of fat-rich products by e-nose technology. These studiesdo not includeassessment of onsetof rancidity in fried food products which will be reported here for the first time.

5.1. Mode of operation of e-nose system

The e-nose system consists of (a) a sample holder, (b) a micro-pump, (c) solenoid valves with automated sequence control, (d) a sensor array and (e) an illumination system and a (f) heating element.E-nose enables generation of VOCs in the headspace of the foodproducts (kept insample holder) by heatingwhich are directed towards the detection system with the

help of a micro-pump and a solenoid valve. The detector comprises of a group of sensors. Metal oxide sensors (MOS) and conducting polymer (CP) sensors are commonly used for quality assessment of food products. Prior to performing the operations such as purging and sample analysis, e-nose should be kept switched on for a minimum of one hour to heat the sensors to facilitate movement of e^-. When in contact with VOCs, this leads to changes in conductivities across the sensors' surfaces and the variations in conductivities are recorded as resistances (R),measured via a transducer on an inter-digitated electrodes i.e., IDE (Tan and Xu, 2020; Li et al., 2010; Tan and Kerr, 2017, 2018a). R values are converted into voltage and subsequent transmission to computational mode occurs via pulse code modulation (PCM) technique.

The requisite airflow is indispensable for analysis of food samples using e-nose system which is maintained using a mini air compressor and serves three purposes: (a) during headspace generation time- a regulated flow of air is maintained on the food samples to ensure an adequate concentration of VOCs in the sample holder; (b) during sampling time- the uniform airflow distribution in the sensor array renders the sensors saturated with VOCs of the food samples; (c) during purging time- sensor heads are cleared with fresh air to enable sensors to return to their base line values (Chatterjee et al., 2015). For obtaining the R values in numerical form, an olfaction software (version number 1.1.)is used which is user-friendly and has programmable sequence control, data logging, alarm annunciation, and data archival.

CP sensors ares commonly used for assessment of food-derived volatile compounds including aldehydes, acetates and alcohols (e.g., butanol) from pears (Matindoust et al., 2017); alcohols from drinks (Tan and Xu, 2020); and alcoholic volatile organic compounds associated with spoiled meat (Tan and Xu, 2020; Khot et al., 2011). MOS are also widely used since these sensors also have several advantagessuch as high sensitivity, high selectivity, and high stability. Several reports on applications of MOS in fried and baked products have been documented wherein MOS have presented good efficiency in classification of fried and baked samples depending on their quality attributes. In the current study, MOS types of sensors were used for assessment of rancidity and freshness status of fried and baked products. Samples (crisps and bread) are subjected to e-nose analysis immediately after frying and baking without any delay.

5.2. MOS

MOS is the most widely used sensor for several fried and baked food products. The surfaces of MOS are coated onto a ceramic substrate such as alumina. Depending on the mode of reaction (reduction or oxidization) occurring on the sensors' surfaces, there are two types of MOS, n-type sensors, doped with group-5 elements such as As or P and p-type sensor, doped with group-13 elements such as B or Ga (Tan and Xu, 2020; Nazemi et al., 2019).

In the current study, n-type MOS were used where e^-s are major carriers. Prior to sample analyses, purging operation is carried out by air. Oxygen in air is highly electronegative and attracted towards the free and excess e^-s of n-type MOS. Thus it creates a film on the surface by adsorption. When sensors come in contact with the volatile organic compounds (VOCs) of food samples, the interaction between the VOCs and the oxygen-filmhappens. Therefore, the density of oxygen-film gets reduced and subsequent changes in the electrical properties i.e., resistance of the sensors occur.The reactions between sensors and VOCs of food samples are described in eq. (1)–(2):

$$\frac{1}{2}O_2 + e^- \rightarrow O^- \qquad (1)$$

$$R(g) + O^-(s) \rightarrow RO(g) + e \qquad (2)$$

where, R(g) is the reducing gas i.e., VOCs.

5.3. Application of e-nose for detection of lipid oxidationin fat-rich foods

In the domain of food science and technology, e-nose has been chiefly applied in four categories: (a) food control, (b) shelf-life investigation, (c) freshness evaluation and (d) authenticity assessment (Peris et al., 2009).

Application of e-nose in food quality assessment over the past ten years has been reported in 25 research documents (as per the report of Scopus web engine on the day this thesis has been compiled). Among these 25 articles, only 2 articles reported on the evaluation of rancidity of oatmeal (Wessling et al., 2001) and French fries (Chatterjee et al., 2014) using MOS.

5.4. Applicability of e-nose in the present work

In our current investigation, e-nose system, ENOVISION Ver.1.Q (developed by M/s Centre for Development of Advanced Computing, Kolkata, India) equipped with eight MOSs (TGS 823, TGS 2620, TGS 816, TGS 830, TGS 2611, TGS 2610, TGS 832 and TGS 2600) was employed to detect the onset of rancidity in DVCO-fried potato crispsw.r.t hexanal (prominent rancidity marker). Firstly, e-nose was calibrated with standard hexanal and thus R value for hexanal ($R_{hexanal}$) was obtained. During sample analyses, the changes in R values of sample (R_{sample}) were measured with respect to $R_{hexanal}$ and were termed as sensor response ($|\Delta R|/R_{hexanal}$). These eight MOS respond broadly to a wide range of VOCs and are reversibleimplying that VOCs which get adsorbed on the sensors' surfaces during sample analyses VOCs again leave the surfaces during air purging. The mode of operation of ENOVISION system ispresented in Figure 7 wherein odor molecules or VOCs are carried by air to the sensor array via solenoid valve (with the help of air pump) and then passed to the pattern recognition system in computer with the help of suction blower. Thereafter,analysis of sensor responses was performed.

Owing to non-specificity of the sensors, machine learning (support vector machine) was employed to screen the sensors on the basis of targeted VOCsnamely, hexanal to allow accurate and robust analysis of rancidity status in fat-rich food products. Chapter-4elaborates on development of an alternate methodology for detection and quantification of remarkably low hexanal concentrations for assessment of onset of rancidity in DFC and SFC, using e-nose technology. Additionally, this unique approach also extended to assessthe progression of rancidity in the sameby quantifying hexanal accurately and rapidly to assure safe, healthy fried products.

5.5. Support vector machine (SVM)

Principle of SVM

SVM is a linear model of supervised machine learning classifier (SMLC), used in classification and regression problems. It is widely used as a pattern recognition tool based on statistical learning theory (SLT) (Vapnik, 1995). The working principle of SVM is to separate the data with known categories or groups with a particular hyperplane, which maximizes the distance from a hyperplane to the nearest point (support vector) in the separated dataset or maximize the margin (Papadopoulou et al., 2013). The hyperplane is constructed using vector

operations, along withLagrange multiplier method under Karush-Kuhn-Tucker (KKT) conditions (Kefi Fatteh et al., 2019). Once the hyperplane is determined, unclassified data are subjected to SVM and classified based on their position to the hyperplane (Kefi Fatteh et al., 2019). SVM algorithm for classification of the sensor responses towards VOCs is presented in a flow chart (Figure 8).

Applicability of SVM in the present work

In the current study,sensor responses were fed to each model of SMLCsuch as decision tree, KNN, discriminate, naïve bays and ensemble classifiers. The best fit model was selected on the basis of higher accuracy, prediction speed, and lower training time. Among all these models investigated with, SVMwas selected which classified the data of sensor responsesinto two classes i.e., low responses group showing insignificantchanges with the changes in concentrations of VOCs and high responses group, showing significant, consistent and distinct changes with changes in concentrations of VOCs and thus helped in identifying the best sensors for detection of rancidity status of DFC as well as SFC.

6. Baking

Another important thermal operationinvestigated upon in the present study was baking. Baking is the key unit operation in bakery, wherein both heat and mass transfers occur simultaneously. It results in the formation of bread/biscuit/cookie/cake with desired characteristics including volume, color, texture and flavor (Manohar, 2014). It comprises of several processesdepicted in Figure 9. Baking imparts different attributes to the baked products via physical and chemical changes. Thus, the baked products aresensorially appealing.

6.1. Attributes induced by baking

Several physical (oven spring) and chemical changes such as starch gelatinization, protein denaturation occur during baking in the temperature range of 60-80 ℃which are responsible for structure development and crust formation in baked products. At a temperature higher than 80℃, loss of moisture happens and subsequently Maillard reaction occurswhen temperature is above 120 ℃ which forms several compounds (Table 7) responsible for color and flavor in baked products. The main precursors of Maillard reaction are amino-acids and reducing sugars.This reaction proceeds via three basic paths.

(1) The initial stage leads to amine condensation to form N-substituted glucosylamine which further undergoes Amadori rearrangement to form ketoseamine. Dehydration (loss of two water molecules) of ketosamine produces reductones and dehydro-reductones.

(2) The intermediate stage involves the production of short chain hydrolytic fission products such as diacetyl, acetol and pyruvaldehyde. Then these undergo Strecker degradation with amino-acids to form aldehydes and by condensation form aldols.

(3) The third stage is formation of a Schiff's base i.e., formation of furfural by loss of three water molecules. A comprehensive summary of early and advanced Maillard reactions are presented in Figure 10.

6.2. Challengesin retention of sweet freshness aroma in bread

During Maillard reaction, condensation of proline and fructose/glucose produces several odorants. Among them, 2-acetyl-1-pyrroline (2-AP) is considered as the key food odorant i.e., key food odorant (KFO; Dunkel et al., 2014) which contributes a sweet aroma ("Popcorn-like aroma") to bread at the time of baking. However, the instability of this KFO causes a significant problem at the commercial level of bread production (Wei et al., 2017).Therefore the challenge lies in retention of KFOin bread which cannot be sensorially perceived 3-4 h post baking (Rychlik and Grosch, 1996). Considerable literatures exist on isolation and identification of aroma and flavor compounds of bread; however, not much emphasis has been laid upon prolonged retention of KFOto augment marketability of bread.**Chapter 5** discusses devolvement of methodologies for enhancing and retaining freshness of bread by use of hurdle technology.

AA formation occurs in parallel with 2-AP while baking. Since AAcontent in bread is ~30 fold lower vis-à-vis that in fried potato crisps, freshness enhancement as well as retentionof the same in breadwas given more emphasis upon. However, the methodology developed not only enhanced the characteristic baked aroma but also concomitantly reduced AA content in the finished product. These have been critically discussed and analyzed in Chapter 5.

6.3.Methods for detection of freshness in bread

Several conventional methods have been reported for identification and quantification of VOCs of bread. Aroma profile of bread is generally determined by sensory evaluation, gas chromatography-olfactometry (GC-O) andstable isotope dilution assayscoupledtoGC-MS.

The salient features of these methods are discussed in Table 8 and their associated drawbacks in Table 9.

These analyses are possible only with highly trained chemists and sensory panelists. Use of e-nose equipped with MOS for analysis of baked products has been reported in literature (Table 10).

6.4.Applicability of e-nose in the present work

To augment rapid analysis of freshness during storage and for futuristic commercial applications involving large sample throughput in bakery industries, in the present study, the state-of-the-art e-nose technology was employed to replace classical GC-FID analysis. All details related to this aspect of the work have been critically enumerated in Chapter 5.

6.4.1. BP-MLP

Principle of BP-MLP

The backpropagation neural network is a multilayered, feedforward neural network and is by far the most extensively used (Lu, 2000). It is also considered as one of the simplest and most general methodsused for supervised training of multilayered neural networks (Lu, 2000). Backpropagation works by approximating the non-linear relationship between the input and the output by adjusting the weight values internally based on a comparison of the output and the target (Marques de Sa et al., 2007).Generally, the backpropagation network has two stages- training and testing. The training will be continuously repeated until the network outputs match termination criteria (Kumar et al., 2015). Typically transfer functions for training ANNs include the sigmoid function, step function, linear combination, and the rectifier (Marques de Sa et al., 2007). The performance of an ANN depends on many factors, such as the number of training pairs, the structure of the ANN (i.e., number of layers and neurons in hidden layer), the selection of a transfer function and activation function, and training termination criteria (Marques de Sa et al., 2007).Figure 12 shows the topology of the backpropagation neural network that includes one input layer, one hidden layer and an output layer; and its mode of operation.

Application of BP-MLP in different food systems

BP-MLP has been used in different food systems owing to its several advantages such as high tolerance to noisy data; ability to classify untrained patterns; well-suited for continuous-valued inputs and outputs; and can handle a wide array of data. Based on literature study

related to ANN-electronic nose, ANNs trained by BP-MLP were found to be useful for-determination of beer quality (Gonzalez Viejo et al., 2020); aging of rice (Rahimzadeh et al., 2019); types of French cheese (Ghasemi-Varnamkhasti et al., 2019); shelf-life analysis of paneer (Ahuja et al., 2012), yogurt (Sofu and Ekinci, 2007) and banana (Sanaeifar et al., 2014); and the presence of food-borne pathogens in foods (Bonah et al., 2019). ANNcombined with PCA has been used to determine the freshness of chicken meat (Timsorn et al., 2014) and the degree of roasting of cocoa beans (Tan and Kerr, 2018b).

Applicability of BP-MLP in the present study

In the current study, e-nose technology for assessment of retention of freshness-aroma i.e., "Popcorn-like aroma" with time has been rendered SMART (specific, measurable, achievable, realistic, time bound) by application of BP-MLP to arrive at a deep learning-model to aid in automation of quality assurance of commercial bread. Development and application of this model have been elaborated in Chapter 5.

The key questions raisedfor the entire study:

1. Can DVCO be used as an alternate healthy frying medium for potato crisps?
2. Is there any requirement to develop a new equation for deriving defuzzified scores (based on sensory outcomes) when (a+c) > 100? Is the new equation adequate to confirm or assure the using of DVCO as a frying medium?
3. Is the strategy developed in this work effective for toxin mitigation in fried potato crisps (fried in DVCO)? How does this strategy differ from the reported ones?
4. Is application of e-nose necessary to predict the onset of rancidity in fried potato crispsw.r.t hexanal?
5. Is the strategy developed in this work effective for freshness ("Popcorn-like aroma") enhancement and prolonged retention of the same in bread? How does this strategy influence other sensorial parameters of freshness-enhanced bread?
6. Is application of e-nose necessary to predict freshness-stability of bread w.r.t 2-AP?
7. How can the e-nose-based freshness-prediction method bemade more 'SMART', faster and accurate while large sample throughput is involved in the industrial scale?

Specific objectives:

Based on these key questions raised,the specific objectives were designed in the following way what have been reported for the first time.

1. Development of a new defuzzified equation to validate sensorial perception of absence of obnoxious odorin DVCO and to explore potential of DVCO as a new frying oil, alternative to linoleic acid-rich soybean oil.

2. Development of astrategy involving hurdle technology to mitigate AA and prevent the formation of 2,4-De in fried potato crisps without compromising their sensorial quality.

3. Development of an e-nose based methodology for rapid and accurate assessment of onset of rancidity in fried potato crisps to circumvent the limitation of GC-FID analyses of the most prominent rancidity molecular marker hexanal when present at an exceedingly low concentrations less than limit of detection (LOD) and limit of quantification (LOQ) of GC; and development of AI-based models for accurate analysis of progression of rancidity of the fried crisps in terms of hexanal content.

4. Development of a strategy involving the enrichment of KFO-precursor content to enhance the freshness aroma in bread at a significant level, assess its concomitant effect on sensory parameters of bread; and application of hurdle technology for prolonged retention of this enhanced freshness aroma to a sensorial-perceptual level in the same.

5. Development of a methodology based on e-nose technologyfor rapid and accurate assessment of freshness in bread by quantification of its KFO forgoing GC analysis.

6. Implementation of ANN to render the e-nose based methodology more robust, fast, accurate and SMART (specific, measurable, achievable, realistic, time bound) to aid in automation of quality assessment of bread on a commercial scale.

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Table 1: Summary of studies related to fuzzy logic applications in food products

Food products	Purpose of using fuzzy logic	Reference
Instant green tea powder and green granules	Ranking of tea samples according to their quality attributes	Sinija et al., 2011
Biscuit	Grading of biscuits on basis of quality	Perrot et al., 1999
Maize	Acceptance with respect to quality (chemical degradation) of maize	Perrot et al., 1999
Millet based bread	Acceptability of bread samples prepared from composite flours vis-à-vis wheat-flour bread	Singh et al., 2012
Tea liquor, prepared with different grade of CTC tea	Grading of tea liquor to detect its strong and weak attributes	Das et al., 2011
Drying of sugar	Fuzzy control of a drying process in the sugar industry based on operator's experiences	Voos et al., 1998
Sausage	Acceptance of sausage during ripening with respect to quality parameters	Curt et al., 2002
Peanut roasting process	Selection of drying process parameters based on final product quality	Davidson et al., 1996
Fried potato wedges	Acceptance of the fried samples during storage	Ghosh et al., 2015
Isomerized hop pallet production	Optimization of production parameters on the basis of desirable hop quality	Alvarez et al., 1999
Infrared roasting of cereal grain	Optimization of roasting conditions on the basis of desired quality of roasted grains	Brown et al., 2001
Soya fortified paneer	Multi-attribute decision making approach and comparison of soya fortified paneer	Uprit et al., 2002
Coffee products	Grading of different coffee products based on sensory parameters	Abdullah and Suriani 2009
Drink formulated from *dahi* (Indian curd)	Grading of differently formulated drinks on the basis of sensory evaluation of the final product	Routray et al., 2011
Mango drinks	Grading of differently formulated drinks on the basis of sensory evaluation of the final product	Jaya and Das, 2003

Table 2: Quality parameters of food products influenced by frying

Quality parameters	Responsible factor(s)/Underlying mechanism(s)	Effects on fried foods	References
Color	Oil degradation Polymerization of triglycerides Process variables (a) Frying temperature (b) Frying time (c) Frying oil composition Melanoidin formation	Brownish color	Leong et al., 2020
Moisture	Increase in surface temperature Evaporation of surface water in the form of water bubbles when surface temperature rises to 100 °C	Crust formation	Isik et al., 2016; Krokida et al., 2001
Uptake of oil	Food characteristics (size, shape, surface roughness and porosity) Oil characteristics (quality and composition of oil) Pre-treatments such as blanching, edible coating, vacuum drying, pulse electric field processing Pore formation owing to moisture evaporation during frying	Variation in oil uptake	Mellema et al., 2003
Texture	Shape, size and true density of food materials Porous structure Frying time and temperature Percentage of shrinkage of total volume (shrinkage initially occurs at the surface, accompanied by the formation of a rigid outer layer and moves inward until the final volume of the fried product is fixed)	Crispy texture	Krokida et al., 2001; Bouchon et al., 2001

Table 3. Acrylamide contents in different food products

Food products	Food product group	Minimum acrylamide (μg/kg)	Maximum acrylamide(μg/kg)
Potatoes	Potato crisps	117	3770
	Chips/French fries	59	5200
Cereal products	Corn crisps	120	220
	Bakery products and biscuits	18	3324
	Ginger bread	< 20	7834
	Bread	< 10	130
	Bread (toast)	25	1430
	Biscuit	337	923
	Breakfast cereals	11	1057
Rice and noodles	Fried noodles	3	581
	Fried rice	< 3	67
	Rice crackers (grilled or fried)	17	500
Fruits and vegetable	Canned black olives	123	1925
	Prune juice	53	267
	Fried vegetables	34	34
Nuts	Nuts	28	339
Fish and meat	Fish and seafood products (crumbed or battered)	< 2	39
	Meat/ poutry products (crumbed, or battered)	< 10	64
Cocoa-based products	Chocolate products	< 2	826
	Cocoa powder	< 10	909
Coffee	Coffee (roasted)	45	975
	Coffee substitute	116	5399
	Coffee extracted/powder	195	4948

N.B.: An average mean intake can be considered to be about 0.4 μg/kg body weight per day (b.wt. day^{-1}), and the average intake for a high-level consumer to be about 1.0 μg/kg b.wt. day^{-1} (Stadler et al., 2009).

Table 4. Effects of various processing conditions and techniques on acrylamide reduction in different food products

Food products	Mitigation process	Acrylamide reduction (%)	Reference(s)
Processing conditions			
Palmolein and soybean oil	Heating at 160 °C for 1.5 min	98.32% and 97.01%, respectively	Daniali et al., 2018
Bread	Baking at 200 °C for 70 min	25.54%	Claus et al., 2008
Green tea	Roasting at 160 °C for 30 min compared to 180°C for 15 min	50%	Mizukami et al., 2008
Potato strips immersed in water for 120 min	Frying at 150 °C, 170 °C, and 190 °C to final moisture contents of 40 g/100g	33%, 21%, and 27%, respectively	Pedreschi et al., 2007
Potato slices immersed in citric acid (10 g/L)	Frying at 150 °C, 170 °C, and 190 °C to final moisture contents of 40 g/100g	~53%	Pedreschi et al., 2007
Potato slices immersed in citric acid (10 and 20 g/L)	Frying at 150 °C to final moisture contents of 1.7 g/100g	70%	Pedreschi et al., 2004
Biscuits	Baking for 15 min at 180 °C instead of 90 °C	50%	Van Der Fels-Klerx et al., 2014
Coffee beans	Roasting at 236 °C for 10 min	38.66%	Summa et al., 2007
Almonds	Roasting for 15 min at 145 °C	78.22%	Lukac et al., 2007
Cocoa beans	Roasting for 2.5 min at 110 °C	84.61%	Farah et al., 2012
Cocoa beans	Roasting at 130 °C for 15 min	55.17%	Farah et al., 2012
Processing techniques			
Potato strips	Microwave pre-cooking at 850 W followed by frying at 190 °C for 30 s	60%	Belgin et al., 2007
Coffee beans	Vacuum roasting at 200 °C, 150 Pa	50%	Anese et al., 2014
Biscuits and Potato chips	Vacuum combined baking at 6.67 Pa, 60 °C for 5-15 min	43% and 18%, respectively	Anese et al., 2010
Potato chips	Vacuum frying at 1333.2 Pa and 125 °C	63%	Granda et al., 2004
Potatoes	Ultrasound pre-treatment (35 kHz and 92.5 W/kg at 42 °C) followed by deep frying at 171 °C	90%	Antunes-Rohling et al., 2018
Chicken coated with rice flour	Microwave frying at 181 °C for 1.5 min	35%	Barutcu et al., 2009
Potatoes	Air frying (at 180 °C)	90%	Sansano et al., 2015
Effect of additives			

Food products	Mitigation process	Acrylamide reduction (%)	Reference(s)
Tortilla chips	Lime (2 g/100 g)	52%	Salazar et al., 2014
Biscuits	Cysteine and glycine (0.36 and 0.2 g/100g)	97.8%	Zou et al., 2015
French fries and chips	Vanadyl sulphate pre-treatment (0.1 M for 60 min)	89.3%	Kalita et al., 2013
Fried potatoes	Solutions of nicotinic acid at 1%	80%	Casado et al., 2010
	Solutions of citric acid at 1%	80%	
	Solutions of glycine at 1%	80%	
	Solutions of NaCl at 2%	90%	
Potato crisps	Acetic acid soaking (at 20 °C for 60 min)	90%	Sansano et al., 2015
Potato slices	L-Asparaginase (8000 U/L from *Bacillus subtilis* B1106)	82%	Kita et al., 2010
Potato chips	L-Asparaginase (300 U/mL from *Fusarium culmorum* ASP-86)	86%	Jia et al., 2013
Sweet Bread	L-Asparaginase (300 U/mL from *Fusarium culmorum* ASP-86)	87%	
French fries	L-Asparaginase (from *Aspergillus oryzae* CCT 3940)	72%	Meghavarnam et al., 2018
Blanching conditions			
Potatoes	Blanching for 3 min at 80 °C	73%	Viklund et al., 2010
Potato crisps	Blanching for 10 min at 70 °C	70%	Mestdagh et al., 2008
French fries	Water blanching and soaking (in 0.5% sodium acid pyrophosphate followed by strip soaking in 0.4% $CaCl_2$)	92.16%	Truong et al., 2014
French fries	Blanching (with 10 U/mL of L-asparaginase at 80 °C for only 4 min)	80.5%	Zuo et al., 2015
Potato chips	Blanching at 68.7-75 °C for 8.8-9.7 min	61.3%	Zhang et al., 2018
Type of fermentation			
Biscuits with flaxseeds (SMF) and lupine (SSF)	Submerged fermentation (SMF) and Solid-state fermentation (SSF)	78% and 85%, respectively	Bartkiene et al., 2016
Wheat bread	Fermented *Helianthus tuberosus* L. tuber	54%	Bartkiene et al., 2013
Instant coffee	Fermentation with Baker's yeast (*Saccharomyces cerevisiae*, 1–2% w/v)	70%	Akıllıoglu et al., 2013
Mixed rye bread	Lactic acid fermentation with *Aspergillus niger* glucoamylase	59.4%	Bartkiene et al., 2013

Food products	Mitigation process	Acrylamide reduction (%)	Reference(s)
Bread	Fermentation with *Pediococcus acidilactici*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Pediococcus pentoseus*	84.2%, 55.6%, 49.2%, and 39.2%, respectively	Nachi et al., 2018
Type of extract			
Fried potato crisps	Proanthocyanidins extracted from cranberry (0.1 mg/mL for 15 min)	44.2%	Qi et al., 2018
Chicken drumsticks and chicken wings	Green tea extract (3%)	43% and 34%, respectively	Demirok et al., 2014
Fried potatoes	Aqueous extracts from green tea, cinnamon, and oregano (1 g/L for 1 min)	62%, 39% and 17%, respectively	Morales et al., 2014
Fried potatoes	Rosemary extract (50 mg/kg)	38%	Urbančič et al., 2014
Cookies	Bamboo leaf antioxidants (0.2 g/kg), and tea polyphenols (0.1 g/kg)	63.9% and 43%, respectively	Li et al., 2012

Table 5: Conventional methods for determination of rancidity in fried food products

Conventional methods	Compounds assessed	Detection technique	Limit of detection (LOD) of the method	Limit of quantification (LOQ) of the method	Standard limit for spoilage	Reference(s)
Titrimetric	Hydroperoxide (primary oxidation product)	PV test	-	-	> 6 ppm for fried potato crisps (as per literature reports)	Frankel, 2012;Petukhov et al., 2014
Titrimetric	Free fatty acid	AV test	-	-	≥ 1.5% (as per FSSAI, 2018)	Bockisch, 1998
Spectrophotometric	MDA (secondary oxidation product)	TBARS test	-	-	-	Zeb and Ullah, 2016
Instrumental	Conductivity of the volatile organic compounds (VOCs) formed by lipid oxidation	Rancimat	-	-	-	Kivevele et al., 2011
Chromatographic	Hexanal (considered as an appropriate rancidity marker compound), heptanal, nonanal, octanal	Gas chromatography -flame ionization detector / mass spectrometry i.e., GC-FID and GC-MS	For GC-FID: 0.89 ppm for hexanal; 0.32 ppm for heptanal; 2.41 ppm for nonanal; 1.33 ppm for octanal	For GC-FID: 2.97 ppm for hexanal; 1.05 ppm for heptanal; 8.05 ppm nonanal; 4.45 ppm for octanal	hexanal concentration > 0.03 ppm for fried potato crisps, measured by automated dynamic headspace sampler coupled to a GC-MS by spiking withstandard hexanal	Aparicio-Ruiz et al., 2018; Agarwal et al., 2018
			For GC-MS: 0. ppm for hexanal; 0.32 ppm for heptanal; 1.14 ppm for nonanal; 0.13 ppm for octanal	For GC-MS: 0.74 ppm for hexanal; 0.60 ppm for heptanal; 0.98 ppm nonanal; 0.44 ppm for octanal		

N.B.: The common analytical approach for detecting the different VOCs originated during lipid oxidation is based on GC. Majority of studies uses FID as detector vis-à-vis MS. Therefore, economical reasons and availability of FID are always weighed when selecting a detector.

Table 6: Drawback(s) in conventional methods for detection of rancidity in fried food products

Purpose(s)	Method(s)	Drawback(S)	Requirement
Assessment of rancidity in fried potato crisps	Conventional and instrumental methods (mentioned in Table 5)	Involvement of human activity which may lead to erroneous results owing to fatigue Need proper training and practices for bringing perfection in analyses (highly subjective, depends on the performer's efficiency) Lack of precision and accuracy in results Invasive in nature Time consuming i.e., no instant outcome for taking immediate decision about the product. Inadequate methods for on-line monitoring the quality of the products for acceptance or rejection. Labour-intensive Cost-intensive Required a large set up for assessment of freshness-aroma or VOCs Complex process No prediction of onset of rancidity in fried products Off-line techniques	Required a method for overcoming of all of these mentioned disadvantages and having ability to provide error-free, accurate, and precise data. It is made possible by electronic nose (e-nose) technology coupled with machine learning
	Sensory evaluation	Time-consuming Expensive since trained professional panelists need to be hired Huge variations in assessment both between and within panelists owing to variations in perception Biasness Stimulus errors Difficult to predict the onset of rancidity owing lack of accuracy in sensorial perception	Required a method for overcoming of all of these mentioned disadvantages and having ability to provide error free, accurate, unbiased decision about the product. It is made possible by fuzzy logic

Table 7: Compounds formed during Maillard reaction having positive effects on baked products

Maillard reaction products (MRPs)	Food products containing MRPs	Odor description
Diacetyl	Baked bread crust	"Popcorn"-like flavor
Furans	-	Caramel-like odor
Pyrazines	Common in all baked products including bread	-

Table 8: Methods for determination of aroma of bread

Method (s)	Mode of operation and salient feature(s)	Reference(s)
Sensory evaluation	Sensory evaluation follows two methods such as discrimination methods and measurement technique. Discrimination method comprises of triangle, duo-trio and pair-comparison tests whereas measurement technique comprises of different scales such as hedonic, just-about-right, face, ordinal, interval and ratio scales. Measurement technique is found to be more reliable for assessing product acceptance which is conducted by trained or semi-trained panelists. Sensory scores on 9-point hedonic scale are assigned for each sensory attribute depending on the perception of panelists.	Stone and Sidel, 2012
GC-O	Gas-chromatography-Olfactometry is a technique to combine the information supplied by chemical characterization and by odor perception. GC-O utilizes a GC-MS system equipped with an olfactory detection port: at the outlet of the GC there is a sniffer mask, where a trained panelist can smell the aroma and provide information about the presence of odor in it. At the end of the GC column, after separation of the chemical compounds in the gas mixture, the sample is divided and an equal flux of it reaches the MS detector and the panelist (Figure 11). The panelist sniffs the gas, and provides a sensorial response in terms of presence and type of odor. This way an olfactogram is obtained which allows correlating the chemical information supplied by the chromatogram and the sensorial perceptions of the panelist.	Delahunty et al., 2006
Stable isotope dilution assays, coupled to GC-MS	It is based on the use of a labeled stable isotope of aroma compound as internal standard, thereby overcoming the limits of structurally different internal standards. This method provides data when their stability and physical properties become similar to those of the compounds to be quantified. The analyte i.e., aroma compound and its isotopic analog can then easily be differentiated by GC-MS, by their different molecular weights.	Grosch, 2001

Table 9: Drawbacks of all conventional methods for determination of onset of rancidity in fried potato crisps and freshness aroma in bread

Purpose(s)	Method(s)	Drawback(S)	Requirement
Assessment of freshness detection in bread	Conventional methods (mentioned in Table 10)	Involvement of human activity which may lead to erroneous results owing to fatigue Need proper training and practices for bringing perfection in analyses (vary person to person depending on the performer's efficiency) Invasive in nature Time consuming i.e., no instant outcome for taking immediate decision about the product Inadequate methods for on-line monitoring of the quality of the products for acceptance or rejection Labour-intensive Cost-intensive Requires a large set up for assessment of freshness-aroma or VOCs Complex process	Requires a method for overcoming all of these mentioned disadvantages and having ability to provide an error free, accurate, unbiased decision about the product.
	Sensory evaluation	Time consuming Expensive method owing to need for hiring trained panelists Huge variation both between and within individuals in assessment owing to variations in perception Biasness Stimulus errors	

Table 10: Applications of e-nose equipped with MOS in bakery products

Food system	E-Nose sensor array system	References
Ingredient differentiation		
Bread	MOS	Sapirstein et al., 2012
Bread	MOS	Torri et al., 2013
Biscuits	MOS	Romani et al., 2006
Baking stage differentiation		
Bread baking aroma	MOS	Ponzoni et al., 2008
Biscuits	MOS	Romani et al., 2012
Differentiation for quality aspects		
Toasted bread	MOS	Ponzoni et al., 2008
Aging		
Bread	MOS	Botre and Gharpure, 2006

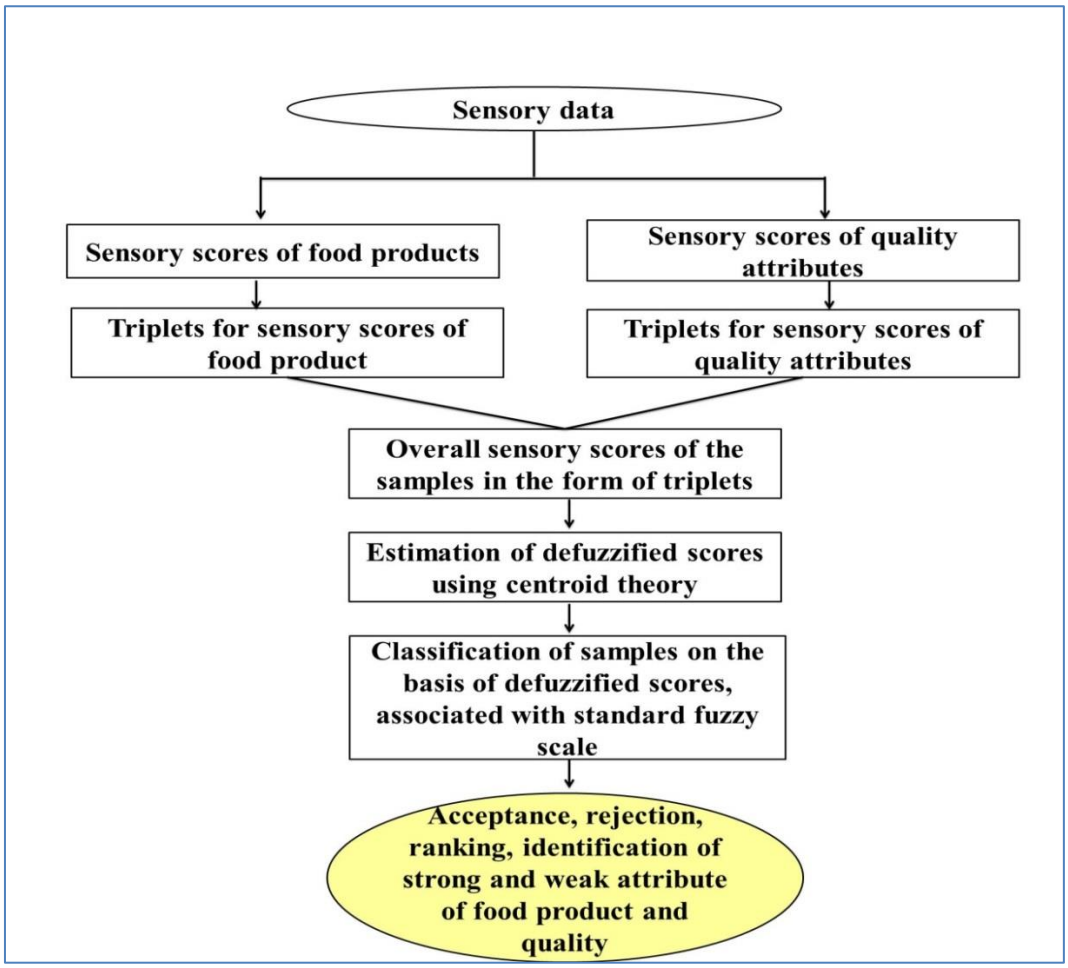


Figure 1. Flowchart showing steps involved in fuzzy logic methodology

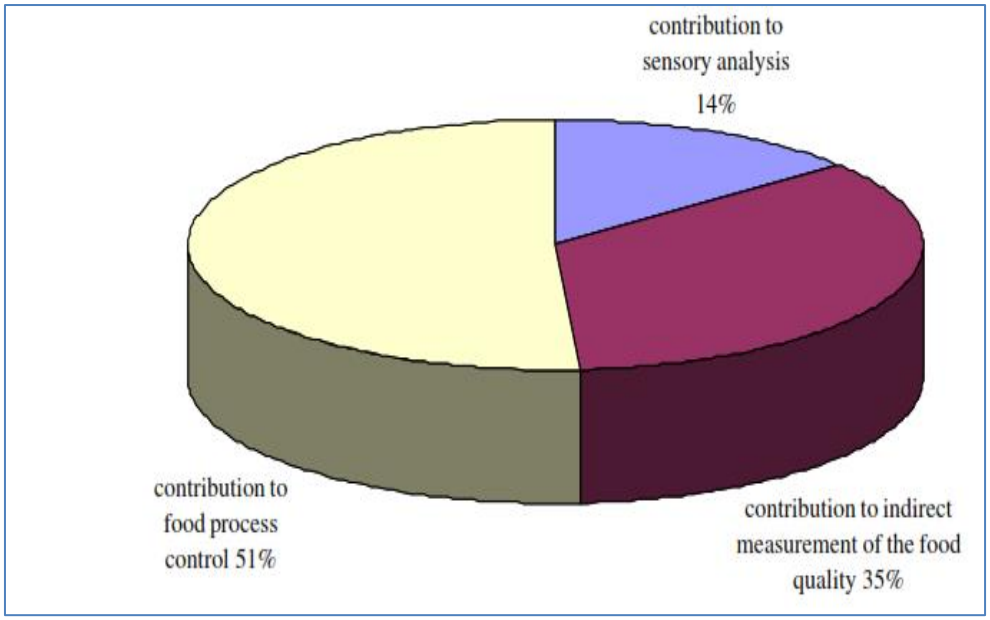


Figure 2. Application of fuzzy logic in different areas of food technology (Perrot et al., 2006)

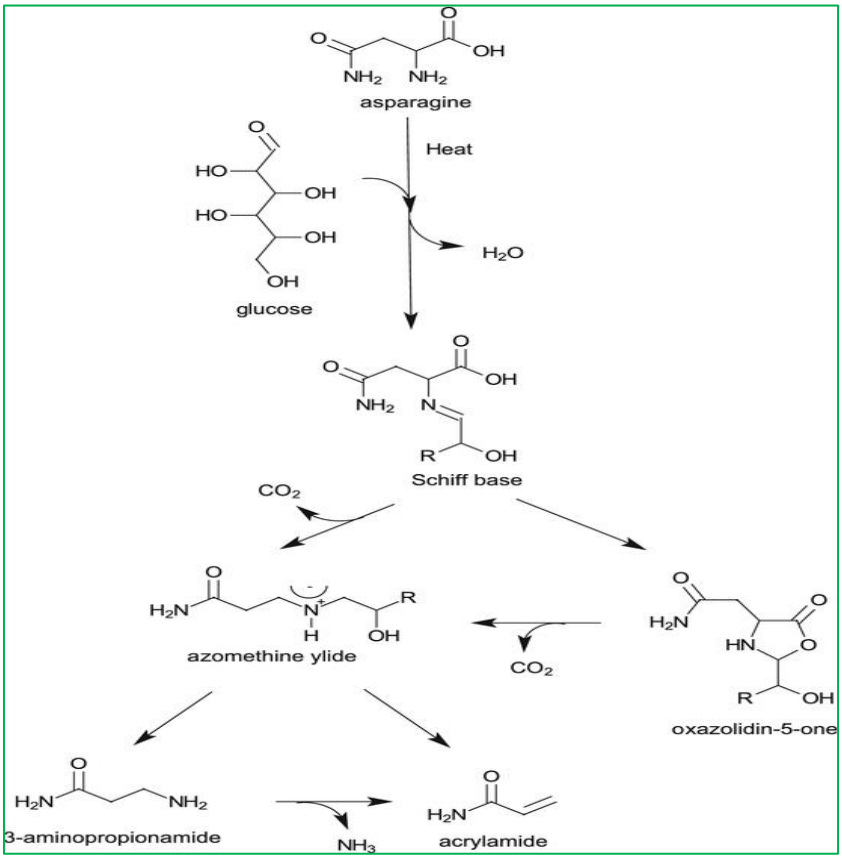


Figure 3. Acrylamide formation mechanism

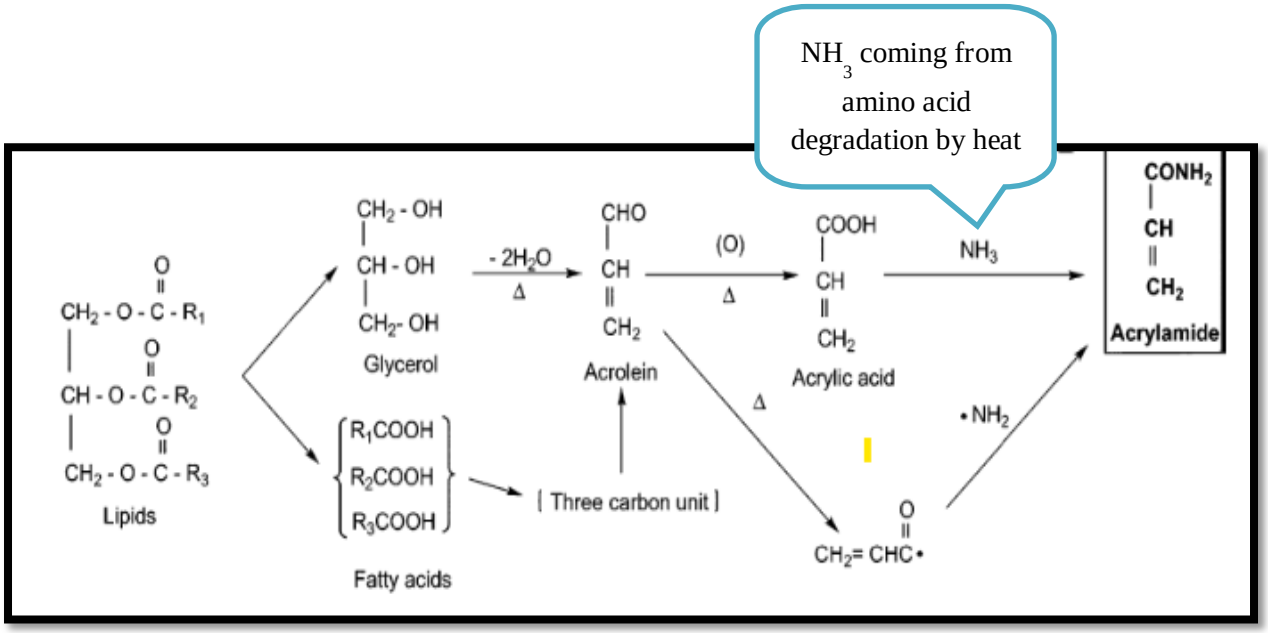


Figure 4. Formation of acrylamide by hydrolysis of triacylglyceride

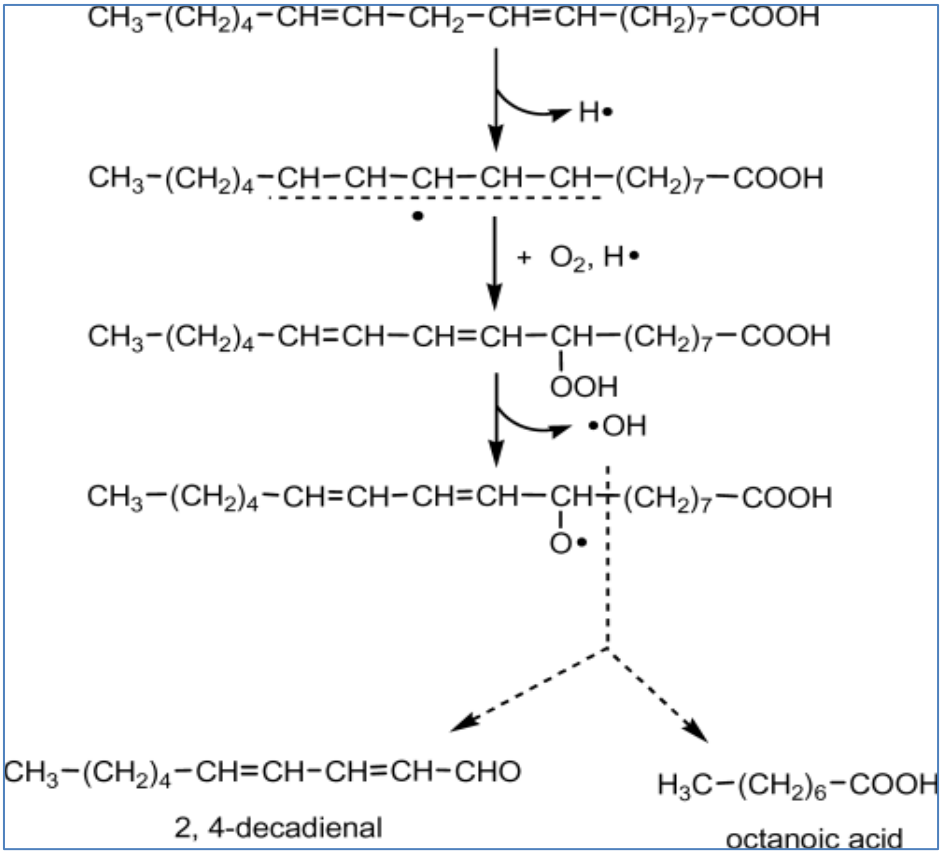


Figure 5. Formation of 2, 4-decadienal by oxidation of linoleic acid

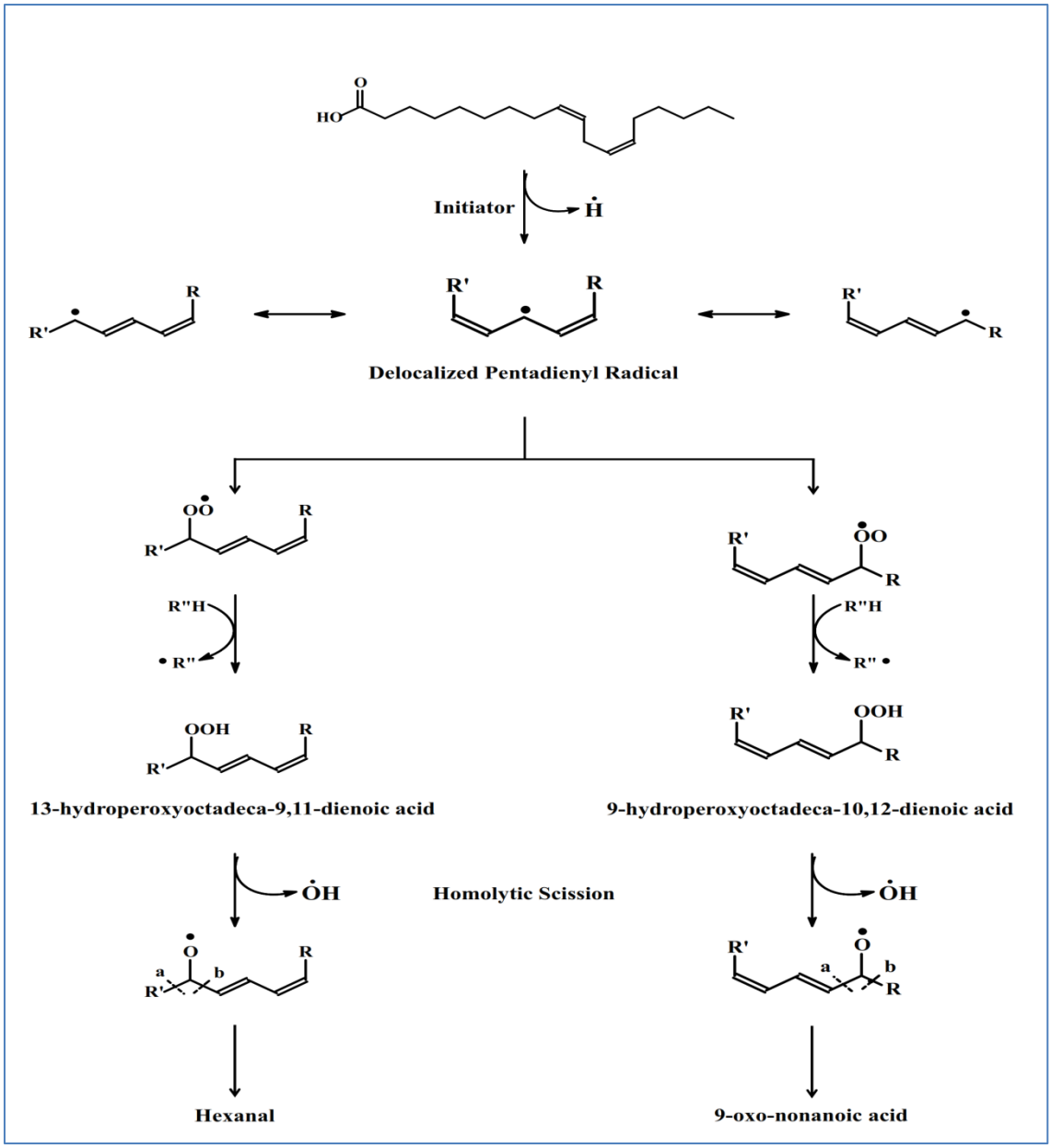


Figure 6. Pathway of autoxidation of linoleic acid to hexanal

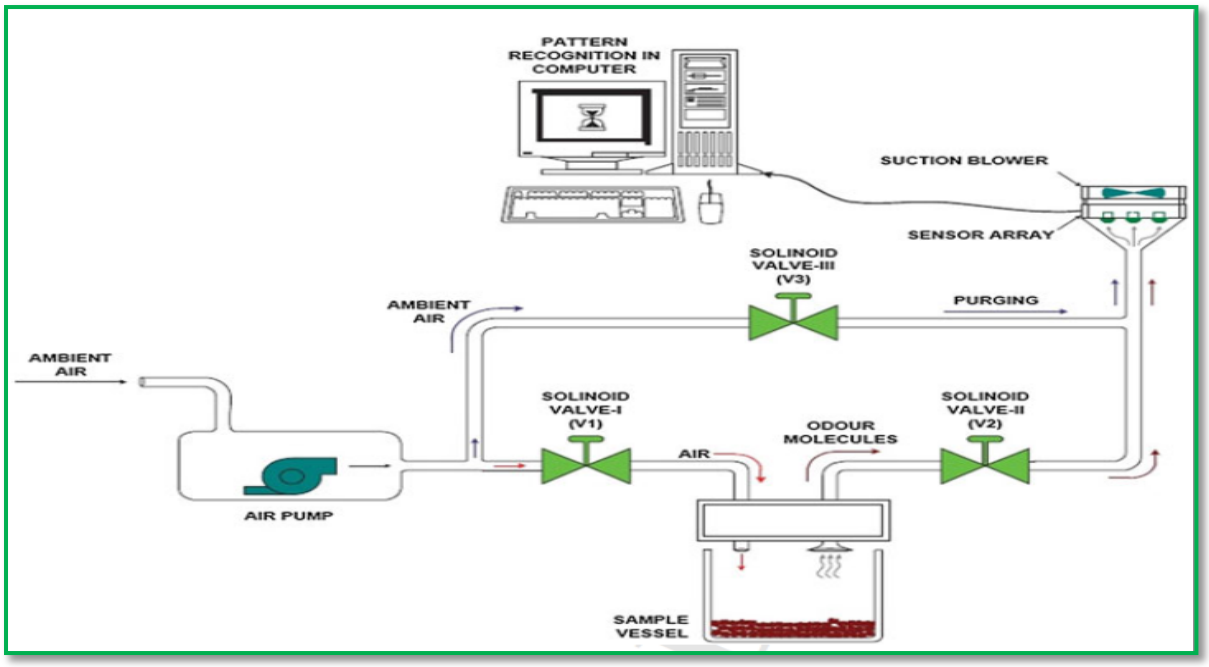


Figure 7. Schematic diagram of customized C-DAC ENOVISION system

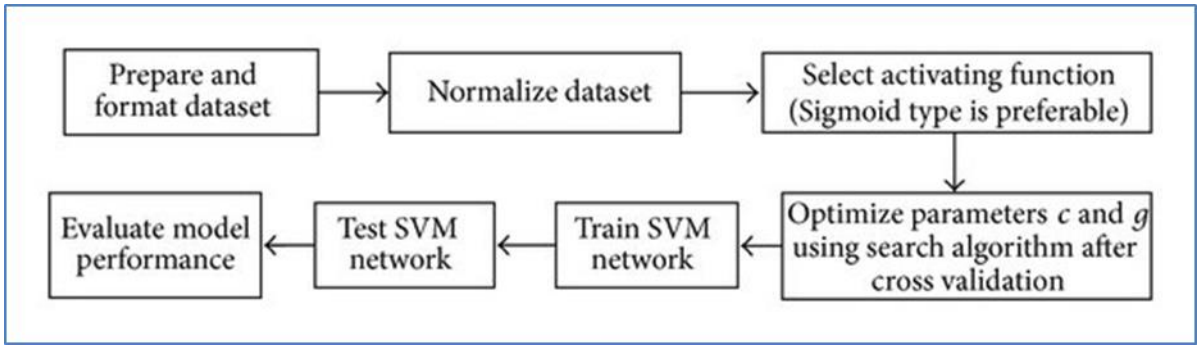


Figure 8. Flow chart of SVM algorithm on predicting classification

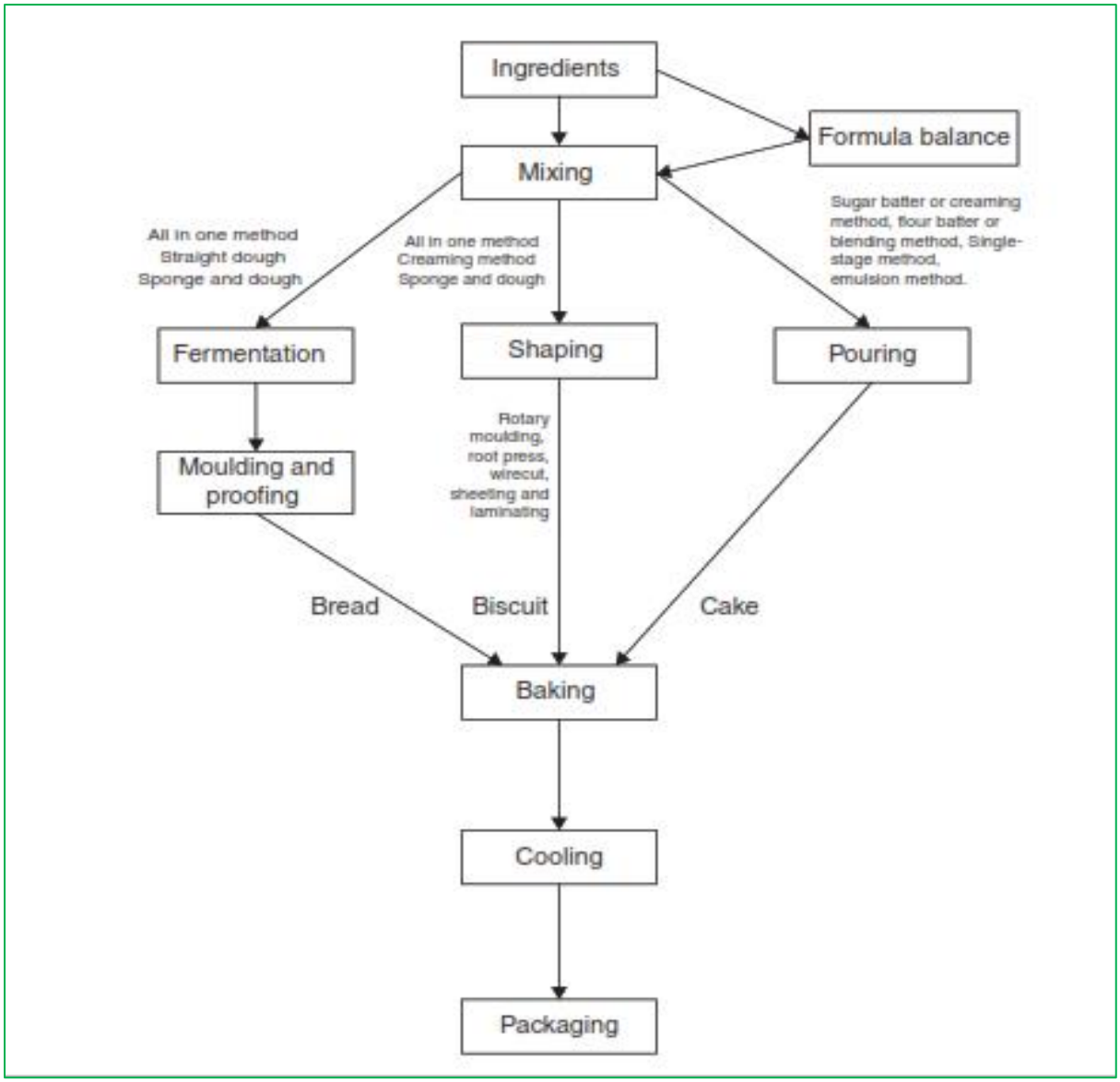


Figure 9. Different unit operations involved in bread making (Ref.: Manohar, 2014)

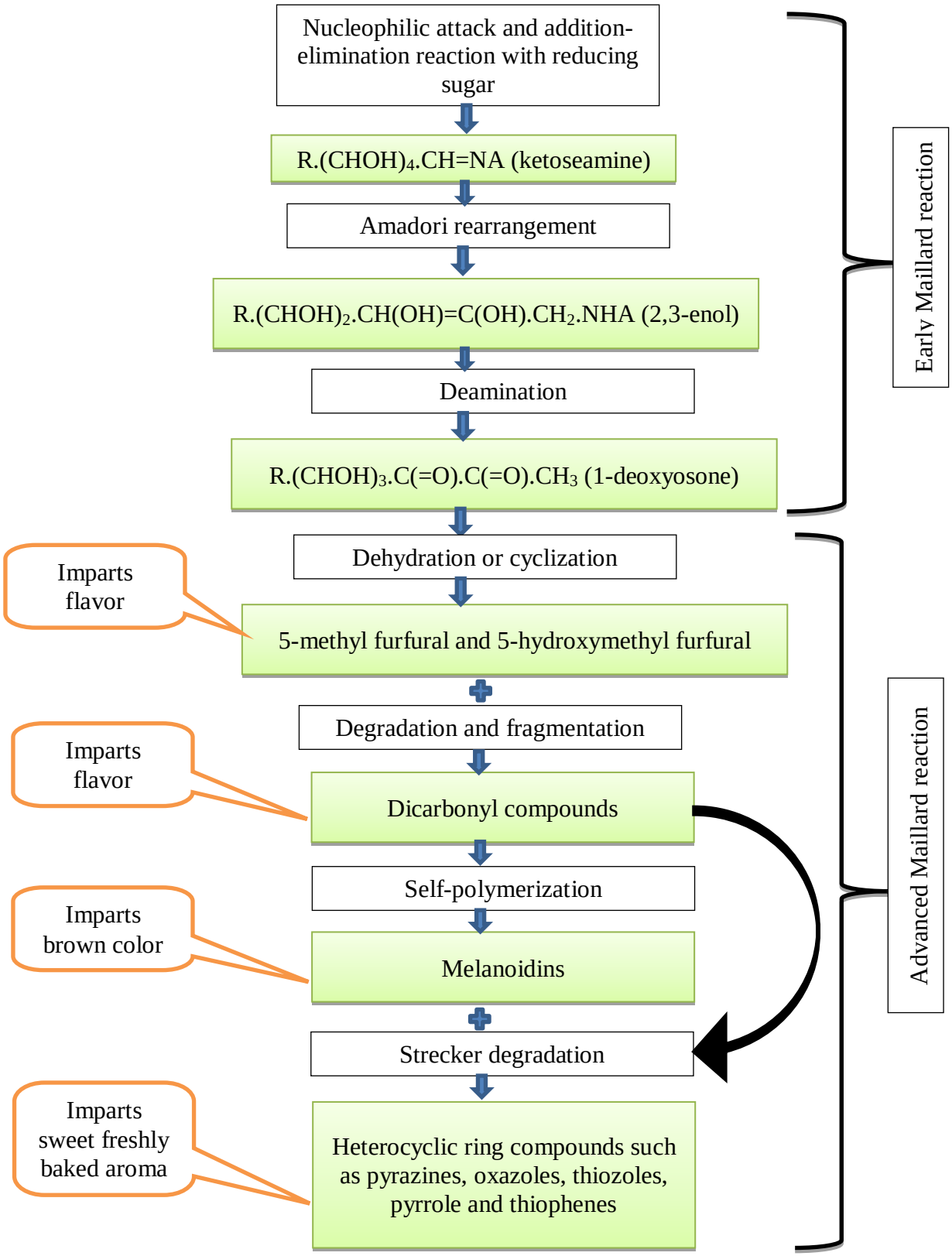


Figure 10: Summary of Maillard reaction

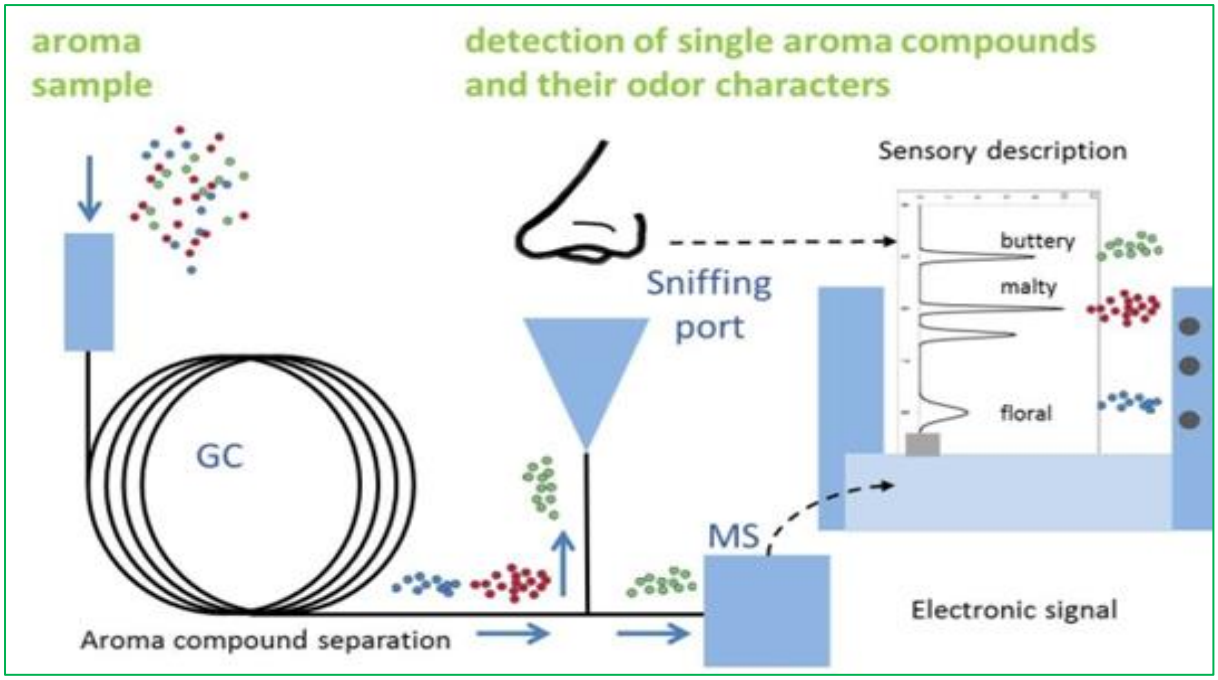


Figure 11. Gas chromatography-olfactometry diagram

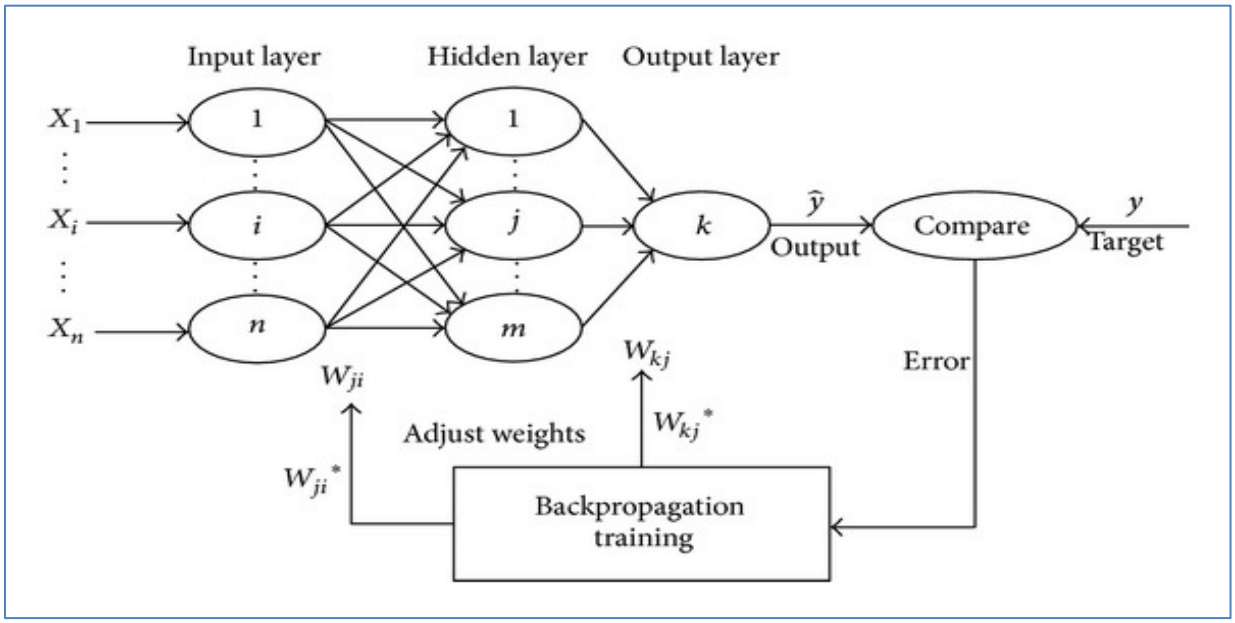
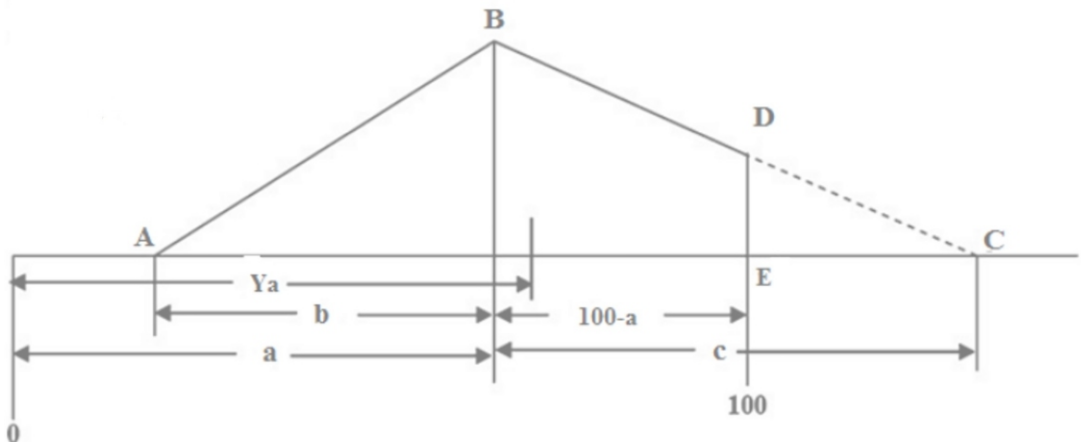


Figure 12.MLP-Multilayer perceptron based on backpropagation technique.



CHAPTER 2:

Development of a new equation in fuzzy logic analysis for ascertaining appropriate dose of gamma irradiation of virgin coconut oil

1. Introduction

Studies from our previous investigation (Ghosh et al., 2016) have established 4.2 kGy to be the appropriate dosage of gamma irradiation for removal of obnoxious rancid-acid-odor of virgin coconut oil (VCO). This dosage was arrived at by sensory evaluation of irradiated oil samples. This study endeavors to establish concurrence of data obtained from sensory evaluation (of deodorized VCO (DVCO) for a storage period of 40 days, at a regular interval of seven days by 30 semi-trained panelists) and fuzzy logic analysis of sensory scores.In due course of work, a special condition was generated wherein the sum of the first and third coordinates of the triplet (a b c)of overall sensory score (SO) was found to be greater than 100 i.e., (a+c) > 100. In accordance with Daset al. (2005), defuzzification of sensory scores can be performed when (a+c) < 100. Under this condition, the centroid theory of triangle is appropriate. When (a+c) is greater than 100, the centroid theory of triangle was not obeyed and thus a new polynomial equation had to be developed as an extension of the centroid theory. This requirement of the new equation [when (a+c) > 100] is justified by a simple example, explained in Appendices 2B.

In view of above, the main objective of the current study was to develop an equation for deriving defuzzified scores when (a+c) > 100. The secondary objective was to validate this new developed equation by the similarity principle and e-nose technologyso as to remove ambiguity, if any in certifying DVCO to be obnoxious odor free post gamma-irradiation.

2. Material and Methods

2.1. Procurement of raw material

A sample of a leading brand of expeller pressed Virgin coconut oil (VCO) (obtained from coconut copra of West coast tall variety coconuts) with no added antioxidant and preservative was used in this study, as had been investigated and reported by Ghosh et al. (2016).

2.2.Preparation of sample for irradiation

Thirty screw LDPE co-polymer capped bottles (500 mL) were sterilized under UV light in a laminar hood. Each bottle was filled with 500 ml VCO and was numbered serially. The bottled oil samples were then irradiated in a Co-60 γ irradiation chamber [(GC 5000; Serial No. GIC 038); source: Cobalt 60 solid (dose rate: 5.201 kGy h^{-1}, displayed on the front panel during automode operation)] of BRIT, Mumbai in the National Instrument Laboratory (NIL)

campus, Jadavpur University at 23±2 ℃ at the pre-selected dose levels of 0.0 kGy (control set, IVCO 1), 4.0 kGy (IVCO 2), 4.2 kGy (IVCO 3), 4.5 kGy (IVCO 4) and 5.0 kGy (IVCO 5), in accordance with the procedure reported by Ghosh et al. (2016). Post irradiation, all samples were stored at room temperature (23±2 ℃) under dark condition in these airtight LDPE co-polymer screw capped bottles for 40 days. Sensory evaluation was conducted for the irradiated oil samples at an interval of seven days for a total period of forty days in accordance with the procedure reported by Ghosh et al. (2016).

2.3.Sensory evaluation of irradiated coconut oil samples

Sensory evaluation of the above irradiated coconut oil samples were conducted inside a university classroom at 24±1 ℃ in bright light. The panel for sensory evaluation consisted of 30 members (in the age group of 21-45 years) including trained students and few semi-trained staff of the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, India. The 30-number panel was sufficient since fuzzy logic reportedly minimizes all the biasing effect, generated due to different consumers' perception (Sinija and Mishra, 2011). Panelists evaluated the samples in terms of aroma, color, homogeneity and flow ability using the standard 5-point hedonic scale [1= not satisfactory, 2 = fair, 3= medium, 4 = good, 5 = excellent]. During testing, the panelists were monitored by the authors. The panelists were served with coffee beans (for smelling) in between to enable them to correctly differentiate the aroma among the different irradiated oil samples. The order of the samples served was same for all the panelists in a particular session but varied between two sessions. Panelists were asked to provide numerical score against each sensory attribute of each sample and also give their preferences against each quality attribute (aroma, color, homogeneity and flowability), following respective scale factors [not at all important (NI), somewhat important (SI), important (I), highly important (HI), extremely important (EI)] for each sample relative to the entire sample set. The main steps of fuzzy modeling of sensory evaluation are: (1) calculation of triplet (S) corresponding to different quality attributes (aroma, color, homogeneity and flowability); (2) relative weightage (Q_{REL}) of particular quality attributes (aroma, color, homogeneity and flowability), (3) overall sensory score (SO); (4) calculation of defuzzified score (Y_a) while (a+c) < 100 and (Yá) when (a+c) > 100 and (5) ranking of quality attributes and grading of samples (Sinija and Mishra, 2011).

2.4. Triangular fuzzy number (TFN) and fuzzy arithmetic operations

Triangular membership function distribution pattern of sensory scale is shown in Figure 1. This represents a 5-point sensory scale viz., poor/not at all important, fair/somewhat important, good/important, very good/highly important and excellent/extremely important. The triplet (a, b, c) associated with sensory scale is a triangular fuzzy number. Here, 'a' (first number of triplets) is called the mean value of the fuzzy number and it denotes the coordinate of abscissa at which value of membership function is 1. Second and third numbers of the triplets 'b' and 'c' are called the left and right spreads respectively, whose membership functions are 0(Wang et al., 2006).

2.5. Triplets for sensory scores of irradiated oil samples and overall quality of samples

Triplets corresponding to specific quality attributes such as aroma, color, homogeneity and flowability were derived from the sum of sensory scores and the triplets associated with sensory scale along with the total number of panelists. For example, for IVCO 1 (0.0 kGy) on day 0, out of 30 panelists, 18 panelists agreed on a 'not significant' score while 12 panelists provided the score 'fair' against the sensory attribute, aroma (Table 1). Therefore, the triplets of the sensory scores for aroma of IVCO 1 (S1A) would be obtained as follows:

$$S1A = (18\ (0\ 0\ 25) + 12\ (25\ 25\ 25) + 0\ (50\ 25\ 25) + 0\ (75\ 25\ 25) + 0\ (100\ 25\ 0))/((18+12+0+0+0)) \quad (1)$$

Similar values for other quality attributes of all samples (during the entire storage period) were obtained (Table 2) using the aforesaid equation (eq. 1). Relative weightage (Q_{REL}) for all sensory attributes (aroma, color, homogeneity and flowability) were calculated by dividing the triplets of each quality attribute with Q_{SUM} as has been described by Das (2005). The triplets of overall sensory scores of all irradiated oil samples for each quality attribute were evaluated using eq. (2). For example, the overall sensory score of IVCO 1 at day 0 (SO10) would be given by:

$$SO10 = S1A \odot QA_{REL} \oplus S1C \odot QC_{REL} \oplus S1H \odot QH_{REL} \oplus S1F \odot QF_{REL} \quad (2)$$

where, S1A, S1C, S1H and S1F represent the triplets corresponding to aroma, color, homogeneity and flowability, respectively, of IVCO 1. QA_{REL}, QC_{REL}, QH_{REL} and QF_{REL} signify the triplets corresponding to relative weightages of aroma, color, homogeneity and

flowability, respectively of irradiated oil samples. In this way, the overall sensory scores for all samples (during storage period) were calculated.

Triplets (a, b, c) representing the overall sensory score (SO) was denoted by Δ XYZ (Figure 3a and Figure 3b). When the value of (a+c) ≤ 100, the Δ XYZ lies within the sensory scale interval [0, 100]. This is represented by Figure 3 (a). Under this condition, the value of Y_a in terms of a, b and c can be expressed by eq. (3) (Zimmermann, 1991), where $Y_a$is the centroid as well as the defuzzified score of ΔXYZ (Figure 3a).

$$Y_a = \frac{(3a-b+c)}{3} \qquad (3)$$

However, under the second condition, i.e. (a+c) > 100, polygon XYMN has to be considered instead of ΔXYZ, to maintain the standard sensory scale in the range 0-100 (Figure 3b). This necessitated development of a new equation for deriving the defuzzified score of any sample when (a+c) > 100.

2.6. Development of new equation for obtaining defuzzified scores

Eq. (3) was not considered or applicable for finding the value of defuzzified scoresof polygon XYMN [condition: (a+c) > 100]. It is well known that the centroid of the polygon represents the defuzzified score of the same. Here, centroid of polygon XYMN was derived using eq. (5) which was obtained from eq. (4).

Centroid of XYMN (Yá) × Area of XYMN (A_n) = (Area of ΔXYO × Centre of gravity ΔXYO) + [(Area of Δ YOC × Centre of gravity of Δ YOC) – (Area of ΔMNZ × Centre of gravity of ΔMNZ)] (4)

$$Yá \times A = \frac{1}{6}\sum_{i=0}^{n-1}(x_i + x_{i+1})(x_i y_{i+1} + x_{i+1} y_i) \qquad (5)$$

As per Figure 3 (b), eq. (5) can be written in the following way:

$$Yá = \frac{1}{6A}[(x_0 + x_1)(x_0 y_1 - x_1 y_0) + (x_1 + x_2)(x_1 y_2 - x_2 y_1) + (x_2 + x_3)(x_2 y_3 - x_3 y_2)] \qquad (6)$$

where,

$$A = ½\,[(x_0 y_1 - x_1 y_0) + (x_1 y_2 - x_2 y_1) + (x_2 y_3 - x_3 y_2)] \qquad (7)$$

It therefore follows that

$$\therefore Ya' = \frac{(x_0+x_1)(x_0y_1-x_1y_0)+(x_1+x_2)(x_1y_2-x_2y_1)+(x_2+x_3)(x_2y_3-x_3y_2)}{3(x_0y_1-x_1y_0)+(x_1y_2-x_2y_1)+(x_2y_3-x_3y_2)} \quad (8)$$

Here, Yáand A are the centroid and area of polygon XYMN, respectively. In Figure 3b, the coordinates of points X, Y, M, N are (x_0 y_0), (x_1 y_1) (x_2 y_2) (x_3 y_3), respectively. In accordance with Figure 3b, the values of these coordinates are as follows: x_0 = (a–b), x_1 = a, x_2 = x_3 = 100 and y_0 = y_3 = 0, y_1 = 1, y_2 = MN. The MN value was obtained from the rule of similarity of triangle, discussed below. Here, Δ YOZ and Δ MNZ are always right angled triangles. Therefore, the rule of similarity was employed here and values of MN were obtained (shown below). The centroid of polygon XYMN was found by putting all the values of coordinates in eq. (8). Thus final equation of Yáof polygon XYMN has been derived and reported by us for the first time and is represented by eq. (9). This equation is not reported in literature. The steps involved to derive this equation were discussed in details in Appendix 2.A.2

$$MN/YO = NZ/OZ$$

$$\therefore MN = \frac{a}{c} + 1 - \frac{100}{c}$$

$$\therefore A = \frac{2ac-bc+a^2+200a-200c+10000}{c}$$

$$\therefore Yá = \frac{(a^3+b^2c+3a^2c-3abc-3\times10^4a-3\times10^4c+2\times10^6)}{3\,(a^2+2ac-bc-2\times10^2a-2\times10^2c+1\times10^4)} \quad (9)$$

3. Results and Discussion

3.1. Defuzzified values for ranking of samples and their quality attribute

The relative importance of four quality attributes (*viz.*, aroma, color, homogeneity and flowability) was obtained from their defuzzified scores. The defuzzified scores were compared with the six point sensory scales of linguistic parameters, i.e., not satisfactory, fair, satisfactory, good, very good and excellent (Figure 2), which are classified by following number ranges: 1-10, 10-30, 30-50, 50-70, 70-90 and 90-100, respectively. In order to judge all the samples on each day of storage with respect to all quality attributes (aroma, color, homogeneity and flowability), defuzzified scores were considered in this comparative study, instead of use of similarity principle (S_m). The defuzzified scores of all samples were calculated using eq. (3) or eq. (9), depending on the value of (a+c). It was observed that for

IVCO 3 on days 21, 28 and 40 had (a+c) values of 101.53, 164.44 and 144.58 respectively [i.e. (a+c) > 100]. For these three cases, eq. (9) was used instead of eq. (3) for finding their respective defuzzified scores.

3.2.Shortcoming of the procedure for ranking of the samples by similarity principle

The overall sensory score (obtained as a single triplet) was fitted into the six point sensory scale (referred to as standard fuzzy scale) employing similarity analysis. Calculation of S_m is very time consuming owing to complexity of its calculation. S_m for the irradiated oil samples were calculated employing overall membership function values of sensory scores and values of membership functions of standard fuzzy scale. Standard fuzzy scale, viz. not satisfactory/not at all necessary, fair/somewhat necessary, medium/necessary, good/important, very good/highly important and excellent/extremely important was designated as F1, F2, F3, F4, F5 and F6, respectively (Figure 3). As per Figure 3, the values of membership functions are defined by a set of 10 numbers, elaborated in (10).

F1 = (1, 0.5, 0, 0, 0, 0, 0, 0, 0, 0)

F2 = (1, 0.5, 0, 0, 0, 0, 0, 0, 0, 0)

F3 = (0, 0, 0.5, 1, 1, 0.5, 0, 0, 0, 0)

F4 = (0, 0, 0, 0, 0.5, 1, 1, 0.5, 0, 0)

F5 = (0, 0, 0, 0, 0, 0, 0.5, 1, 1, 0.5)

F6 = (0, 0, 0, 0, 0, 0, 0, 0, 0.5, 1)

(10)

The membership function of a triplet (a b c) has been presented graphically in Figure 3 wherein the value of membership function is 1 and 0 for the triplet (a b c) under the condition of value of abscissa ‘a’ and less than ‘(a-b)’ or greater than ‘(a+c)’, respectively. The value of membership function, B_x at x = 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 obtained from overall sensory scores of the irradiated coconut oil samples were calculated from eq. (11). After calculating the B_x values (as a set of ten values) for each sample, $S_m$were derived using eq. (12). Determination of S_m comprises of the following steps: step1: determination of B_x values for each sample; step 2: multiplication of matrix F with transpose matrix of F ($F \times F^T$); step 3: multiplication of matrix F with transpose matrix of B ($F \times B_x^T$) and step 4: multiplication of matrix B with transpose matrix of B ($B_x \times B_x^T$). Thus $S_m$valueswere

calculated under six categories of sensory scales, employing these steps. The highest S_m was considered and its corresponding category described the quality of sample. This approach clearly demonstrates that determination of defuzzified scores using eq. (9) is relatively simpler than determination of $S_m$values. Thus the advantage of using eq. (9) [condition: (a+c) > 100] lies in its simplicity.

$$B_X = \frac{X-(a-b)}{b} \text{ for } (a-b) < x < a$$

$$B_x = \frac{(a+c)-x}{c} \text{ for } a < x < (a+c)$$

$$B_x = 0 \text{ for } x < (a-b) \text{ and } x > (a+c) \quad (11)$$

$$S_m(F, B) = \frac{F \times B^T}{\text{Max}(F \times F^T \text{ and } B \times B^T)} \quad (12)$$

3.3.Validation of the newly developed equation

The conclusion derived from the results of newly developed equation [eq. (9)] was validated from results of similarity value and also by odor profile analysis of the irradiated oil samples using electronic nose (e-nose) (ENOVISION, C-DAC). The signal responses ($|\Delta R|/R$) of the irradiated coconut oil samples, obtained from e-nose analysis were already reported (Ghosh et al., 2016). Validation by S_m was carried out by standard fuzzy and sensory scales.

3.4.Ranking of quality attributes of irradiated coconut oil samples based on defuzzified scores

The triplets of sensory scores for four quality attributes (aroma, color, homogeneity and flowability) in general were calculated using eq. (1). Sensory scores, triplets associated with these scores of quality attributes of irradiated coconut oil samples and relative weightages of all quality attributes are presented in Table 2. Defuzzified scores for four quality attributes of each sample were calculated using eq. (3) (Table 3). The $Y_a$values for aroma and homogeneity were obtained as 75.00 and 50.00, respectively. These results illustrated that aroma had received the highest importance in sensory acceptance followed by sample homogeneity. The defuzzified scores for other two quality attributes (color and flowability) were 8.33 and 25, respectively. Therefore, the following trend of preference of quality attributes of the irradiated coconut oil samples can be arrived at using linguistic representations of standard sensory scale.

Aroma (highly important) > homogeneity (important) >flowability (somewhat important) > color (not at all important)

3.5.Defuzzified scores of deodorized coconut irradiated oil samples and samples ranking

The sensory scores of five samples w.r.t four quality attributes (aroma, color, homogeneity and flowability) on each day of storage have been presented in Table 1.The triplets associated with sensory scores were determined using eq. (1). Defuzzified scores for the stored irradiated coconut oil samples were evaluated using eq. (3) or eq. (9), depending on the values of (a+c). For example, triplets of overall sensory score of IVCO 1 on day 0 (SO10) were found to be (24.17 32.08 45.56) from eq. (2). Here, (a+c) = 69.73 (< 100). Therefore, eq. (3) was employed for finding defuzzified scores of IVCO 1 on day 0. For IVCO 3, eq. (3) was applicable up to day 14. From day 21 onwards, (a+c) values were greater than 100. Triplets of overall sensory score ($SO3_{21}$) of IVCO 3 on day 21 were (45.28 47.92 56.25). The value of (a+c) was 101.53 (> 100). Therefore, under this condition, Eq. (9) was used instead of Eq. (3) to determine the defuzzified scores (Y_a') of the same.

In case of IVCO 1 and 2, defuzzified scores (Y_a) declined gradually up to day 40 (Table 4), meaning that sample acceptance with respect to all quality attributes deteriorated with time. $Y_a$values of IVCO 1 (27.22) and 2 (26.16) were foundto be insignificant ($p > 0.05$) on day 21 onwards. It implied that the above two samples reached almost saturation level of deterioration on the said day. For IVCO 4 and 5, the defuzzified scores declined in a similar manner as those of IVCO 1 and 2 with a variation in slope (Figure 4). From Table 4, it was found that the defuzzified scores of IVCO 4 and 5 were obtained under category ‘satisfactory’ up to day 14, which came down to 29.40 and 28.98, respectively on day 21 under category ‘fair’. Therefore, 4.5 kGy and 5.0 kGy dose levels did not show effective deodorization effects in coconut irradiated oil samples with time, especially with respect to its rancid-acid aroma. Comparing the defuzzified scores of all the five samples on each day of storage study, it was observed that only IVCO 3 experienced a continuous improvement with time, implying that 4.2 kGy dose level had effectively deodorized the oil sample with time. The defuzzified scores for IVCO 3 became the highest on day 28 i.e. 74.71 and it started to decrease on day 40 i.e., 70.21. However, both were under the category of ‘very good’. The defuzzified scores for the rest of the samples were found to be below 50 throughout the study period and therefore they were under categories of ‘fair’ or ‘satisfactory’. Thus, it was validated that 4.2 kGy irradiated coconut oil sample alone had the highest good sensory acceptance specifically with respect to

deodorization (aroma) on day 28. Afterwards again flavor reversion started to occur which resulted in decrease in defuzzified scores. This observation is in consonance with the inference of the studies using electronic nose (Ghosh et al., 2016).

3.6.Validation of new developed equation by similarity principle

Values of overall membership function (B_x) and similarity values of five samples (IVCO -1, 2, 3, 4, 5) on each storage day were calculated using eq. (11) and eq. (12), respectively. For example, the triplet of overall sensory score of IVCO 1 at day 0 was found as (24.17 32.08 45.56), i.e. a = 24.57, b = 32.08, c = 45.56. Using the value of this triplet in eq. (11), the values of membership function for IVCO 1(at day 0) was obtained as B10 = (0.5582 0.8700 1.0000 0.8720 0.6525 0.4330 0.2135 0.0000 0.0000 0.0000). Similar values for other samples at each day were evaluated using same equation (eq. 10) and were presented in Table 5. Subsequently, S_m of all five samples (IVCO-1, 2, 3, 4, 5) was calculated using eq. (12) (Table 6). The S_m values of both IVCO 1 and 2 were found maximum under "satisfactory" category throughout the period of the study. The similar trend was followed by IVCO 4 and 5 from day 21. IVCO 3 had the highest S_m under "good" category up to day 21. On day 28, S_m value was found to be maximum and therefore attested the VCO sample to "very good" category. Although $S_m$value of IVCO 3 on day 40(0.6400) was found under category of "very good", the value of S_m was lower ($p < 0.05$) than that of 28 days (0.8151). These findings (obtained from defuzzified scores and $S_m$analyses) revealed that IVCO 3, i.e. VCO irradiated at 4.2 kGy had no objectionable odor at the end of 28 days of storage at 23 ± 2 °C and therefore was sensorially highly accepted. This was in agreement with the findings, obtained by Ghosh et al. (2016) who reported that complete deodorization in this sample occurred at the day of 28 which was owing to absence of octanoic acid (due to radiolysis) in the same. The e-nose analysis, conducted by Ghosh et al. (2016) revealed that TGS 823 was used for evaluation of deodorization index of rancid-acid values ($DI_{rancid\text{-}acid}$) and this sensor provided the highest response on day 28 of storage for IVCO 3.

3.7. Validation of new developed equation against our previous finding by electronic nose technology

The new developed equation (eq. 9) for deriving defuzzified scores under condition (a+c) > 100, unambiguously concluded that VCO sample irradiated at 4.2 kGy on day 28 of storage was the best quality sample, especially with respect to deodorization which was in complete

agreement with the findings by e-nose technology and sensory evaluation (Ghosh et al., 2016).

4. Conclusion

From the above discussion, it can be concluded that the results of similarity value and the electronic nose analyses were in agreement with that obtained by the new developed equation of fuzzy logic analysis. As per the new equation, the defuzzified scores were evaluated and it's corresponding remarks implied that IVCO 3 was regarded as the best with respect to (w.r.t) deodorization (quality attribute: aroma) on day 28 in the storage-period. Similarity value analysis also remarked IVCO 3 as 'very good' w.r.t deodorization (quality attribute: aroma) on day 28 in accordance with six point standard fuzzy scale. This conclusion was further proved by the e-nose signal responses ($|\Delta R|/R$) of the irradiated coconut oil samples. This new developed equation can be widely adopted for ranking food samples unambiguously, rapidly and reliably, without any conflict with similarity value and e-nose approaches. It evades the complexity in evaluating defuzzified scores of samples, compared to similarity values approach.

This obnoxious odor free DCVO oil was successively used as a frying oil and investigated for its quality parameters vis-à-vis common frying oils.

Novelty:

This is the first report on development of an equation to derive defuzzified scores when the sum of the first and third coordinates of the triplet *(a b c)* of overall sensory score is greater than 100, i.e., (a+c) > 100. This newly developed equation has been applied to reaffirm that 4.2 kGy is the best dose of gamma irradiation for the purpose of deodorization of VCO.

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Table1. Panelists' preference for specific quality attributes of irradiated coconut oil samples and corresponding triplets

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
Aroma							
IVCO 1	0	18	12	0	0	0	$S1A_0$ = (10.00 10.00 25.00)
	7	16	14	0	0	0	$S1A_7$ = (11.67 11.67 25.00)
	14	18	12	0	0	0	$S1A_{14}$ = (10.00 10.00 25.00)
	21	19	11	0	0	0	$S1A_{21}$ = (9.17 9.17 25)
	28	20	10	0	0	0	$S1A_{28}$ = (8.33 8.33 25.00)
	40	20	10	0	0	0	$S1A_{40}$ = (8.33 8.33 25.00)
IVCO 2	0	12	18	0	0	0	$S2A_0$ = (15.00 15.00 25.00)
	7	15	15	0	0	0	$S2A_7$ = (12.50 12.50 25.00)
	14	17	13	0	0	0	$S2A_{14}$ = (10.83 10.83 25.00)
	21	18	12	0	0	0	$S2A_{21}$ = (10.00 10.00 25.00)
	28	19	11	0	0	0	$S2A_{28}$ = (9.17 9.17 25.00)
	40	20	10	0	0	0	$S2A_{40}$ = (8.33 8.33 25.00)
IVCO 3	0	0	17	13	0	0	$S3A_0$ = (35.83 25.00 25.00)
	7	0	11	19	0	0	$S3A_7$ = (40.83 25.00 25.00)
	14	0	10	20	0	0	$S3A_{14}$ = (41.67 25.00

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
	21	0	8	22	0	0	25.00) $S3A_{21}$ = (43.33 25.00 25.00)
	28	0	0	0	1	29	$S3A_{40}$ = (99.17 25.00 0.83)
	40	0	0	0	7	23	$S3A_{28}$ = (94.17 25.00 5.83)
IVCO 4	0	0	16	14	0	0	$S4A_0$ = (36.67 25.00 25.00)
	7	0	21	9	0	0	$S4A_7$ = (32.50 25.00 25.00)
	14	0	30	0	0	0	$S4A_{14}$ = (25.00 25.00 25.00)
	21	17	13	0	0	0	$S4A_{21}$ = (10.83 10.83 25.00)
	28	18	12	0	0	0	$S4A_{28}$ = (10.00 10.00 25.00)
	40	19	11	0	0	0	$S4A_{40}$ = (9.17 9.17 25.00)
IVCO 5	0	0	17	13	0	0	$S5A_0$ = (35.83 25.00 25.00)
	7	0	24	6	0	0	$S5A_7$ = (30.00 25.00 25.00)
	14	0	30	0	0	0	$S5A_{14}$ = (25.00 25.00 25.00)
	21	18	12	0	0	0	$S5A_{21}$ = (10.00 10.00 25.00)
	28	19	11	0	0	0	$S5A_{28}$ = (9.17 9.17 25.00)
	40	20	10	0	0	0	$S5A_{40}$ = (8.33 8.33 25.00)

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
Color							
IVCO 1	0	0	17	13	0	0	$S1C_0$ = (35.83 25.00 25.00)
	7	0	18	12	0	0	$S1C_7$ = (35.00 25.00 25.00)
	14	0	17	13	0	0	$S1C_{14}$ = (35.83 25.00 25.00)
	21	18	12	0	0	0	$S1C_{21}$ = (10.00 10.00 25.00)
	28	19	11	0	0	0	$S1C_{28}$ = (9.17 9.17 25.00)
	40	19	11	0	0	0	$S1C_{40}$ = (9.17 9.17 25.00)
IVCO 2	0	0	19	11	0	0	$S2C_0$ = (34.17 25.00 25.00)
	7	0	19	11	0	0	$S2C_7$ = (34.17 25.00 25.00)
	14	0	18	12	0	0	$S2C_{14}$ = (35.00 25.00 25.00)
	21	11	19	0	0	0	$S2C_{21}$ = (15.83 15.83 25.00)
	28	12	18	0	0	0	$S2C_{28}$ = (15.00 15.00 25.00)
	40	12	18	0	0	0	$S2C_{40}$ = (15.00 15.00 25.00)
IVCO 3	0	0	18	12	0	0	$S3C_0$ = (35.00 25.00 25.00)
	7	0	13	17	0	0	$S3C_7$ = (39.17 25.00 25.00)
	14	0	12	18	0	0	$S3C_{14}$ = (40.00 25.00 25.00)
	21	0	5	20	5	0	$S3C_{21}$

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
	28	0	0	0	2	28	= (50.00 25.00 25.00) $S3C_{40}$
	40	0	0	5	15	5	= (98.33 25.00 1.67) $S3C_{28}$ = (62.50 20.83 16.67)
IVCO 4	0	0	16	14	0	0	$S4C_0$ = (36.67 25.00 25.00)
	7	0	11	19	0	0	$S4C_7$ = (40.83 25.00 25.00)
	14	0	11	19	0	0	$S4C_{14}$ = (40.83 25.00 25.00)
	21	0	13	17	0	0	$S4C_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S4C_{28}$ = (38.33 25.00 25.00)
	40	0	15	15	0	0	$S4C_{40}$ = (37.50 25.00 25.00)
IVCO 5	0	0	17	13	0	0	$S5C_0$ = (35.83 25.00 25.00)
	7	0	10	20	0	0	$S5C_7$ = (41.67 25.00 25.00)
	14	0	15	5	0	0	$S5C_{14}$ = (20.83 16.67 16.67)
	21	0	13	17	0	0	$S5C_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S5C_{28}$ = (38.33 25.00 25.00)
	40	0	15	15	0	0	$S5C_{40}$ = (37.50 25.00 25.00)
Homogeneity							
IVCO 1	0	0	15	15	0	0	$S1H_0$

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
	7	0	15	15	0	0	= (37.50 25.00 25.00) $S1H_7$
	14	0	18	12	0	0	= (37.50 25.00 25.00) $S1H_{14}$
	21	0	13	17	0	0	= (35.00 25.00 25.00) $S1H_{21}$
	28	0	13	17	0	0	= (39.17 25.00 25.00) $S1H_{28}$
	40	0	13	17	0	0	= (39.17 25.00 25.00) $S1H_{40}$ = (39.17 25.00 25.00)
IVCO 2	0	0	19	11	0	0	$S2H_0$ = (34.17 25.00 25.00)
	7	0	16	14	0	0	$S2H_7$ = (36.67 25.00 25.00)
	14	0	17	13	0	0	$S2H_{14}$ = (35.83 25.00 25.00)
	21	0	19	11	0	0	$S2H_{21}$ = (34.17 25.00 25.00)
	28	0	21	9	0	0	$S2H_{28}$ = (32.50 25.00 25.00)
	40	0	21	9	0	0	$S2H_{40}$ = (32.50 25.00 25.00)
IVCO 3	0	0	14	16	0	0	$S3H_0$ = (38.83 25.00 25.00)
	7	0	13	17	0	0	$S3H_7$ = (39.17 25.00 25.00)
	14	0	14	16	0	0	$S3H_{14}$ = (38.33 25.00 25.00)
	21	0	7	19	4	0	$S3H_{21}$ = (47.50 25.00

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
	28	0	0	0	3	27	25.00) $S3H_{40}$ = (98.33 25.00
	40	0	0	5	25	0	25.00) $S3H_{28}$ = (70.83 25.00 20.00)
IVCO 4	0	0	17	13	0	0	$S4H_0$ = (35.83 25.00 25.00)
	7	0	15	15	0	0	$S4H_7$ = (37.50 25.00 25.00)
	14	0	11	19	0	0	$S4H_{14}$ = (40.83 25.00 25.00)
	21	0	13	17	0	0	$S4H_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S4H_{28}$ = (38.33 25.00 20.00)
	40	0	15	15	0	0	$S4H_{40}$ = (37.50 25.00 25.00)
IVCO 5	0	0	16	14	0	0	$S5H_0$ = (36.67 25.00 25.00)
	7	0	16	14	0	0	$S5H_7$ = (36.67 25.00 25.00)
	14	0	14	16	0	0	$S5H_{14}$ = (38.33 25.00 25.00)
	21	0	13	17	0	0	$S5H_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S5H_{28}$ = (38.33 25.00 20.00)
	40	0	15	15	0	0	$S5H_{40}$ = (37.50 25.00 25.00)
Flowability							
IVCO 1	0	0	12	18	0	0	$S1F_0$ = (40.00 25.00 25.00)

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
	7	0	17	13	0	0	$S1F_7$ = (35.83 25.00 25.00)
	14	0	16	14	0	0	$S1F_{14}$ = (36.67 25.00 25.00)
	21	0	14	16	0	0	$S1F_{21}$ = (38.33 25.00 25.00)
	28	0	14	16	0	0	$S1F_{28}$ = (38.33 25.00 25.00)
	40	0	14	16	0	0	$S1F_{40}$ = (38.33 25.00 25.00)
IVCO 2	0	0	15	15	0	0	$S2F_0$ = (37.50 25.00 25.00)
	7	0	18	12	0	0	$S2F_7$ = (35.00 25.00 25.00)
	14	0	15	15	0	0	$S2F_{14}$ = (37.50 25.00 25.00)
	21	0	14	16	0	0	$S2F_{21}$ = (38.33 25.00 25.00)
	28	0	15	15	0	0	$S2F_{28}$ = (37.50 25.00 25.00)
	40	0	15	15	0	0	$S2F_{40}$ = (37.50 25.00 25.00)
IVCO 3	0	0	13	17	0	0	$S3F_0$ = (39.17 25.00 25.00)
	7	0	15	15	0	0	$S3F_7$ = (37.50 25.00 25.00)
	14	0	14	16	0	0	$S3F_{14}$ = (38.33 25.00 25.00)
	21	0	7	20	3	0	$S3F_{21}$ = (46.67 25.00 25.00)
	28	0	0	0	10	20	$S3F_{40}$

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory scores
	40	0	0	16	14	0	= (91.67 25.00 8.33) $S3F_{28}$ = (61.67 25.00 25.00)
IVCO 4	0	0	18	12	0	0	$S4F_0$ = (35.00 25.00 25.00)
	7	0	18	12	0	0	$S4F_7$ = (35.00 25.00 25.00)
	14	0	18	12	0	0	$S4F_{14}$ = (35.00 25.00 25.00)
	21	0	14	16	0	0	$S4F_{21}$ = (38.33 25.00 25.00)
	28	0	15	15	0	0	$S4F_{28}$ = (37.50 25.00 25.00)
	40	0	16	14	0	0	$S4F_{40}$ = (36.67 25.00 25.00)
IVCO 5	0	0	20	10	0	0	$S5F_0$ = (33.33 25.00 25.00)
	7	0	18	12	0	0	$S5F_7$ = (35.00 25.00 25.00)
	14	0	19	11	0	0	$S5F_{14}$ = (34.17 25.00 25.00)
	21	0	15	15	0	0	$S5F_{21}$ = (37.50 25.00 25.00)
	28	0	14	16	0	0	$S5F_{28}$ = (38.33 25.00 25.00)
	40	0	15	15	0	0	$S5F_{40}$ = (37.50 25.00 25.00)

Table 2. Panelists' preference for quality attributes of irradiated coconut oil samples in general and corresponding triplets

Quality attributes	NI	SI	I	HI	EI	Triplets of sensory score	triplets of relative weightage
Aroma	0	0	0	0	30	(75.00 25.00 25.00)	(0.50 0.17 0.17)
Colour	30	0	0	0	0	(0 0 25.00)	(0.00 0.00 0.17)
Homogeneity	0	0	30	0	0	(50.00 25.00 25.00)	(0.33 0.17 0.17)
Flowability	0	0	0	0	0	(25.00 25.00 25.00)	(0.17 0.17 0.17)

Table 3. Defuzzified score of quality attributes of irradiated coconut oil samples

Quality attributes	Q (for relative importance)			Defuzzified scores
	a	b	c	
Aroma	75	25	25	75.00
Colour	0	0	25	8.33
Homogeneity	50	25	25	50.00
Flowability	25	25	25	25.00

Table 4. Defuzzified scores of irradiated coconut oil sample

Time	Sample no.	SO			(a+c)	Y_a/ $Y_{a'}$	Remarks
		a	b	c			
Day 0	IVCO 1	24.17	32.08	45.56	69.73	28.66	Fair
	IVCO 2	25.14	34.44	45.14	70.28	28.70	Fair
	IVCO 3	37.22	43.89	49.72	86.94	39.17	Satisfactory
	IVCO 4	36.11	42.92	49.03	85.14	38.15	Satisfactory
	IVCO 5	35.69	42.64	48.61	84.30	37.69	Satisfactory
Day 7	IVCO 1	24.31	32.50	45.00	69.31	28.47	Fair
	IVCO 2	24.31	32.78	44.72	69.03	28.29	Fair
	IVCO 3	39.74	45.26	52.01	91.75	41.99	Satisfactory
	IVCO 4	34.58	42.50	49.31	83.89	36.85	Satisfactory
	IVCO 5	33.06	41.94	48.89	81.95	35.57	Satisfactory
Day 14	IVCO 1	22.78	31.11	44.58	67.36	27.27	Fair
	IVCO 2	23.61	31.94	44.86	68.47	27.92	Fair
	IVCO 3	40.00	44.72	51.39	91.39	42.22	Satisfactory
	IVCO 4	31.94	41.81	48.61	80.55	34.21	Satisfactory
	IVCO 5	30.97	41.25	44.72	75.69	32.13	Satisfactory
Day 21	IVCO 1	24.03	31.53	41.11	65.14	27.22	Fair
	IVCO 2	22.78	31.25	41.39	64.17	26.16	Fair
	IVCO 3	45.28	47.92	56.25	**101.53**	**48.04**	Satisfactory
	IVCO 4	24.86	32.64	46.25	71.11	29.40	Fair
	IVCO 5	24.31	31.94	45.97	70.28	28.98	Fair
Day 28	IVCO 1	23.61	30.97	40.83	64.44	26.90	Fair
	IVCO 2	21.67	30.28	40.69	62.36	25.14	Fair
	IVCO 3	97.36	73.06	67.08	**164.44**	**74.71**	*Very good*
	IVCO 4	24.03	31.81	45.69	69.72	28.66	Fair
	IVCO 5	23.75	31.39	45.69	69.44	28.52	Fair
Day 40	IVCO 1	23.61	30.97	40.83	64.44	26.90	Fair
	IVCO 2	21.25	29.72	40.56	61.81	24.86	Fair
	IVCO 3	80.97	62.78	63.61	**144.58**	**70.21**	*Very good*
	IVCO 4	23.19	30.97	45.14	68.33	27.92	Fair
	IVCO 5	22.92	30.56	45.14	68.06	27.78	Fair

Table 5. Overall membership function values of irradiated coconut oil samples

Storage day	Values of overall membership functions (B_x)									
Day 0										
IVCO 1	0.5582	0.8700	1	0.8720	0.6525	0.4330	0.2135	0	0	0
IVCO 2	0.6001	0.9273	1	0.8238	0.5974	0.3710	0.1446	0	0	0
IVCO 3	0.3798	0.6076	0.8354	1	0.9440	0.7430	0.5418	0.3407	0.1395	0
IVCO 4	0.3916	0.6246	0.8576	1	0.9206	0.7170	0.5127	0.3087	0.1048	0
IVCO 5	0.3975	0.6320	0.8665	1	0.9113	0.7060	0.4998	0.2941	0.0884	0
Day 7										
IVCO 1	0.5597	0.8674	1.1751	0.8736	0.6513	0.4291	0.2069	0	0	0
IVCO 2	0.5635	0.8685	1.1736	0.8728	0.6492	0.4255	0.2019	0	0	0
IVCO 3	0.3578	0.5802	0.8025	1.1717	0.9783	0.7850	0.5916	0.3983	0.2049	0.0116
IVCO 4	0.3750	0.6058	0.8366	1.1412	0.9418	0.7423	0.5429	0.3434	0.1440	0
IVCO 5	0.3566	0.5859	0.8152	1.1594	0.9616	0.7638	0.5661	0.3683	0.1705	0
Day 14										
IVCO 1	0.5892	0.9106	1.2321	0.8380	0.6137	0.3894	0.1651	0	0	0
IVCO 2	0.5739	0.8870	1.2001	0.8576	0.6346	0.4117	0.1888	0	0	0
IVCO 3	0.3659	0.5930	0.8201	1.1575	0.9586	0.7597	0.5609	0.3620	0.1631	0
IVCO 4	0.3428	0.5692	0.7956	1.1772	0.9810	0.7848	0.5886	0.3924	0.1962	0
IVCO 5	0.3418	0.5696	0.7974	1.1744	0.9782	0.7820	0.5858	0.3896	0.1934	0
Day 21										
IVCO 1	0.5550	0.8721	1	0.8547	0.6115	0.3683	0.1250	0	0	0
IVCO 2	0.5910	0.9110	1	0.8255	0.5839	0.3424	0.1007	0	0	0
IVCO 3	0.2637	0.4724	0.6811	0.8898	1	0.9161	0.7383	0.5605	0.3828	0.2049
IVCO 4	0.5447	0.8511	1	0.8888	0.6726	0.4564	0	0	0	0
IVCO 5	0.5519	0.8650	1	0.8762	0.6586	0.4412	0	0	0	0
Day 28										
IVCO 1	0.5605	0.8834	1	0.8435	0.5986	0.3537	0.1087	0	0	0
IVCO 2	0.6146	0.9448	1	0.7953	0.5495	0.3038	0.0580	0	0	0
IVCO 3	0	0	0.0780	0.2149	0.3518	0.4886	0.6255	0.7624	0.8993	1.1097
IVCO 4	0.5589	0.8733	1	0.8693	0.6505	0.4316	0.2127	0	0	0
IVCO 5	0.5620	0.8805	1	0.8632	0.6443	0.4255	0.2066	0	0	0
Day 40										
IVCO 1	0.5605	0.8834	1.2063	0.8435	0.5986	0.3537	0.1087	0	0	0
IVCO 2	0.6215	0.9579	1.2944	0.7843	0.5377	0.2912	0.0446	0	0	0
IVCO 3	0	0.0288	0.1881	0.3474	0.5067	0.6660	0.8253	0.9845	1.1438	0.8580
IVCO 4	0.5741	0.8970	1.2199	0.8491	0.6276	0.4061	0.1845	0	0.0000	0
IVCO 5	0.5772	0.9045	1.2317	0.8432	0.6216	0.4001	0.1786	0	0.0000	0

Table 6. Similarity values of irradiated oil samples and their ranking

Sample no.	Storage day	Six categories					
		Not significant	**Fair**	**Satisfactory**	**Good**	**Very good**	**Excellent**
IVCO 1	0	0.0000	0.3394	1.0398	0.9010	0.3914	0.0430
	7	0.0000	0.4011	1.0428	0.9040	0.3878	0.0420
	14	0.0000	0.4367	1.0965	0.8968	0.3598	0.0343
	21	0.0000	0.4303	1.1190	0.9340	0.3469	0.0269
	28	0.0000	0.4420	1.1403	0.9352	0.3361	0.0238
	40	0.0000	0.4420	1.1403	0.9352	0.3361	0.0238
IVCO 2	0	0.0000	0.4522	1.1215	0.8959	0.3464	0.0306
	7	0.0000	0.4032	1.0457	0.9038	0.3850	0.0451
	14	0.0000	0.4167	1.0655	0.9001	0.3762	0.0385
	21	0.0000	0.4610	1.1543	0.9171	0.3239	0.0220
	28	0.0000	0.4603	1.2100	0.9119	0.2909	0.0132
	40	0.0000	0.5088	1.2303	0.9103	0.2794	0.0102
IVCO 3	0	0.0000	0.2074	0.6471	0.8290	0.5846	0.1856
	7	0.0000	0.1879	0.5976	0.8037	0.5987	0.2012
	14	0.0000	0.1980	0.6268	0.8221	0.5924	0.1923
	21	0.0311	0.2539	0.5440	**0.6437**	0.4593	0.1563
	40	0.0000	0.0000	0.0902	0.4151	***0.8151***	0.5244
	28	0.0000	0.0913	0.3100	0.5371	***0.6400***	0.2947
IVCO 4	0	0.0000	0.2071	0.5883	0.9461	0.8608	0.3136
	7	0.0000	0.2058	0.6469	0.8294	0.5850	0.1864
	14	0.0000	0.1842	0.5972	0.8131	0.6043	0.2012
	21	0.0000	0.4080	1.0790	0.9622	0.3328	0.0000
	28	0.0000	0.4521	1.1041	0.9539	0.3231	0.0000
	40	0.0000	0.4220	1.0759	0.8993	0.3733	0.0380
IVCO 5	0	0.0000	0.2245	0.6912	0.8484	0.5686	0.1711
	7	0.0000	0.1942	0.6215	0.8222	0.5961	0.1945
	14	0.0000	0.1847	0.6003	0.8153	0.6044	0.2012
	21	0.0000	0.4178	1.0951	0.9580	0.3270	0.0000
	28	0.0000	0.4295	1.1114	0.9516	0.3206	0.0000
	40	0.0000	0.4270	1.0846	0.8983	0.3693	0.0373

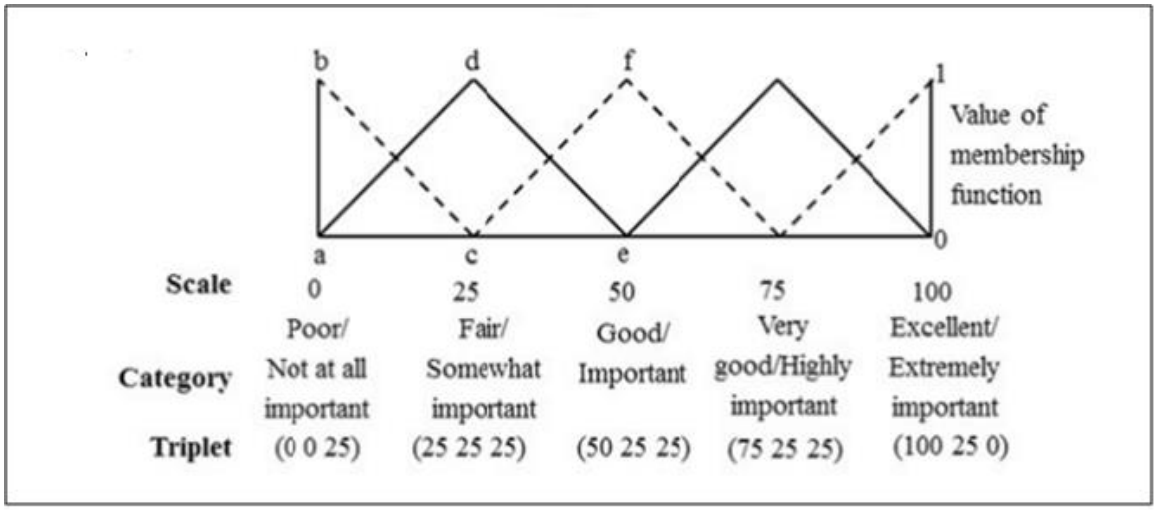


Figure 1. Distribution pattern of five point sensory scale associated with corresponding

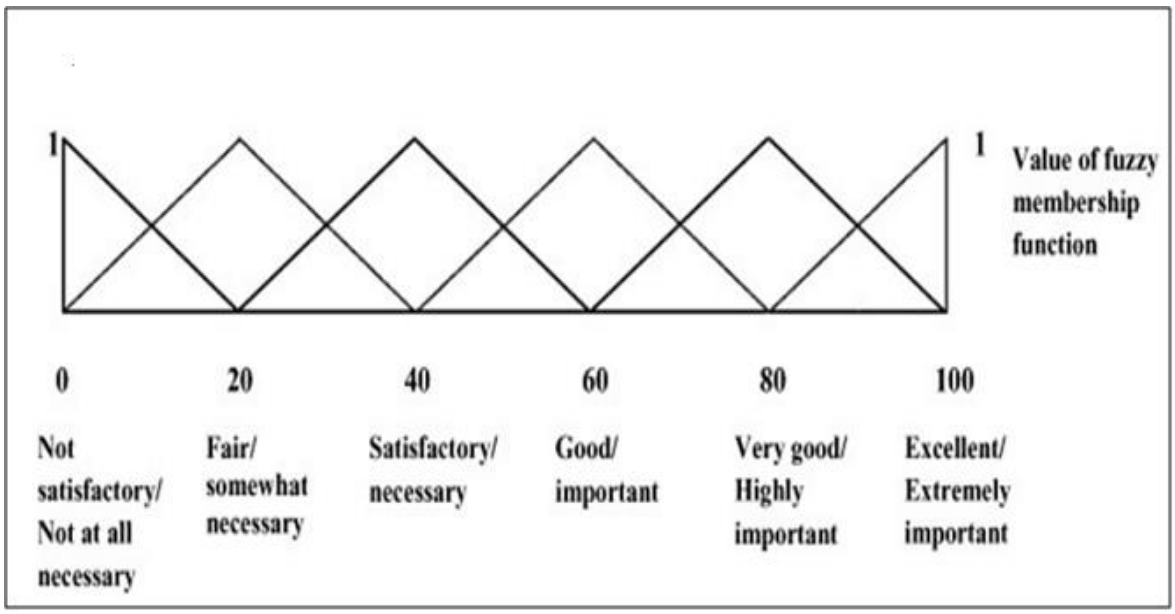


Figure 2. Standard Fuzzy scale

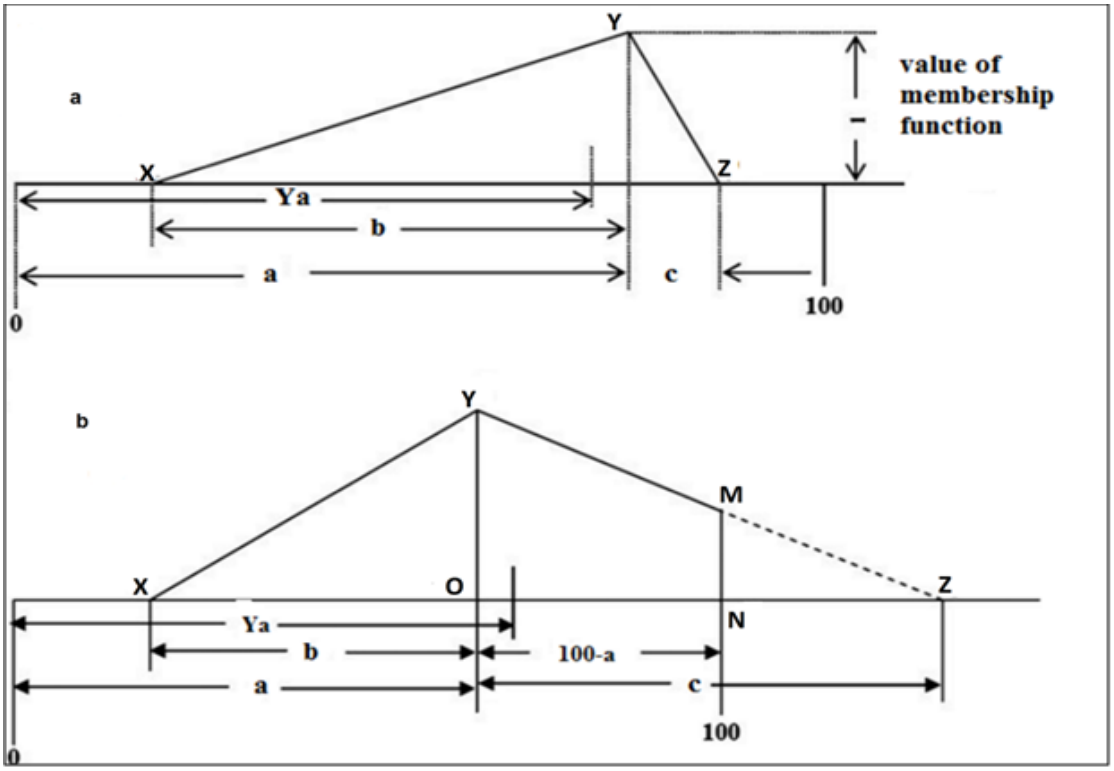


Figure 3.(a)Graphical view of overall sensory score as triangle ABC when the value of (a+c) is less than 100, the triangle XYZ lies within the sensory scale interval [0, 100], (b) Graphical view of overall sensory score as polygon XYMN when the value of (a+c) is greater than 100, a part of the triangle XYZ lies beyond the interval 0 to 100

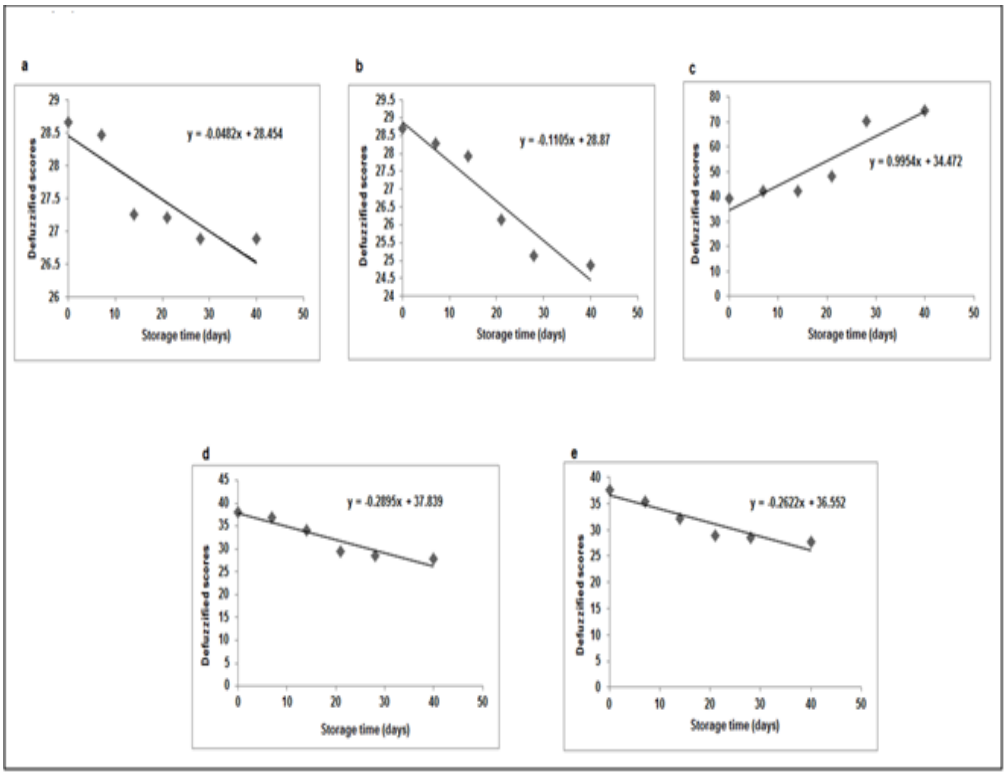
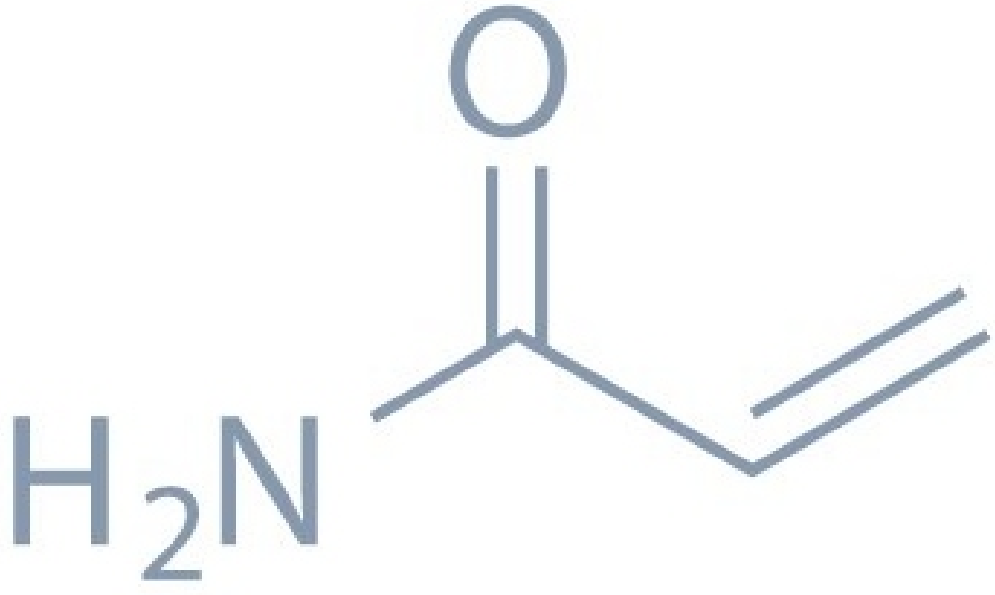


Figure 4. Plot of defuzzified scores of irradiated coconut oil samples with storage time (days) for (a) IVCO 1(0 kGy), (b) IVCO 2 (4.0 kGy), (c) IVCO 3 (4.2 kGy), (d) IVCO 4 (4.5 kGy), and (e) IVCO 5 (5.0 kGy).



CHAPTER 3:

Acrylamide mitigation and 2,4-decadienal elimination in fried potato-crisps

1. Introduction

AA (2-propene amide) is labeled as a human carcinogen-cum-neurotoxin by the International Agency for Research on Cancer, Lyon, France since 2002 (Claus et al., 2008). The Swedish National Food Administration, Sweden and the University of Stockholm, Stockholm, Sweden reports the formation of AA in carbohydrate-rich foods at processing temperatures higher than 120 °C, as a product of Maillard reactions between reducing sugar(s) and free amino-acid (L-asparagine). Fried potato-crisps are one of the most commonly consumed snacks globally. Among several strategies implemented for mitigation of AA in fried potato products, aqueous pre-treatments such as rinsing, soaking and blanching are reportedly effective (Pedreschi et al., 2004). Many additives have proved to be useful in pre-treatment of potatoes prior to frying. For example, addition of 2% citric acid or 1% NaCl or ~2% $CaCl_2$ to soaking solutions lowers subsequent AA formation in fried potato chips by 60% (Rydberg et al., 2003), 75% (Mestdagh et al., 2007) and 85% (Gökmen et al., 2007), respectively. Another strategy for mitigation of AA in food products (fried and baked) is the use of the enzyme asparaginase which effectively reduces AA in fried and baked food products by 99% (Zyzak et al., 2009). However, all the above-mentioned methodologies adversely affect sensory quality of the fried products and therefore could not be adopted successfully.

Other approaches of mitigation of AA formation in fried potato products include reducing the formation of AA using amino-acids and modification of thermal processing conditions (Eriksson, 2005). Addition of amino-acids in glucose-asparagine model system could successfully mitigate AA formation (Koutsidis et al., 2009). Nine amino acids- histidine, alanine, methionine, glutamic acid, aspartic acid, proline, phenylalanine, valine and arginine could individually reduce AA by 22-78% in the glucose (0.4%)-L-asparagine (0.18%) model system heated at 160 °C (Elder et al., 2007). Similar experiment on mitigation of AA formation was conducted with 5.0% aqueous suspension of waxy maize starch accompanied by reactants glucose (50 mmol/kg) and amino-acids (mentioned earlier) (25 or 75 mmol/kg) to elucidate the effect of each amino-acid on AA formation (Koustsidis et al., 2009). The findings of Koustsidis et al. (2009) complemented the findings of Elder et al. (2007) that a AA formation is strongly correlated with the presence of amino-acid addition. Kim et al. (2005) reported that 0.5% glycine and 3.0% lysine in combintion during pre-treatment of potatoes could mitigate AA formation in potato chips by more than 70% and 80%, respectively. AA in french fries can be reduced by 40% using 2% glutamine (Bråthen and Knutsen, 2005). A maximum concentration of 3.0% (w/d.w of potato flakes) of L-cysteine,

glycine, glutamine and lysine have been used for thermally processed foods such as potato chips and bread. However, none of these reported methods could reduce AA by more than 90% and compromised the fried chips organoleptically. To the best of the knowledge, there is no published literature on alleviation of AA in fried potato-crisps by addition of L-proline accompanied by multiple stage treatments.

The commonly used oil for deep frying potato-crisps is soybean oil due to its high smoke point (216 to 252 ℃) and reasonable price (Serna-Saldivar and Chuck-Hernandez, 2019). Being linoleic acid-rich oil (C18:2; 51%) (Thomas et al., 2016), it produces a potentially toxic aldehyde, namely z,z-2,4-decadienal (2,4-De) through oxidation of C18:2 in oil which eventually concentrates in the fried products (Boskou et al., 2006). In the current investigation, deep frying was carried out using deodorized-virgin-coconut oil (DVCO) instead of using soybean oil. DVCO was prepared in our laboratory by deodorizing VCO using 4.2 kGy dose of gamma-irradiation and subsequent storage of the irradiated oil at 23±2 ℃ for 28 days, in accordance with the method elaborated by Ghosh et al. (2016). There is ample evidence in literature that the predominant presence of medium chain fatty acid (MCF), principally lauric acid (C12:0; 47.8%) (Narayanankutty et al., 2018; Ghosh et al., 2014) in VCO would never support the formation of any toxic aldehyde. DVCO was preferred over VCO since the latter renders a typical rancid-acid odor which limits acceptability of VCO-fried products at large.

The current investigation reports for the first time on the application of L-proline amino acid with unique treatment combinations for mitigation of acrylamide and minimizing content of 2,4-De in DVCO-fried potato-crisps. The study examined the feasibility of formulating fried potato-crisps using exclusive treatment combinations for delivering safe fried products while preserving their sensory appeal.

2. Material and Methods

2.1. Materials

Potato tubers (Kufri jyoti) were purchased from a local super market of Jadavpur, Kolkata, India. Soybean oil (M/s Adani Wilmar Ltd., Haldia, India) was purchased from a local super market and DVCO, prepared in our "Green Technology Laboratory" following the procedure developed by Ghosh et al. (2016), were used for the current study. A potato cutter was procured from M/s Jas Enterprises, Ahmedabad, India. Frying was carried out in a countertop

electric deep fat fryer procured from M/s Shiva Kitchen Appliances, Kolkata, India. All chemicals and solvents used were of AR grade. Speciality chemicals such as acrylamide (~99% pure), 2,4-decadienal (> 90% pure) were procured from M/s Sigma-Aldrich, Bengaluru, India and M/s Aldrich, Steinheim, Germany respectively. Food grade L-proline was procured from Indiamart. HPLC grade solvents namely *n*-hexane, acetonitrile, methanol, phosphoric acid, 2-propanol and deionized water were procured from M/s Merck, Mumbai, India. SPE-QuEChERS (Solid phase extraction-Quick, Easy, Cheap, Effective, Rugged, and Safe) method was performed for extraction of AA from bread samples and purification of the same using QuEChERS extraction kit (p/n 5982-5850), purchased from M/s Agilent Technologies, California, USA.

2.2. Preparation of conventional set of fried potato-crisps

Fresh potato tubers were washed under running water for 5 min, peeled and sliced to average dimensions of 70 × 5 × 10 mm using a potato cutter. The sliced tubers were washed in distilled water and then surface water was removed using tissue papers. Thereafter the dried slices of potatoes were subjected to deep frying in DVCO at 170 °C for 3 min [deep frying conditions; surface: volume (S/V) = 1.0 cm^{-1}] in an electric deep fat fryer (2270-2500 W, 50 Hz, 220-230 V).

2.3. Preparation of experimental control (EC) set of fried potato-crisps

Fresh potato tubers were washed under running water for 5 min, peeled and sliced to average dimensions of 70 × 5 × 10 mm using a potato cutter. The sliced tubers were washed in distilled water and then surface water was removed using tissue papers. Thereafter the dried slices of potatoes were subjected to deep frying in soybean oil at 170 °C for 3 min (deep frying conditions; surface: volume,S/V = 1.0 cm^{-1}) in an electric deep fat fryer (2270-2500 W, 50 Hz, 220-230 V). The purpose of this EC set was to verify the method of extraction of 2,4-De via identification by high resolution-mass spectrometry (*vide infra*) and make a comparative study on rancidity with DVCO-fried potato crisps (discussed in Chapter-4).

2.4. Preparation of treated and control sets of fried potato-crisps

2.4.1. Aqueous pre-treatments of potato slices

It is well known that incorporation of aqueous pre-treatment steps *viz.* rinsing, soaking and blanching of potato slices contribute to reduction in AA formation (Table 1). Rinsing and

soaking of potato slices up to 90 min can reduce sugar content by 20% via leaching of precursors from the surfaces of cut potatoes (Pedreschi et al., 2004). Blanching is reportedly known to be more effective compared to the former processes in reducing the contents of AA precursors (L-asparagine and reducing sugar) (Jackson and Al-Taher, 2005). In this current study, steps of aqueous pre-treatment, *viz*. immersion and blanching, time and temperature of treatments of potato slices were optimized through several preliminary trials on basis % loss of precursors and texture. The rinsed potato slices were immersed in distilled water for 40 min and subsequently blanched at 70 °C for 20 min. Post blanching, the potato slices were subjected to further treatments.

2.4.2. Treatment by L-proline followed by frying under modified conditions

The aqueous pre-treated sets of potato slices were then soaked in L-proline of different concentrations (Table 2). The temperature of the soaking solution was maintained at 80 °C and the time of immersion was 30 min (time-temperature for immersion was optimized based on texture of potato slices through preliminary trials). Aqueous immersion-solution was drained off and then air-dried for 25 min at ambient condtions (23±2 °C, 70% RH) and finally tray-dried at 60 °C for 130 min under pre-set drying conditions (fixed through several preliminary trials) to a moisture content of ~40 % [moisture analysis of the potato slices and crisps of three different sets including conventional (*vide infra*), control and treated sets (prior to frying and post frying) conducted in accordance with AOAC methods (1998).The dried potato slices were then fried in DVCO under varying deep frying conditions (s/v = 1.0 cm^{-1}).

2.4.3. Preparation of control sets of fried potato crisps

Non-treated set of potato slices fried under modified and optimized deep frying conditions (s/v = 1.0 cm^{-1}) has been designated as the control set. The importance of control set was to find the impact of modified deep frying conditions on % acrylamide mitigation and physicochemical characteristics of the fried product, vis-à-vis the conventional frying conditions (170 °C for 3 min). Percent acrylamide mitigation was calculated as [(quantity of AA in conventional set – quantity of AA in treated set)*100 / (quantity of AA in conventional set)].

2.5. Quantification of asparagine and reducing sugar contents in aqueous-pretreated and control/conventional set of potato slices

AA precursors were quantified to study the effects of aqueous pre-treatment on potato slices.

Analyses of L-asparagine and reducing sugar contents in potato tuber, control/conventional set of potato slices (washed in water) and aqueous pre-treated potato slices were performed (Table 1) according to the methods described by Boos et al. (1996) and McFeeters' (1993) respectively.

2.6. AA extraction from conventional, control and treated fried potato-crisps using SPE-QuEChERS method

In this SPE-QuEChERS extraction method, all the parameters (quantity of sample and ratio of water-acetonitrile for extraction of AA) were optimized. A sample (grounded) of ~15 g was transferred into a 50-mL Falcon tube. Then 5 mL of *n*-hexane was added and vortexed for 5 min followed by addition of 20 mL mixed solution of water and acetonitrile (ACN) in 4:1 ratio. Thereafter Bond Elut QuChERS salt packet was added after addition of each solvent and was again vortexed for 1 min. The tubes were centrifuged for 1 min at 5000 x g. The upper layer of hexane was discarded and 1 mL of aqueous-ACN layer was pipetted out into 2 mL miocrocentrifugal vial pre-packed with 50 mg PSA and 150 mg $MgSO_4$. The sample was vortexed for 1 min followed by centrifugation at 3000 x g for 5 min. The filtrate was collected through 25 µ filter-paper. It was transferred into amber-colored screw capped glass vials in an inert atmosphere of nitrogen, stored at 4 ℃ in the dark and subjected to high resolution mass spectrometry (HR-MS) and reverse phase-high performance liquid chromatography (RP-HPLC) for qualitatitive and quantitative analyses of AA, respectively.

2.7. Extraction of 2,4-De from conventional, optimized treated (T-22) and EC sets

Total 5 batches of conventional and EC sets of fried potato-crisps were successively deep fried in the same oil (implying that the same oil was subjected to repeated frying for 5 times without replenishment by fresh oil as is practiced industrially) at 170 ℃ for 3 min in DVCO and soybean oil, respectively to to find the effect of frying oil on formation of the potent toxin, 2,4-De i.e., assess the presence of in the same. Frying operation was performed in duplicate for each batch of sample in each of these two oils. The cooking oil was allowed to cool prior to each new frying operation. The excess oil was drained off from the fried samples using cross-linked S.S wire mesh. Samples of each batch was packaged in LDPE-bags under nitrogen flushing, marked accordingly and stored at –24 ℃ for further analysis. T-22 set was also considered to assess the presence of this potential toxin.

The method for extraction of 2,4-De reported by Boskou et al. (2006) was adopted with few modifications. A quantity of fried potato crisps (25 g) (conventional and EC sets) was extracted three times with HPLC-grade methanol (50 mL) and the extracts were combined. The combined extract was concentrated to 5 mL using vigreaux column. The concentrated methanol extract of potato fries was subjected to qualitative and quantitative analyses of 2,4-De therein by HR-MS and RP-HPLC, respectively.

2.8. Qualitative analysis of AA and 2,4-De by HR-MS

HR-MS is invaluable to prove the identity of AA and 2,4-De. In this current study, standard acrylamide (concentration: 200 µg/mL) in acetronitrile and standard 2,4-De in methanol were pumped to HR-MS (Model: QTOF Micro YA263) at a flow rate of 5 µL/min. Orbitrap MS equipped with a heated electrospray interface, was operated in the positive mode and scanned the ions in the m/z range of 50−200 in both of the cases. The resolving power was set to 50000 full width at half-maximum as reported by Troise et al., (2014). The interface parameters were: spray voltage, 4.0 kV; capillary voltage, 41.0 V; skimmer voltage, 13 V; capillary temperature, 300 °C; heater temperature, 250 °C; sheath gas flow, 35 (arbitrary units); and auxiliary gas flow, 5 (arbitrary units). These conditions were maintained for the SPE-QuEChERS and methanolic extracted sample solution to identify AA (Figure 1a) and 2,4-De (Figure 1b), respectively.

2.9. Quantification of AA in fried potato-crisps by RP-HPLC

Quantification of AA in the purified extract of defatted (AOAC Method 3540) conventional, control and in different treated sets of fried potato-crisps was performed by RP-HPLC analysis. The RP-HPLC method for detection of AA reported by Khoshnam et al. (2010) was adopted with few modifications. The HPLC system (100V-240V 50 / 60 Hz 28 W; MD 2015 plus, M/s JASCO, Easton, US) consisted of a reversed-phase C18 column (25 cm × 0.46 cm I.D.) with a HPLC pump (PU 2080 plus, M/s JASCO Easton, US) and an injector with a 20 µL sample loop. Retention time of acrylamide was determined using HPLC-PDA detector (M/s JASCO MD 2015 plus, Easton, US) at 200 nm. The mobile phase (HPLC grade water) was purged at a constant flow rate of 1 mL/min. A standard curve of pure AA (10-200 µg/L) was prepared by dissolving a pure standard of AA (Figure 6a) in HPLC grade deionized water. Dilutions were made immediately before injection into LC column for quantification. The limit of detection (LOD) was determined to be 10 µg/L. The peak of standard AA and

AA in the sample under optimized operating conditions are presented by Fig. 6 (a) and (b), respectively.

2.10. Hierarchical cluster analysis (HCA) analysis of AA contents obtained different treatment conditions

In this study, HCA was employed as an 'unsupervised classification' method to assess the association of % similarity in AA mitigation among 27 different treatment conditions (Table 2). The results of HCA are presented in a dendogram.

2.11. Sensory evaluation of fried potato-crisps

Sensory evaluation by 30 semi-trained panelists (comprising of University staff, faculty and students) were carried out for treated sets of fried potato-crisps in an air-ventilated room under white light. Unsalted crackers and water were supplied to panelists for refreshing their palates before tasting subsequent samples. Frying time and temperature contributes to sensory perception of the fried product, including it's texture. In the current investigation, a total of 27 runs were performed under nine time-temperature combinations (Table 1) which differed from conventional deep frying conditions (*vide supra*). Sensory evaluation of the treated fried potato crisps wherein ≥ 98% AA mitigation had occurred was considered for the evaluation. A total of six quality attributes such as color, odor, texture, taste, after-taste and overall acceptability of the aforementioned sample sets were adjudged on the basis of the standard 9-point hedonic scale (Stone and Sidel, 2012).

2.12. Optimization of the treatment conditions (concentration of L-proline, frying time and temperature) for AA mitigation in fried potato-crisp

The process for % AA mitigation in fried potato-crisps was verified using full factorial design with three parameters and response surface methodology (RSM). Three factors namely % L-proline (g/100g of blanched potato slices), frying time (min) and temperature (℃) were considered as independent variables and % AA mitigation in fried potato-crisps as the response variable (Table 2 and 3). Preliminary trials suggested that significant percentage of AA mitigation ($p < 0.05$) was obtained when the potato slices were treated with L-proline ranging from 1%-2% (w/w), at modified deep frying temperature i.e., 140-160 ℃ for time varying from 4-6 min. Significant changes in % AA mitigation ($p < 0.05$) was found when % L-proline, frying temperature and time were varied at 0.5% (w/w) (1, 1.50 and 2%), 10 ℃ (140, 150 and 160 ℃) and 1.0 min (4.0, 5.0 and 6.0 min) intervals, respectively. Treatment

conditions were optimized on basis of % AA mitigation and overall acceptability (objective sensory evaluation, performed by 15 semi-trained panelists).

2.13. Validation of AA content in conventional and T-22 sets of fried potato crisps by liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis

The quantification of AA in conventional and T-22 sets of fried potato crisps were verified by LC–MS/MS (company name) using C18 column (L×I.D. 150 mm×3 mm, 5 µm particle size and 125 Å pore size) and electrospray ionization (ESI) according to the method reported by Mulla *et al*. (2017). The operating conditions of ESI were: capillary voltage of 3.0 kV, cone voltage of 24 V, desolvation temperature of 500 °C and desolvation gas flow was 1000 L/h. The mobile phase comprised of aqueous solution of 0.1% formic acid. The MS data were acquired in the positive mode and AA was detected in multiple reactions monitoring (MRM) mode. The transition of MRM was monitored from 71.66 to 54.80 at 8 V.

A standard curve of AA (10-200 µg/L) was prepared by dissolving a pure standard of the same in HPLC grade water. Dilutions were made immediately before injection into LC–MS/MS analysis. The LOD was determined as 0.6 ng/g.

2.14. Quantification of 2,4-De in fried potato-crisps by RP-HPLC

The methanol based extraction of 2,4-De from conventional, T-22 and EC sets of fried potato-crisps and its quantification (Table 4) were performed by RP-HPLC using HPLC-PDA in accordance with the method described by Boskou et al. (2006) which followed a few modification at the time of performance. Retention time of 2,4-De (Figure 8a) was determined using HPLC-PDA detector (M/s JASCO MD 2015 plus, Easton, US) at 280 nm. Mobile phase consisting of methanol and 2-propanol with gradient elution on C18 column at a flow rate of 1 mL/min was used. A standard curve of pure 2,4-De (20-250 µg/L) was prepared by dissolving a pure standard of the same (> 90% purity) in HPLC grade methanol. Dilutions were made immediately before injection into LC column for quantification. The LOD was determined as 0.3 µg/g.

2.15. Analysis of fatty acid profile of frying oils by gas-chromatography (GC)

Fatty acids in frying oil influence the formation of 2,4-De in fried potato crisps. Therefore, analyses of fatty acid profile of DVCO (used for frying T-22 set) (Figure 9a) and soybean oil (used for frying EC set) (Figure 9b) were important to consonant the findings, obtained from

toxins analyses of fried potato crisps. It was performed on GC (Trace GC 700; Thermofischer Scientific) equipped with with TR-1 capillary column (30 m × 0.32 mm, i.d. 0.25 μm) and flame ionization detector (FID) by preparation of methyl ester of fatty acids i.e., FAMEs in accordance to Seneviratne and Dissanayake (2011). The injector and detector temperatures were set at 250 ℃ and 260 ℃, respectively. N_2 was used as the carrier gas at a flow rate of 1 ml min^{-1}. The oven temperature was programed as follows: 70 ℃ (3 min hold), 70-100 ℃ at 5 ℃ min^{-1}, then increasing at 10 ℃ min^{-1} to 150 ℃ and 150-200 at 10 ℃ min^{-1} and final hold at 260 ℃ for 15 min. FAME (1 μL; dissolved in n-hexane) was injected into GC in splitless mode for analyses. Identification of FAMEs in two oils was performed using the standard 37-component FAME mix [butyric acid methyl ester ($C_{4:0}$) − nervonic acid methyl ester ($C_{24:1n9}$)] of M/s Supelco Analytical, St. Louis, MO, USA. The fatty acid composition was reported by the normalization method and expressed as % relative composition of individual fatty acids.

2.16. Characterization of physical properties of conventional, control and T-22 set of fried potato-crisps

2.16.1. Assessmet of color

The Commission Internationale de I'Elcairage (CIE) color values (L*, a* and b* values) of the conventional, control and T-22 sets of fried potato-crisps were determined with a CM-5 spectrophotometer (M/s Konica Minolta Inc., Osaka, Japan) at a 10° inclination from the light source. The color co-ordinates of these fried potato-crisps were calibrated against a standard white plate (100% calibration). Chroma (C*) values and hue (h) angles were calculated (Table 5) using standard equations (1) and (2) (Chatterjee and Bhattacharjee, 2015).

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$\text{Hue} = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

2.16.2. Analysis of texture

The texture profile analysis (TPA) of the conventional, control and T-22 sets of fried potato-crisps were conducted using texture analyzer (TA.XT Express; TA XTplus Texture Analyzer Stable Micro System) with a 5 g load cell (P/5) and 5 mm diameter of cylindrical probe. The conditions for performing TPA of fried potato-crisps were: pre-test: 1.0 mm/s; test: 0.5 mm/s; post-test: 10.0 m/s; distance: 2.0 mm; trigger force: auto; data acquisition: 400 ppb. The

texture of the potato-crisps was quantified in terms of hardness, fracturability as f(crispiness), adhesiveness, cohesiveness and chewiness (Table 6). Texture analysis data were subjected to a one-way ANOVA to determine how well the texture analyzer differentiated the control and T-22 set of fried potato crisps on basis of the five textural attributes. Correlations between the TPA parameters and sensory scores (related to texture judgment) of T-22 set of fried potato-crisps were also evaluated using Pearson's correlation matrix (Serna-Saldivar and Chuck-Hernandez, 2019).

2.16.3. Pearson's correlation study of texture of fried potato crisps

Fifteen numbers of semi-trained panelists comprisisng faculty members and research scholars willingly performed objective sensory evaluation for three textural attributes (which can be perceived sensorially) such as hardness, crispiness and mouthfeel of T-22, conventional and control sets of fried potato crisps (Figure 10). Since sensorial assessment of these three parameters is crucial, selected semi-trained panelists were only allowed to conduct it. Pearson's correlation analysis (Table 8) between the results of these sensory attributes of texture (acquired from sensory evaluation) and texture analyzer (instrumental tests) was performed. Pearson's correlation analysis was conducted using SPSS 20.0 software (M/s IBM).

2.17. DPPH assay of T-22 and EC sets

The antioxidant property of T-22 and EC sets were determined by DPPH assay in accordance with Sarkar et al. (2021) and reported as IC_{50} values (mg/mL).

2.18. Fuzzy logic analysis of T-22, conventional and EC sets of fried potato crisps

In the current study, objective sensory evaluation of T-22, conventional and EC sets (control set was exempted from this study because purpose of this set was to investigate the effect of modified frying conditions solely on AA content in the final products) was performed by the same panel following the same process (mentioned in section 2.11.). This evaluation is highly important to draw the final decision about the acceptability of T-22. Since the panel members were semi-trained, there might be a possibility of biasness and sensory-perceptual variation in their decision about the final acceptance or rejection of the product. To circumvent these and to validate the sensory scores, fuzzy logic analysis was performed as per the methods, described by Das et al. (2005). Panelists were asked to provide tick (√) mark to appropriate scale factor i.e., "not satisfactory," "fair," "medium," "good," and "excellent" for the quality

attributes such as color, odor, texture, taste and after-taste and also asked to rank the quality attributes of these samples by again giving a tick (√) mark to appropriate scale factor, *viz*. "not at all important," "somewhat important," "important," "highly important," and "extremely important". These data (Table 9.1) of each set were processed using fuzzy membership function and generated relative weightage of the quality attributes which ultimately produced crisp overall sensory score for each set. Using centroid rule (based on triangular fuzzy number and fuzzy arithmetic operations), defuzzified score for each set (Table 9.2) was calculated and ranking of samples was performed accordingly. Based on the highest defuzzified score, the highest overall acceptability of the products was judged.

2.19. Producing cost analysis of fried potato-crisps

Producing cost analysis of control, conventional and T-22 set of fried potato crisps (Table 10) was carried out to find the effect of processing and treatment conditions on the same and adoptability by industry. The calculations of energy requirement for different unit operations are discussed in appendices 2C.

2.20. Statistical analysis

In this investigation, HCA was performed using SPSS 20.0 software (M/s IBM). Statistical analysis for ascertaining optimum treatment conditions (based on % acrylamide mitigation in fried potato-crisps) was conducted by one-way analysis of variance (ANOVA), response surface methodology (RSM) and regression modeling using STATISTICA 8.0 software (M/s Statsoft, OK, USA). The ridge analysis for saddle point analysis was performed using R 3.4.3 software. A $p \leq 0.05$ was used to verify the significance of the tests.

3. Results and Discussion

3.1. Determination of AA precursors (L-asparagine and reducing sugar) in potato tuber, control/conventional set of potato slices and blanching water

It was found that quantities of L-asparagine and reducing sugar varied in both aqueous pre-treated and control/conventional set of potato-crisps (from data of Table 1) at significant levels ($p < 0.05$). The aqueous pre-treatments resulted in loss of precursors of AA by 43.29% for L-asparagine and 45.77% for reducing sugar.These results suggested that aqueous pre-treatments are potential methods to reduce the contents of precursors significantly ($p < 0.05$) which in turn resulted in effective mitigation of AA in the final fried products.

3.2. Qualitative analysis of AA and 2,4-De by HR-MS

The lens voltage was optimized by setting the mass to 72.0 (theoritical values of m/z ~ 72.03217-72.03261) and 81.0 by manual tuning for acrylamide and 2,4-De, respectively. In case of standard AA solution, peak having m/z value 72.0320 was identified (Figure 1a) and the same was also detected in SPE-QuEChERS extracted sample (conventional set). Similarly, peak having m/z value 81 (also found consonance with the findings of Živković et al., 2021) represents the standard 2,4-De and the same (Figure 1b) was detected in methanolic extract of sample (EC set).

3.3. Classification of treatment conditions causing significant AA mitigation by hierarchical cluster analysis

Four individual clusters (I, II, III and IV) were obtained from cluster analysis based on the correlation coefficient distances among them (Figure 2). Cluster I presents a similarity level having the least acceptable level (73.04%-78.74%) of % AA mitigation and is associated with treatments 2, 3, 5, 6, 8, 9, 15 and 18. This association is probably influenced by the effects of combinations of lowest % L-proline concentration (1%) coupled with moderate frying temperature (150 °C); and lowest % L-proline concentration (1%) with highest frying temperature (160 °C). Cluster II represents a similarity level higher than 80% AA mitigation by grouping together the treatments 1, 4, 7, 11, 12, 14 and 17 and possesses third highest acceptable level of % AA mitigation (80.62% - 84.32%). The high similarity level is probably related to the consequence of combination of moderate concentration of % L-proline (1.5%) and lowest frying temperature (140 °C) and opposite combination of these factors (% L-proline: 1% and frying temperature: 150 °C). Cluster III is associated with treatments 19, 22 and 25, representing a high similarity level (> 95% AA mitigation). It possesses the highest acceptable level of % AA mitigation (97.79 - 99.03%). This association is influenced by the effect of combination of highest % L-proline concentration (2%) and lowest frying temperature (140 °C). Cluster IV includes treatments 10, 13, 16, 20, 21, 23, 24, 26 and 27, signifying greater than 85% AA mitigation implying second highest acceptable level (87.48%-92.07%). The high similarity level is possibly related to the consequence of combination of moderate concentration of % L-proline (1.5%) and highest frying temperature (160 °C) and opposite setting of these two factors (% L-proline: 2% and frying temperature: 150 °C).These findings suggested that the combination of highest % L-proline with lowest frying temperature yielded the most desirable result of highest (> 95%) acrylamide mitigation

in the fried potato-crisps. These findings were further validated through RSM optimization of treatment conditions to achieve maximum possible mitigation of AA under given experimental conditions. In cluster III, among T-19, T-22 and T-25 sets, T-19 and T-22 were chosen since they exhibited > 98% AA mitigation. On the basis of % AA mitigation, T-19 (99.03%) was adjudged to be the best.

3.4. Optimum conditions of treatment for mitigation of AA in treated set of fried potato-crisps

Table 2 provdies the % AA mitigation in fried potato-crisps subjected to 27 different treatment combinations obtained by varying of % L-proline, deep frying temperature and deep frying time. ANOVA study (Table 3) revealed that % AA mitigation in treated set of fried potato-crisps increased as % L-proline amount (X_1) ($p = 0.0000$) increased and frying time (X_2) ($p = 0.0371$) and temperature (X_3) ($p = 0.0000$) decreased. Among 27 treated fried potato crisps, T-19 and T-22 sets were received the foremost priority owing to have more than 98% AA mitigation (evident from HCA). The maximum % AA mitigation (99.03%; T-19) was obtained in 2% L-proline pre-treated samples when fried for 4 min at 140 °C in DVCO. However the texture (crispiness) of the same was not adequate and received poor sensory score. Consequently, overall acceptability of T-19 set became low. The second highest % AA mitigation (98.82%) was obtained under following conditions: pre-treatment with 2% L-proline, 5 min frying time and 140 °C frying temperature. The fried potato-crisps under these conditions received appreciable and the highest sensorial acceptability with respect to all sensory attributes. Considering good sensory acceptability and > 98% AA mitigation, the combination of 2% L-proline, 5 min frying time and 140 °C frying temperature (T-22 set) was regarded as the best for mitigation of AA under the given experimental conditions. Therefore, T-22 set won over T-19 set.

The above finding indicates that % AA mitigation in low frying (deep) temperature increased with increasing concentration of % L-proline used in pre-treatment and with decreasing frying time. Therefore, high % L-proline as well as reduced frying time and temperature played important roles in reducing AA formation in potato crisps.

3.5. Generation of response curves

The response surfaces of % AA mitigation in relation to the % L-proline concentrations (w/w), frying temperature and time are shown in stereoscopic figures (Figure 3a–c). The %

L-proline concentrations (w/w), frying temperature and time were fixed at their middle values of 1.50 %, 150 °C and 5 min in respective stereoscopic figures (Figure 3a–c) and showed significant ($p < 0.05$) effects on % AA mitigation, evidenced by the test statistics for the regression models (*vide supra*).

3.6. Regression modeling of mitigation of AA in treated set of fried potato-crisps

The second-order polynomial equation that fitted the coded variables is stated as follows:

$$Y = B_0 + \sum B_i X_i + \sum B_{ii} X_i^2 + \sum B_{ij} X_i X_j \qquad (3)$$

where, Y represents the response variables (% AA mitigation in treated set of potato-crisps), B_0, B_i, B_{ii}, and B_{ij} are constants and regression coefficients of the model, and X_i and X_j are independent variables in coded forms. The expanded model includes linear, quadratic and cross-product terms as shown below (with intercept):

$$Y = 189.26 + 83.09\ X_1 – 7.35\ X_2 – 1.43\ X_3 + 4.06\ X_1^2 – 0.46\ X_2^2 + 0.003\ X_3^2 – 3.53\ X_1X_2 – 0.45\ X_1X_3 + 0.11\ X_2X_3 \qquad (4)$$

The significance of the investigated factors and their interactions were examined. It was seen that there was a significant ($p < 0.05$) dependence of % AA mitigation (response variable) in fried potato-crisps on % L-proline concentration (X_1) ($p = 0.000$), frying time (X_2) ($p = 0.0371$) and temperature (X_3) ($p = 0.000$). However, all the second-order terms (X_{12}, X_{22}, X_{32}) of the three processing parameters did not have significant effects on the same. The two-level interactions showed that % L-proline concentration and frying temperature (the cross-product term X_1X_3) ($p = 0.000$) were found to be the most significant followed by interdependence of % L-proline concentration and frying time (the cross-product term X_1X_2) ($p = 0.0196$). However, the interdependence of frying time and temperature (X_2X_3) did not have significant ($p = 0.0957$) effect on the response variable. ANOVA study showed a good F value for the cross-product term X_1X_3 ($F = 15.4098$), signifying that this interaction was very important (Table 3). The cross-product term X_1X_2 showed a moderate F value (5.93) representing interactions among the variables to be significant. However, a poor F value (2.92) was obtained for the remaining interaction (X_2X_3) indicating that its effect on the response variable was negligible.

A plot of observed vs. predicted values of responses (Y) (Figure 4) showed a close fit of the observed values with the predicted ones. Thus, a statistically significant multiple regression

relationship (r = 0.91) between the independent variables and the responding variable could be established. The complete quadratic model showed a very good fit.

3.7. Analysis of response surfaces

The response surfaces have been shown in the stereoscopic figures [Figure 3 (a), (b) and (c)]. From the test statistics for the regression models (F-test and ANOVA), it can be reasonably concluded that % L-proline and frying temperature in combination had the most significant effect (p = 0.000) on % AA mitigation.

3.8. Optimization of mitigation of acrylamide in treated set of fried potato-crisps

The stationary point obtained following the method of Montgomery (2001) was X_{1S}= 1.28%, X_{2S} = 6.34 min and X_{3S}= 142.52 °C. Percentage AA mitigation at this stationary point was found to be 79.66.

3.9. Characterizing the Response Surface

In this study, the eigen values determined in accordance with the method described by Montgomery (2001) were 4.6842, –0.0080 and –1.0704 for %L-prolin, frying time and temerature, respectively. Different signs of eigen values indicated X_S to be a saddle point.

3.10. Optimal conditions of mitigation of AA

Since the conditions of the saddle point (X_{1S} = 1.28%, X_{2S} = 6.34 min and X_{3S} = 142.52 °C) cannot be considered as optimum conditions for maximum mitigation of AA in treated set of fried potato-crisps, a ridge analysis was performed to identify the point of maxima. Ridge analysis (Figure 5) indicated that % L-proline (X_1) if increased along with decreasing frying time (X_2) and temperature (X_3), % AA mitigation would be maximum. It revealed the optimum conditions for obtaining the point of maxima to be: X_1 = 2.02%, X_2 = 4.63 min, X_3 = 139.77 °C. The predicted % AA mitigation at this point was found to be as 98.28 and the experimental value was also found to be 98.82% under conditions of 2% L-proline pre-treatment, 5 min frying time and 140 °C frying temperature. Therefore the findings based on the concept of canonical ridge analysis are strongly in accordance with the experimental findings.

3.11. RP-HPLC analyses of AA of fried potato crisps

Quantification of AA in extracts of conventional, control and 27 different treated sets of fried potato-crisps (obtained by SPE-QuEChERS extraction method) were carried out by RP-HPLC. AA was quantified using standard curve having high regression coefficient (R^2 = 0.9984). The concentration of AA in the conventional set (Figure 6b) of fried potato-crisps was 1739.12 μg/kg (8695.6 μg/L) which post application of modified frying conditions alone (for control set) and along with the combination treatment i.e., T-22 set (Figure 6c), reduced to 1182.60 (5922.6 μg/L) and 20.52 μg/kg (102.94 μg/L), respectively. Compared to the conventional set, 38.10% and 98.82 % (~99%) mitigation of AA occurred in the control and T-22 sets. Therefore, it can be said that modified frying conditions played one of the crucial roles in controlling AA level. This combination treatment strategy proved to be effective in reducing AA content by ~99% (98.82%) without compromising sensory acceptability of the fried potato-crisps.

3.12. LC-MS/MS analysis of AA of conventional and T-22 sets for validation purpose

The quantification of AA were validated by LC-MS/MS analysis for conventional and T-22 sets (Figure 7a and b). AA was quantified using standard curve (performed in LC-MS/MS), having correlation coefficient 0.9971 (r). The concentration of AA of conventional set of fried potato crisps were obtained as 1739.28 μg/kg (8696.40 μg/L), but after employing the combined treatment it alleviated to 20.54 μg/kg (103.04 μg/L). Compared to conventional set, 98.82% (approx. 99%) mitigation of AA occurred in T-22 set of fried potato crisps. The results of LC-MS/MS was in consequence with the findings of RP-HPLC and thus, validated the %reduction of AA in T-22 set. Therefore, this strategy was proved to be an effective method in mitigating AA in fried potato crisps by ~99%.

3.13. 2,4-De contents in conventional, T-22 and EC sets of fried potato-crisps

2,4-De contents are expressed as mean values of μg decadienal per g fried potato crisps. Table 4 reveals that 2,4-De in each batch of T-22 (Figure 8b) and conventional (Figure 8c) sets could not be quantified by RP-HPLC vis-à-vis EC set (Figure 8d) since the concentration of this compound was lower than with its LOD (considered as 0 μg/g). However, this toxic aldehyde was detected in each batch of fried crisps of EC set, fried successively in the same batch of frying oil (soybean oil). Significantly higher quantity (11-15 μg/g) of this toxic aldehyde detected in fried potato-crisps during 3 to 5 (times) repeated frying was influenced

by successive thermal oxidation of C18:2 present (Thomas et al., 2016) in soybean oil. On the contrary, this phenomenon was not supported by DVCO in repeat frying process since DVCO contains insignificant amount of C18:2 (*vide infra*).

3.14. GC-FID analyses of fatty acid profile of DVCO and refined soybean oil

FAME-GC of DVCO (Figure 9a) revealed that it contains low degree of linoleic acid (C18:2; 1.8%); whereas, soybean oil (Figure 9b) contains 50.9% linoleic acid (C18:2), confirmed by FAME-GC-FID analysis of the same. Thus, it was verified that DVCO is linoleic acid-lean oil vis-à-vis soybean oil and justified the findings about absence 2,4-De in conventional and T-22 sets.

3.15. Physical characteristics of fried potato-crisps

3.15.1. Color profiles of conventional, control and T-22 sets

The color profiles of conventional, control and optimized treated sets are represented by L*, a*, and b* values, chroma and hue angles (Table 5). It was found that T-22 set was brighter and more yellowish color with higher L* (L*: 68.68±4.90) and b* values (b*: 13.40±0.68), compared to those in the conventional and control sets. The T-22 set of fried potato-crisps had significantly higher ($p = 0.0001$) chroma value, implying higher color intensities. The hue angle of T-22 set of fried potato-crisps was in the range of 88.53±6.02° indicating golden yellowish color of the samples which increases sensory appeal of the product. These results corroborated well with the sensory scores.

3.15.2. Texture analysis of conventional, control and T-22 set of fried potato-crisps

Chauvin et al. (2010) established that crispness, hardness and fracturability were significantly correlated (Pearson's correlation coefficient 'r' values ranging from0.80 to 0.90) with each other. Crispiness is a prime factor among all textural attributes of fried potato crisps. But, in double compression test, crispiness cannot be quantified directly from TPA graph. Fracturability encompasses crispiness and can be measured directly from TPA graph. Fracturability is defined as the tendency of a material to shatter, crack, crumble or fail upon the application of a relatively small magnitude of force. It is usually displayed by a product of high degree of hardness and low degree of cohesiveness (Walter et al., 2002). Thus crispy foods require less force to fracture. Hardness and fracturability are diretly correlated with each other whereas crispiness is inversely proportional to fracturability and hardness(Chauvin

et al., 2010). Comparative and quantitative assessments of TPA of conventional, control and T-22 sets of fried potato crisps (Table 6) revealed that there was no significant difference ($p > 0.05$) between conventional and T-22 sets in all five textural attributes. The conventional and T-22 sets had significantly lower ($p < 0.05$) hardness and fracturability (implying higher crispiness) vis-à-vis the control set of fried potato crisps (Table 6). This was due to difference in the moisture content of treated potato slices before and after frying which were influenced by drying (prior to frying) and frying conditions, respectively. It is evident from the results of moisture analysis (Table 7) that conventional and control sets were found to have higher and comparable ($p > 0.05$) moisture contents prior to frying, which differed significantly ($p < 0.05$) from that of the T-22 set. On the basis of final moisture content after frying, it is seen that conventional deep frying conditions (170 ℃ for 3 min) led to a significant moisture loss of ~ 54.4%, higher than that of the control and T-22 sets (~19.5%). These observations showed that higher frying temperature (170 ℃ at 3 min) could more effectively remove moisture than lower frying temperature i.e., 140 ℃ at 5 min (optimized deep frying condition). This was attributed to similar moisture contents (31.9-32.2 %) of conventional and T-22 set of fried potato crisps and thus accounted for similar texture profile of the same [excessive lower or higher moisture content of fried product (</> 32%) hampered the texture, color and overall acceptability of the product]. The control set of fried potato crisps however, possessed higher moisture content i.e., ~56.4% (post frying) since the same did not undergo drying prior to frying unlike T-22 set and was fried at a comparitively lower temperature (140 ℃). Consequently, cohesiveness and chewiness of conventional and T-22 set of fried potato crisps were lower than that of the control set. Adhesiveness being indirectly proportional to cohesiveness, therefore followed an opposing trend in each case. The values of textural parameters were statistically different ($p < 0.05$) between control and conventional (or considering T-22 set) sets. Therefore, it is evident that moisture content and frying conditions strongly influenced the physicochemical properties of the fried potato products effectively. These findings unambiguously suggest that potato crisps should be fried at 140 ℃ for 5 min immediately after drying to a moisture content of ~32.2%, for better texture appeal and safety (98.82% AA mitigation).

3.15.3. Correlation between sensory and texture analyses data

The statistical correlations between sensory (hardness, crispiness and mouthfeel) and instrumental (hardness, fracturability, adhesiveness, cohesiveness) analysis for texture were performed which revealed that sensorial hardness was strongly and positively ($r = 1.000$)

correlated with that of instrumental analysis. Sensorial crispiness and hardness were negatively correlated with instrumental parameters such as fracturability (r = –0.989) and adhesiveness (r = –0.977), respectively and sensorial mouthfeel was negatively correlated (r = –0.995) with cohesiveness (instrumental parameter) (Table 8). These results sucessfully proved that lower cohesiveness, hardness and fracturability of fried potato crisps improved mouthfeel of the same and thus validated the sensory scores (Figure 10) for textural attributes of the conentional,T-22 and control sets of fried potato-crisps.

3.16. DPPH assay of T-22 and EC sets

IC_{50} values of T-22 set was lower (8.25 ± 0.20 mg/ml) when compared to EC set (26.88± 0.15 mg/ml). This observation implies that antioxidant potential of T-22 set is higher ($p < 0.05$), compared to that of EC set.

3.17. Fuzzy logic analysis of T-22, conventional and EC sets of fried potato crisps

The acceptability of T-22, conventional and EC sets (Figure 11) of fried potato crisps (Table 9.2) with respect to overall quality were assessed using the defuzzified scores associated with the standard decision scales (Chakraborty et al., 2011). T-22 set received the highest defuzzified score (categorized as ‘good’) followed by conventional, EC sets, respectively. This was attributed to development of attractive golden yellow color as well as unique odor-cum-flavor over and above the characteristic ones possibly owing to addition of L-proline during pre-treatment.

3.18. Producing cost analysis of fried potato crisps

It is evident from Table 10 that producing cost (Rs./g of fried potato crisps) of T-22 set is significantly lower ($p = 0.001$) than that of conventional set. Although different unit operations such as blanching, drying were adopted in the preparation of T-22 set which incur some cost for energy, the ultimate producing cost becomes lower ($p = 0.001$) owing to less oil absorption. Blanching causes surface gelatinization of stach in potato slices which probabaly leads to less oil absorbtion during frying (Troncoso and Zúñiga, 2009). Consequently, cost involved in total oil absorbtion of T-22 set was found as ~52% less compared to that of conventional set. This reduction in cost for total oil absorbtion influenced the final and net producing cost of T-22 set which leads to ~27% reduction in the same.

3.19. Combined effect of three treatments on AA formation in treated set of potato-crisps and effect of usage of DVCO as cooking-medium on 2,4-De content in fried crisps

The combined effects of four treatments viz. aqueous pre-treatments treatment with L-proline amino acid, drying and modified frying conditions effectively mitigate the AA content of fried potato crisps ensuring better sensory appeal of the same. This could be attributed to three different reasons. Firstly, the aqueous pre-treatments profoundly reduced the AA content in the said food matrix because these treatments reduced free L-asparagine and glucose (main precursors of AA formation) significantly. Secondly, immersion of potato slices in the solution of 2% L-proline limited the AA formation in T-22 set. AA has two reactive sites *viz*. the conjugated double bond and the amide group. Therefore, L-proline formed adduct (Figure 12) with AA via alkylation of amide group (Koutsidis et al., 2009). It was reported that L-proline promotes this alkylation reaction profoundly due to the increased nucleophilicity of its secondary amine group. Consequently L-proline amino-acid played a crucial role in mitigating AA in treated set of fried potato-crisps by promoting Michael addition reaction with the same (Figure 12). Thirdly, the influence of moisture content (Table 7) of potato slices (prior to frying) in relation to AA formation and overall acceptability (judged on basis of five sensory attributes, mentioned earlier) was investigated. Figure 11 shows ~40% to be the optimum moisture content of potato slices (prior to frying) considering both AA formation and sensory acceptability. Moisture is known to impact the chemical route, namely, hydrolysis of imine (intermediates of Maillard reaction) as well as molecular mobility of chemical constituents associated with the formation of AA (Ciesarová et al., 2006). The frying of T-22 set potato slices having ~40% moisture content (prior to frying) resulted in mimimum AA formation accompanied by the highest overall acceptability (Figure 13). Finally, modified deep frying conditions of 140 ℃ for 5 min instead of conventional deep frying condition (170 ℃ for 3 min) contributed to lower AA formation. However, the lower frying conditions compared to optimal one (< 140 ℃ and 5 min) rendered the fried crisps organoleptically unacceptable especially in terms of texture. On increase of frying time beyond 5 min at 140 ℃, the textural characteristics improved (sensorically judged) with concomitant increase of AA content in the final products.

Holistically, considering all parameters, this study established that the following conditions: aqueous pre-treatments [rinsing, soaking and blanching (at 70 ℃ for 20 min)]; addition of 2% L-proline; drying at 60 ℃ for 130 min; 140 ℃ frying temperature and 5 min frying time effectively reduced AA formation by 98.82% (~99%). This set of condition also improved the sensory attributes (in terms of color profile, aroma and taste) of the product significantly ($p <$

0.05). In addition, low frying time and temperature (140 ℃ for 5 min) and usage of DVCO as frying oil did not allow formation of the potential toxic aldehyde i.e., 2,4-De in fried potato-crisps and made it antioxidant-rich. In addition to these, reduced producing cost (compared to that of conventional set) makes the chance of adoptability of this treatment process by industry high. Outcome of this work if adopted by food processing industries for manufacture of fried crisps would certainly aid in mitigating the health-debilitating effects associated with these toxicants.

4. Conclusion

The new combination treatment approach for AA mitigation in successfully contributed to ~99% AA mitigation with concomitant enhancement of organoleptic properties (characteristic color and unique flavor) of fried potato-crisps, which undoubtedly holds promise of utilization in snack food industries. The potato-crisps with minimum AA content, absence of 2,4-De, rich in antioxidant property and excellent sensory appeal could be a novel fried product for global consumers. DVCO as a new frying oil is worthy of being considered industrially to be a healthier alternative to the globally most consumed linoleic-acid rich soybean oil.

After proving DVCO as a safe oil vis-à-vis other common frying oils, the next chapter focused on storage stability of T-22 set vis-à-vis EC set i.e., influence of DVCO on storage stability of fried products vis-à-vis other common frying oils and also development a fast and accurate detection technique, coupled with e-nose to predict the rancidity status of fried products w.r.t. the rancidity molecular marker (viz. hexanal) forgoing GC analysis.

Novelty:

The novelty of the present work is in-

- development of a methodology based on hurdle technology including aqueous pre-treatment, L-prolin treatment along with modified frying conditions and using of DVCO as frying oil (as a replacement of commonly used frying oils) which could successfully mitigate acrylamide by 99% with concomitant prevention of 2,4-decadienal formation in the most popular fried snack (potato fries) and improvement of its sensorial properties and in
- establishing DVCO as a healthy frying oil alternative to linoleic acid-rich soybean oil

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Table 1. Analyses of L-asparagine and reducing sugar contents

Particulars	L-Asparagine content (g/kg)	Reducing sugar content (g/kg)
Potato tuber (*kufri jyoti*)	2.31±0.12	2.25±0.11
Control/conventional set of potato slices (without aqueous pre-treatment except rinsing)	2.25±0.03	2.19±0.05
Aqueous pre-treated set (with aqueous pre-treatment including rinsing, soaking and blanching)	1.31±0.02	1.22±0.03

Limit of quantification of reducing sugar: 1.0 g/kg; Limit of quantification of L-asparagine: 0.5g/kg
All results are expressed in mean±SD

Table 2. Experimental results of % acrylamide mitigation of treated set of fried potato crisps (full factorial design)

Treatment no.	Concentration of L-proline (% w/w)	Frying time (min)	Frying temperature (°C)	Acrylamide mitigation (%)
T-1	1.0	4.0	140	84.32±0.04
T-2	1.0	4.0	150	76.88±0.04
T-3	1.0	4.0	160	74.57±0.07
T-4	1.0	5.0	140	83.72±0.06
T-5	1.0	5.0	150	75.49±0.07
T-6	1.0	5.0	160	73.77±0.03
T-7	1.0	6.0	140	83.12±1.09
T-8	1.0	6.0	150	74.95±0.08
T-9	1.0	6.0	160	73.04±0.05
T-10	1.5	4.0	140	90.04±0.05
T-11	1.5	4.0	150	82.99±0.04
T-12	1.5	4.0	160	80.62±1.0
T-13	1.5	5.0	140	88.60±0.09
T-14	1.5	5.0	150	81.75±0.08
T-15	1.5	5.0	160	78.74±0.07
T-16	1.5	6.0	140	87.48±0.04
T-17	1.5	6.0	150	80.89±0.05
T-18	1.5	6.0	160	78.01±0.06
T-19	2.0	4.0	140	99.03±0.07
T-20	2.0	4.0	150	92.07±0.08
T-21	2.0	4.0	160	89.96±0.09
T-22	**2.0**	**5.0**	**140**	**98.82±0.03**
T-23	2.0	5.0	150	91.75±0.06
T-24	2.0	5.0	160	88.76±0.05
T-25	2.0	6.0	140	97.79±0.04
T-26	2.0	6.0	150	90.90±0.07
T-27	2.0	6.0	160	87.92±0.05

All the results are expressed in mean±SD
[Bold] in Table 1 signifies the optimum experimental conditions of L-proline concentration (%w/w) and frying operations

Table 3. ANOVA study of surface methodology

Effect	Degree of freedom	Yield of acrylamide on extraction (g/ kg of dry potato crisp) SS	Yield of acrylamide on extraction (g/ kg of dry potato crisp) MS	Yield of acrylamide on extraction (g/ kg of dry potato crisp) F	Yield of acrylamide on extraction (g/ kg of dry potato crisp) P
X_1	1	586.665	586.665	102.5962	0.0000
X_1^2	1	16.688	16.688	2.9185	0.095730
X_2	1	26.687	26.687	4.6670	0.037119
X_2^2	1	2.886	2.886	0.5048	0.481744
X_3	1	1416.119	1416.119	247.6489	0.00000
X_3^2	1	1.516	1.516	0.2651	0.609597
$X_1 X_2$	1	33.957	33.957	5.9384	0.019608
$X_1 X_3$	1	88.117	88.117	15.4098	0.000352
$X_2 X_3$	1	16.715	16.715	2.9231	0.095477
Error	38	217.294	5.718		
Total	**47**	**3190.753**			

Table 4. Quantity of 2,4-decadienal of EC, conventional and T-22 sets of fried potato crisps

No. of frying	EC set	Conventional set	T-22 set
	Deep frying (μg/g)		
1	4 ± 0.01^a	0	0
2	8 ± 0.02^b	0	0
3	11 ± 0.05^c	0	0
4	14 ± 0.08^d	0	0
5	15 ± 0.07^d	0	0

a, b, c, d Different letters in a column indicate significant differences at $p < 0.05$.
All the results are expressed in mean±SD on basis of three standard deviations of the mean value.
'0 μg/g' implies the quantity is less than LOD (0.3 μg/g)

Table 5. Color parameters analysis of control and treated set of potato crisps

Color Parameters	Conventional set of fried potato crisps	Control set of fried potato crisps	Treated (T-22) set of fried potato crisps
L^*	59.31 ± 4.50^b	65.68 ± 4.55^c	68.68 ± 4.90^c
a*	0.62 ± 0.03^b	-0.30 ± 0.05^c	-0.50 ± 0.07^c
b*	8.51 ± 0.64^b	10.40 ± 0.57^c	13.54 ± 0.68^c
Chroma	8.53 ± 0.35^b	10.40 ± 0.56^c	13.55 ± 0.63^c
Hue	78.02 ± 7.65^b	84.32 ± 5.10^c	$88.53\pm6.02^{0\ c}$

L*, a*, b* values of potato crisp (control and treated) are mean ±SD.
Colorl parameters of potato crisp (control and treated) are mean ±SD.
a, b, c Different letters in a row indicate significant differences at $p < 0.05$.

Table 6. TPA parameters analyses of conventional, control and treated sets of potato crisps

Treatment no.	TPA parameters					Average sensory scores (w.r.t six sensory attributes)
	Hardness (g)	Fracturability (g)	Adhesiveness (g-s)	Cohesiveness	Chewiness (g)	
Conventional set	3.96 ±0.03[b]	1.62 ±0.02[b]	–1.38 ±0.03[b]	0.41 ±0.02[b]	2.13 ±0.02[b]	8.5[b]
Control set	4.15 ±0.04[c]	2.03 ±0.04[c]	–1.11 ±0.02[c]	0.48 ±0.03[c]	2.60 ±0.02[c]	7.0[d]
T-22	3.93 ±0.03[b]	1.59 ±0.02[b]	–1.37 ±0.03[b]	0.40 ±0.02[b]	2.11 ±0.03[b]	8.7[b]

N.B.: Hardness α Fracturability α 1/crispiness; Hardness α cohesiveness α chewiness; cohesiveness α 1/adhesiveness; Fracturability = hardness × cohesiveness; Textural parameters of potato crisp (control and treated) are mean ±SD. b-z Different letters in a column indicate significant differences at $p < 0.05$; sensory scores based on attribute, texture

Table 7. Moisture analysis of conventional, control and treated sets of potato slices and crisps

Parameters	Conventional set	T-22 set	Control set
Final moisture content prior to frying	~70±1 %[a]	~40±1 %[b] (after tray drying)	~70±1 %[a]
Frying condition	170° c for 3 min	140° c for 5 min	140° c for 5 min
Moisture content after frying	~31.9±1 %[a]	~32.2±1 %[a]	~56.4±1 %[b]
%moisture loss during frying operation	~54.4%	~19.5%	~19.5%

Table 8. Pearson's correlation between instrumental and sensory values of textural parameters of control and optimized treated set of fried potato crisps

		Sensory hardness values	**Instrumental hardness values**
Sensory hardness values	Pearson correlation	1	1.000**
	Sig. (2-tailed)		0.000
	N	2	2
Instrumental hardness values	Pearson correlation	1.000**	1
	Sig. (2-tailed)	0.000	
	N	2	2
		Sensory crispiness values	**Instrumental fracturability values**
Sensory crispiness values	Pearson correlation	1	–0.989**
	Sig. (2-tailed)		0.000
	N	2	2
Instrumental fracturability values	Pearson correlation	–0.989**	1
	Sig. (2-tailed)	0.000	
	N	2	2
		Sensory hardness values	**Instrumental adhesiveness values**
Sensory mouthfeel values	Pearson correlation	1	–0.977**
	Sig. (2-tailed)		0.000
	N	2	2
Instrumental adhesiveness values	Pearson correlation	–0.977**	1
	Sig. (2-tailed)	0.000	
	N	2	2
		Sensory mouthfeel values	**Instrumental cohesiveness values**
Sensory hardness values	Pearson correlation	1	–0.995**
	Sig. (2-tailed)		0.000
	N	2	2
Instrumental cohesiveness values	Pearson correlation	–0.995**	1
	Sig. (2-tailed)	0.000	
	N	2	2

Table 9.1. Scale factor and relative importance of different attributes of samples

Attribute	Scale factor					Relative importance				
Color	**N.S**	**Fair**	**Medium**	**Good**	**Excellent**	**Not at all imp.**	**Somewhat imp.**	**Imp.**	**Highly imp.**	**Extremely imp.**
T-22	0	0	0	0	30	0	0	0	30	0
Conventional	0	10	10	10	0	0	0	0	30	0
EC	0	12	18	0	0	0	0	0	30	0
Odor										
T-22	0	0	0	0	30	0	0	0	0	30
Conventional	0	0	15	15	0	0	0	0	0	30
EC	0	0	21	9	0	0	0	0	0	30
Texture										
T-22	0	0	0	20	10	0	0	0	30	0
Conventional	0	0	0	18	12	0	0	0	30	0
EC	0	0	3	27	0	0	0	0	30	0
Taste										
T-22	0	0	0	10	20	0	0	0	30	0
Conventional	0	0	0	20	10	0	0	0	30	0
EC	0	0	4	26	0	0	0	0	30	0
After taste										
T-22	0	0	0	30	0	0	0	30	0	0
Conventional	0	0	0	30	0	0	0	30	0	0
EC	0	0	1	29	0	0	0	30	0	0

Table 9.2. Defuzzified scores of nine different freshness enhanced bread samples obtained from fuzzy logic analysis

Sample	Overall sensory score (SO)			(a+c)	Defuzzified scores	Category
	a	**b**	**c**			
T-22	94.23	53.85	26.92	121.15	79.61	Good
Conventional	69.62	46.60	37.56	107.18	66.01	Good
EC	60.19	43.59	39.17	99.36	58.72	Medium

Table 10. Producing cost of conventional, control and T-22 set

Sl. no.	Raw material	Rate/kg (Rs.)	Unit	Quantity / batch			Amount (Rs.)/batch		
				Conventional set	Control set	T-22 set	Conventional set	Control set	T-22 set
1	Potato	17	kg	2	2	2	34	34	34
2	DVCO oil absorption	700 (per L)	ml	460	260	240	322	182	168
3	L-proline	1000	g	-	-	40	-	-	40
4	Cost for power consumption due to blanching at 70 ℃	10	KWh	-	-	0.11	-	-	1
5	Cost for power consumption during heating of water to 80 ℃	10	KWh	-	-	0.13	-	-	1
6.	Cost for power consumption during drying at 60℃	10	KWh	-	-	0.69	-	-	7
7	Cost for power consumption during frying	10	KWh	0.26 (170 ℃)	0.21 (140 ℃)	0.21 (140 ℃)	3	2	2
Total cost (round off)							359	218	253
Net yield/batch (g)							1384	1820	1360
Cost Rs./ g							**0.26**[a]	**0.12**[c]	**0.19**[b]

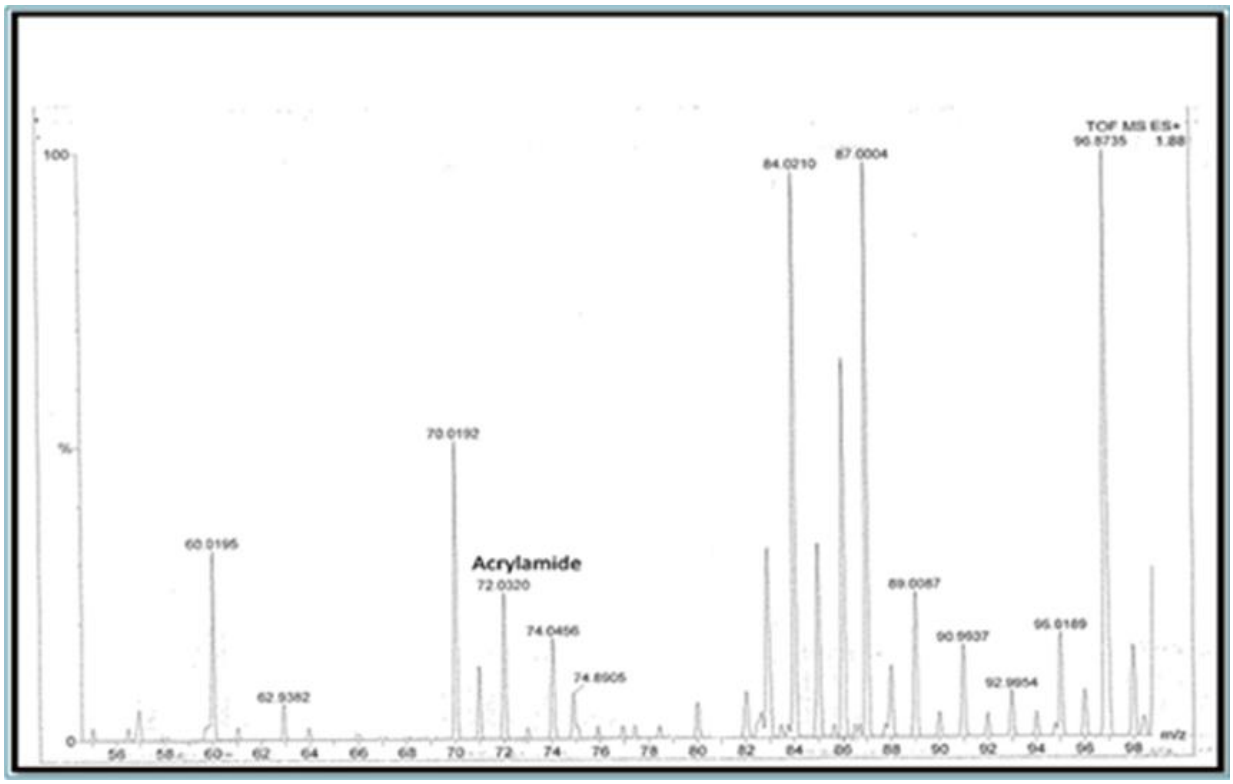


Figure 1a. Qualitative analysis of acrylamide of conventional set of fried potato crisps by HR-MS.

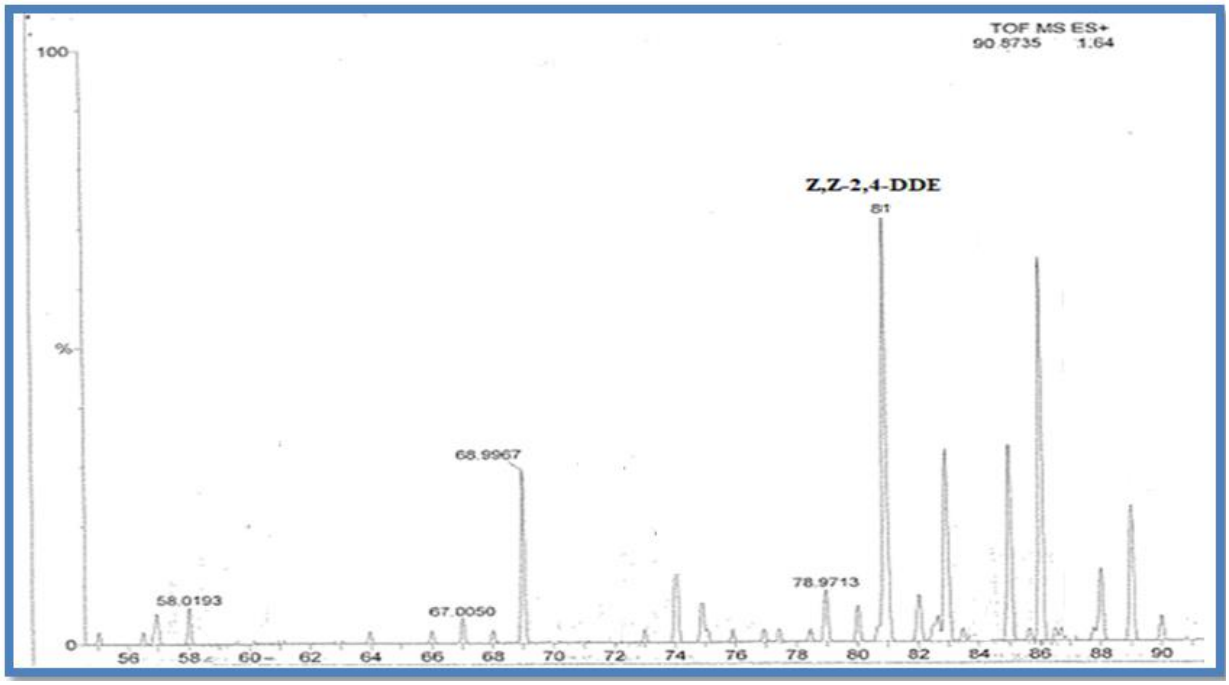


Figure 1b. Qualitative analysis of 2,4-decadienal of EC set of fried potato crisps by HR-MS.

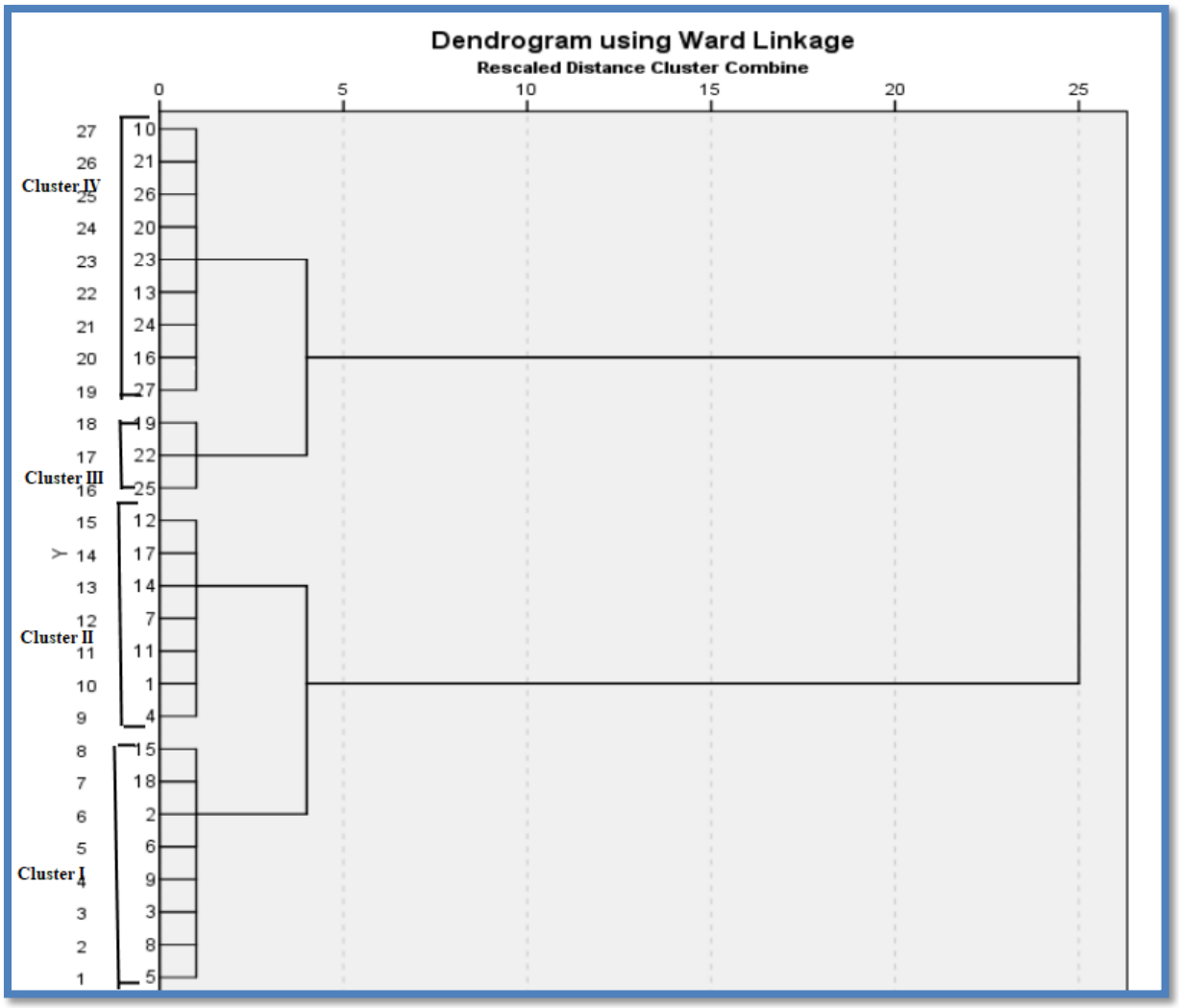


Figure 2. Hierarchical cluster analysis of different treatment conditions based on % acrylamide mitigation (Dendogram)

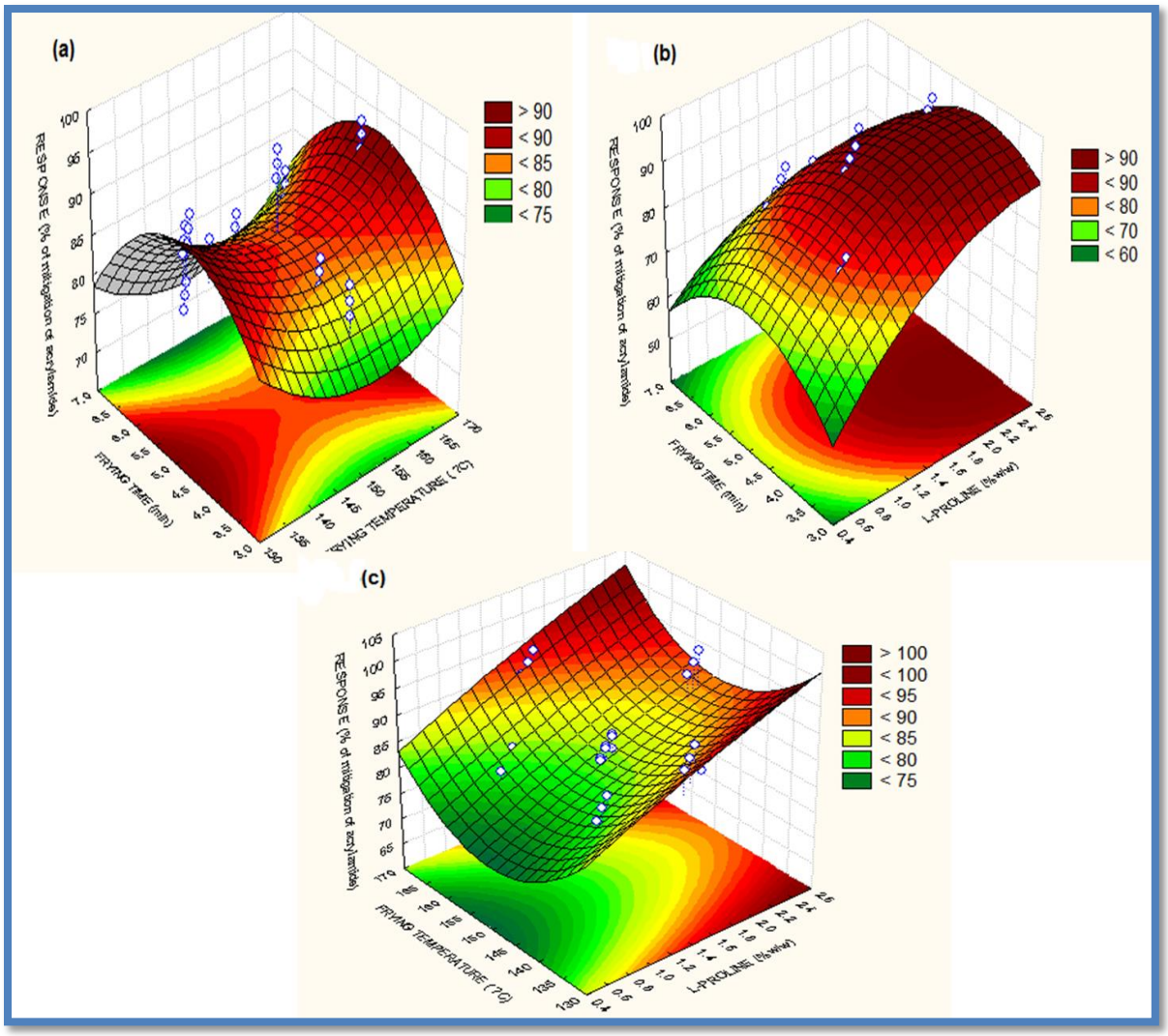


Figure 3. Plot of response surface indicating percentage of acrylamide mitigation in treated set of fried potato-crisps (a) as a function of frying time and frying temperature at 1.50% (w/w) L-proline concentration, (b) as a function of frying time and L-proline concentration at 150°C frying temperature, (c) as a function of frying temperature and L-proline concentration at 5 min frying time

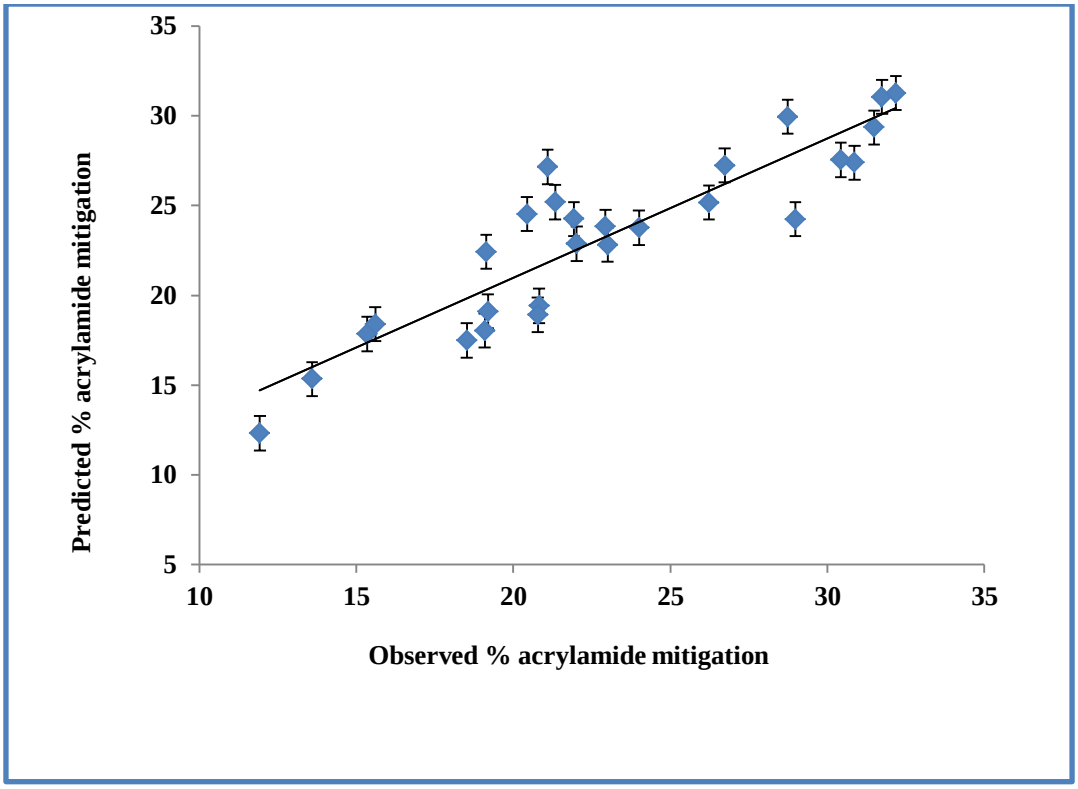


Figure 4. Predicted % of acrylamide mitigation vs. experimental % of acrylamide mitigation (R^2=0.910)

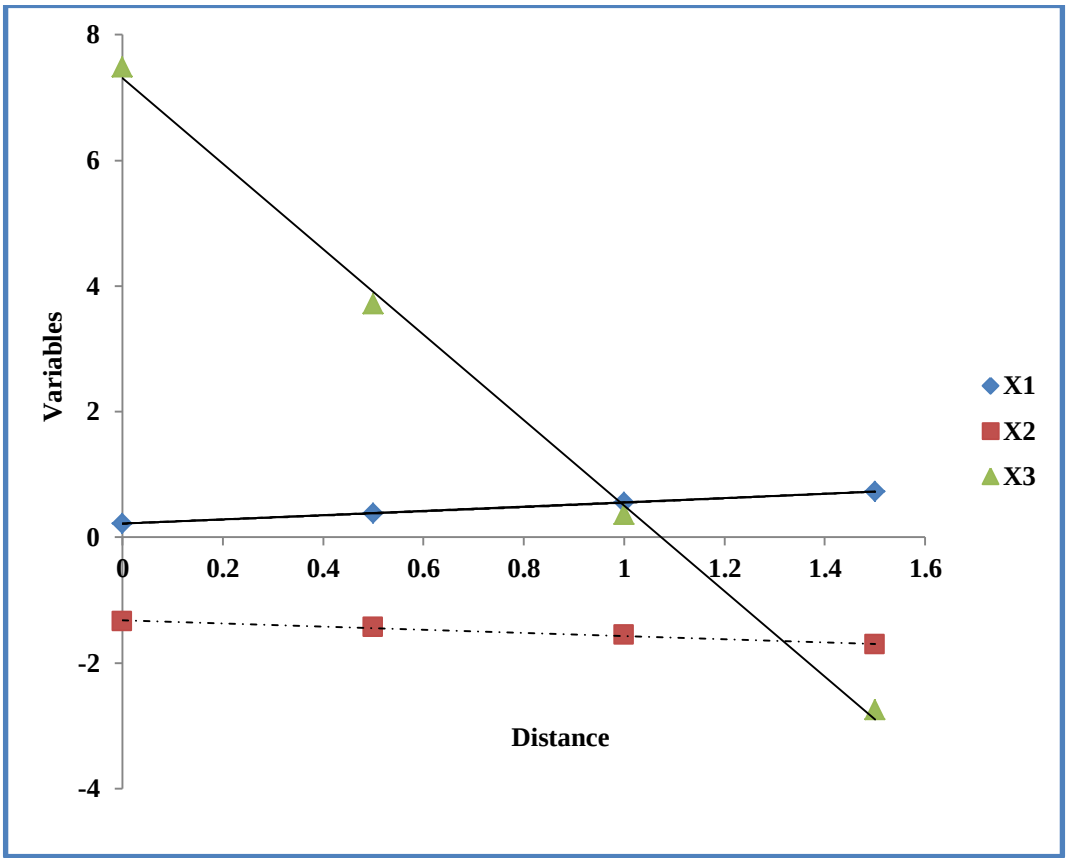


Figure 5. Ridge analysis of treatment conditions

(variable: %L-proline, X_1; frying time, X_2 and temperature, X_3)

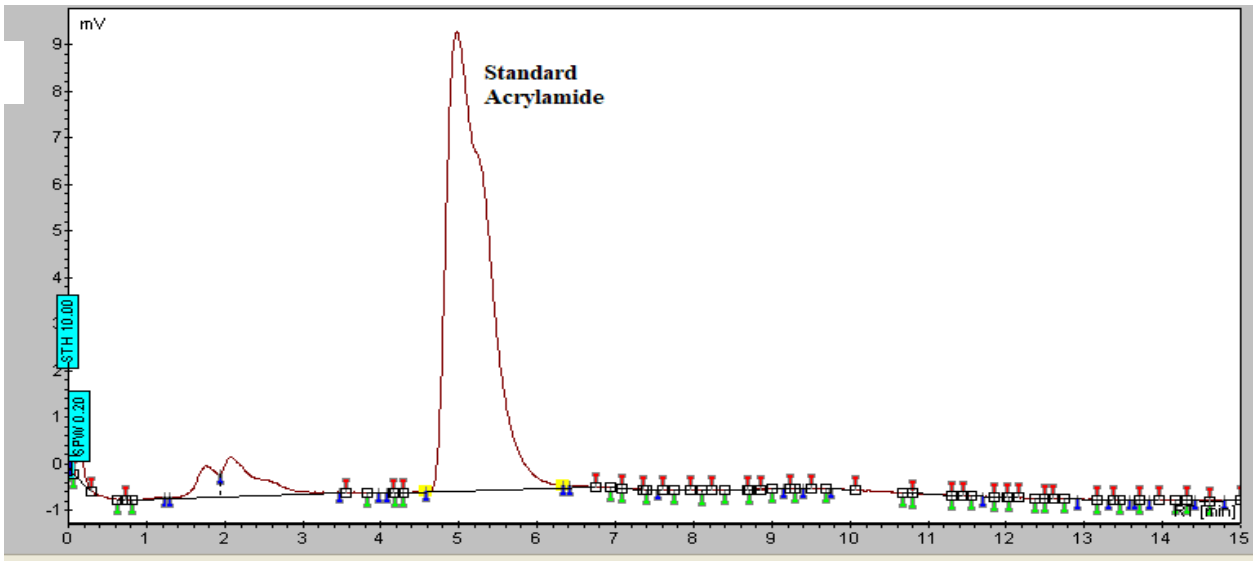


Figure 6a. HPLC analysis of standard acrylamide

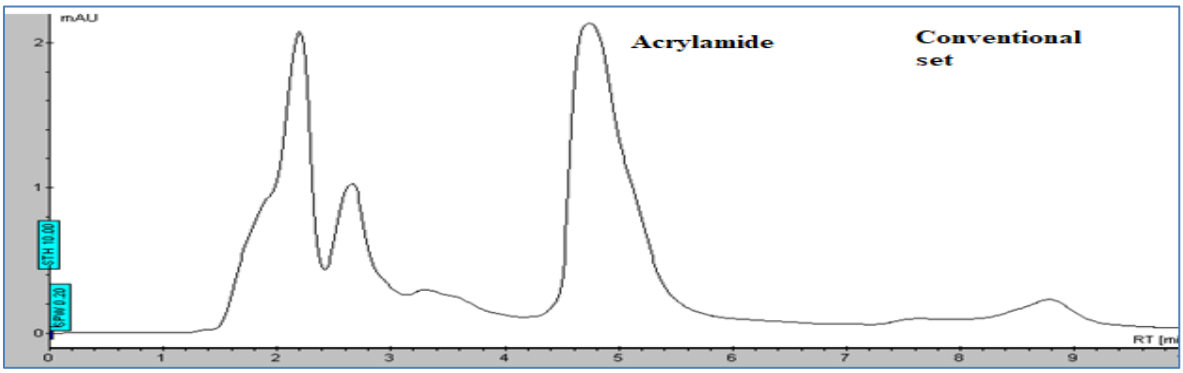


Figure 6b. HPLC analysis of acrylamide in conventional set

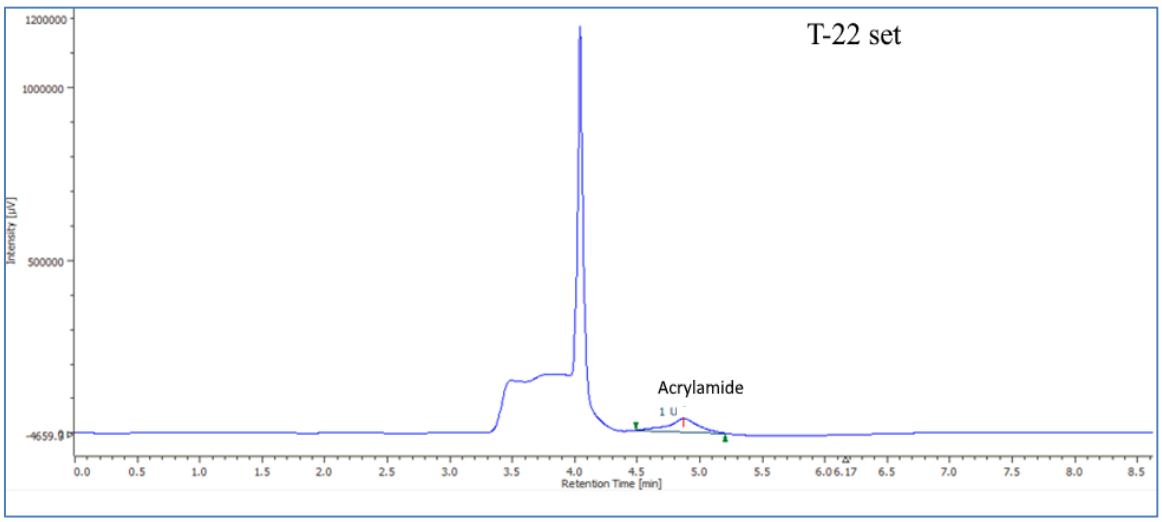


Figure 6c. HPLC analysis of acrylamide in T-22 set

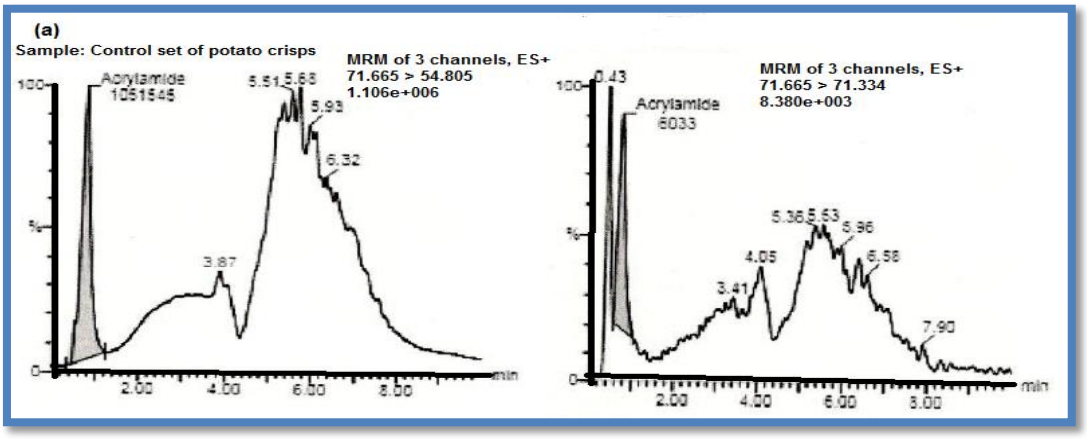


Figure 7a. LC-MS/MS analysis of acrylamide in conventional set

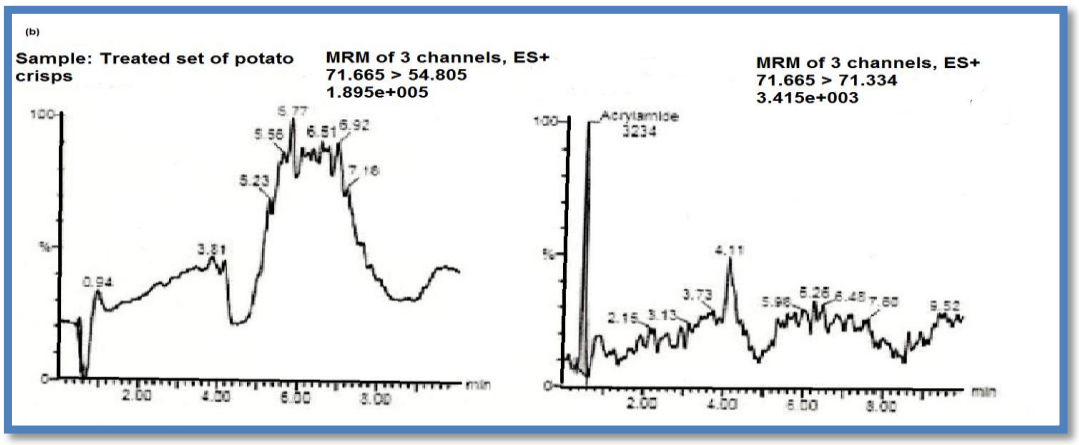


Figure 7b. LC-MS/MS analysis of acrylamide in T-22 set

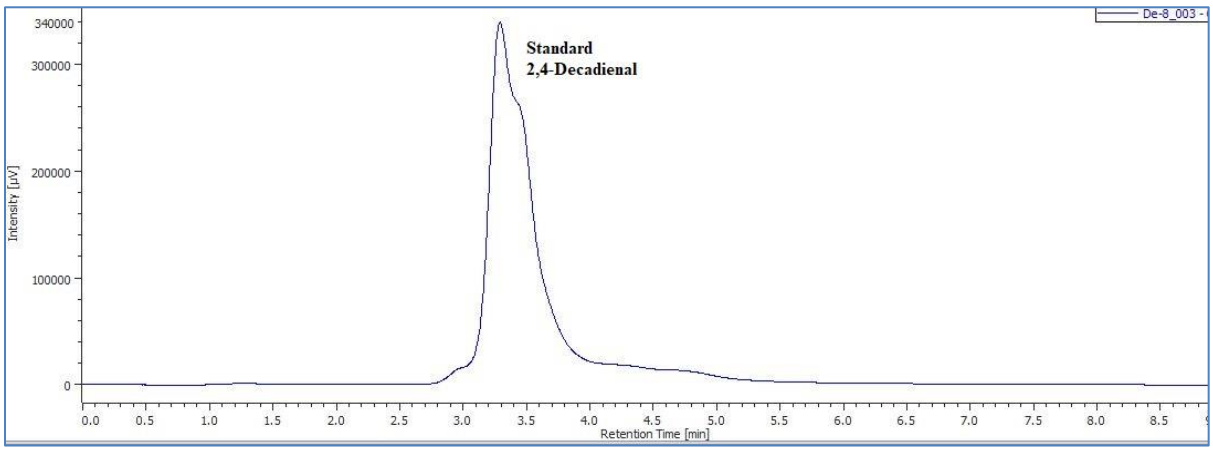


Figure 8a. HPLC analysis of standard 2,4-decadienal

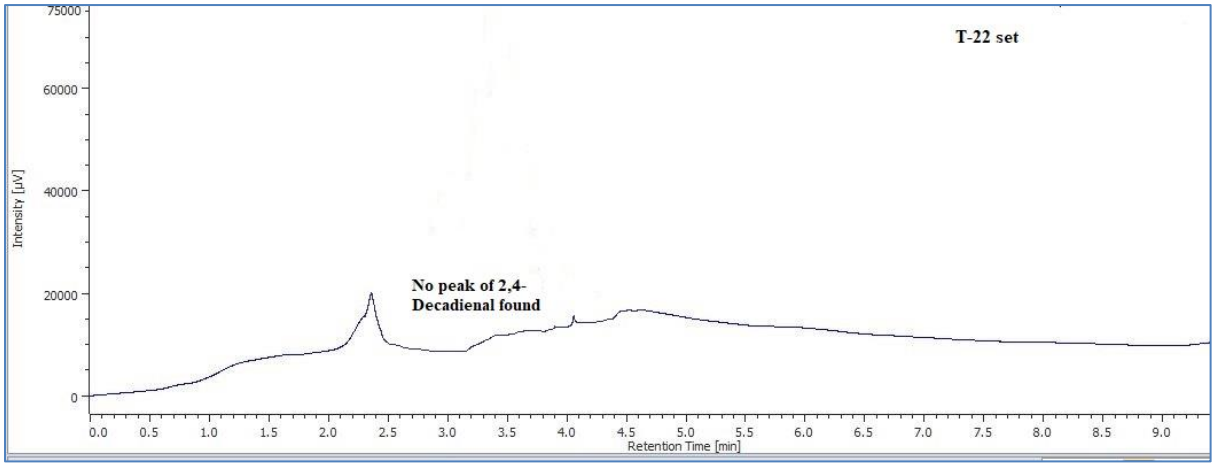


Figure 8b. HPLC analysis of 2,4-Decadienal in T-22 set (after 5 times of repeated frying)

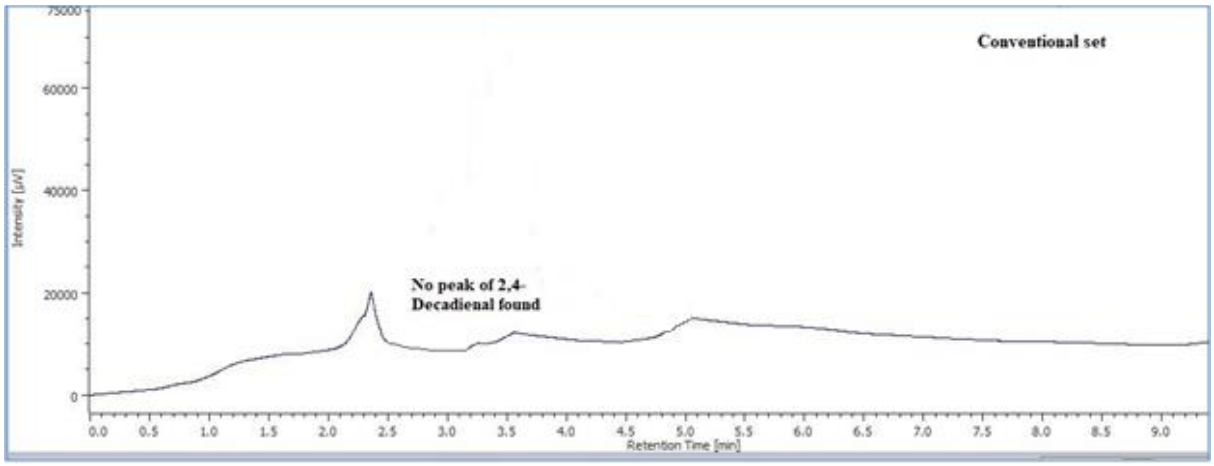


Figure 8c. HPLC analysis of 2,4-Decadienal in conventional set (after 5 times of repeated frying)

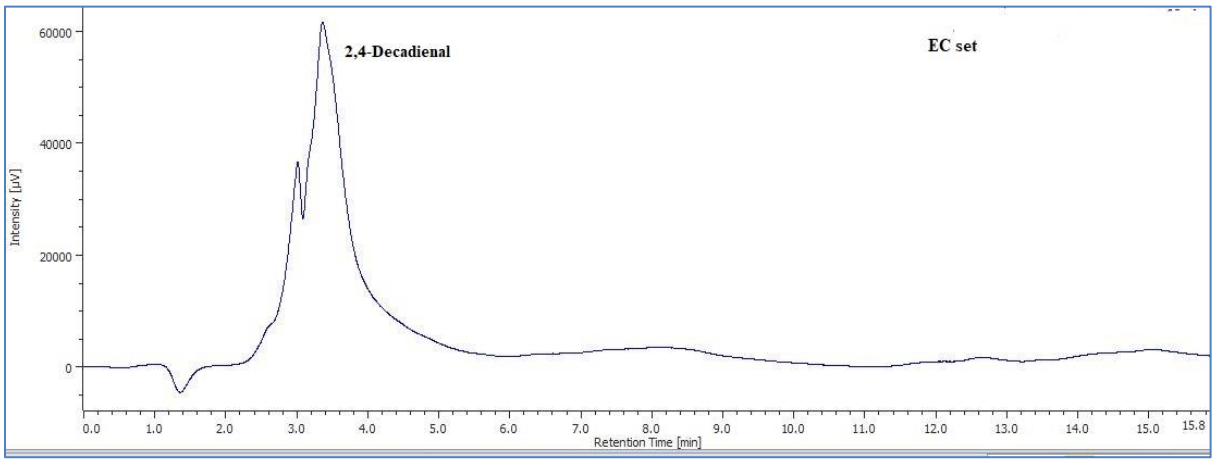


Figure 8d. HPLC analysis of 2,4-Decadienal in EC set (after 5 times of repeated frying)

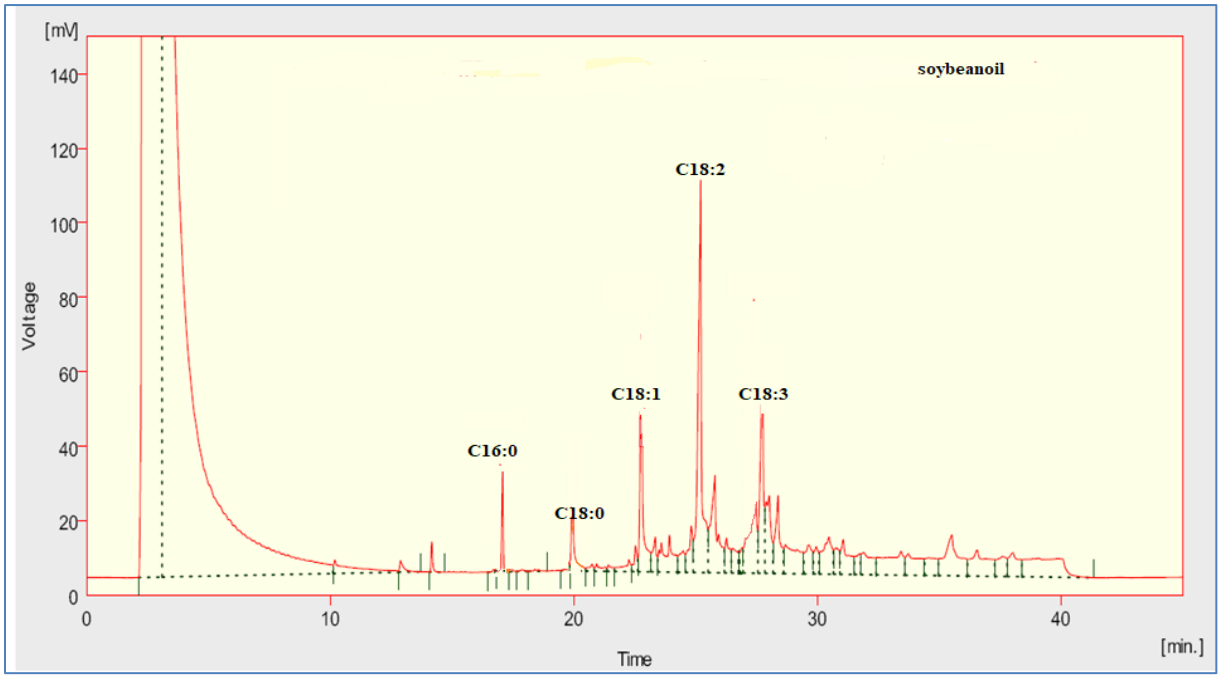


Figure 9a. FAME-GC of soybean oil

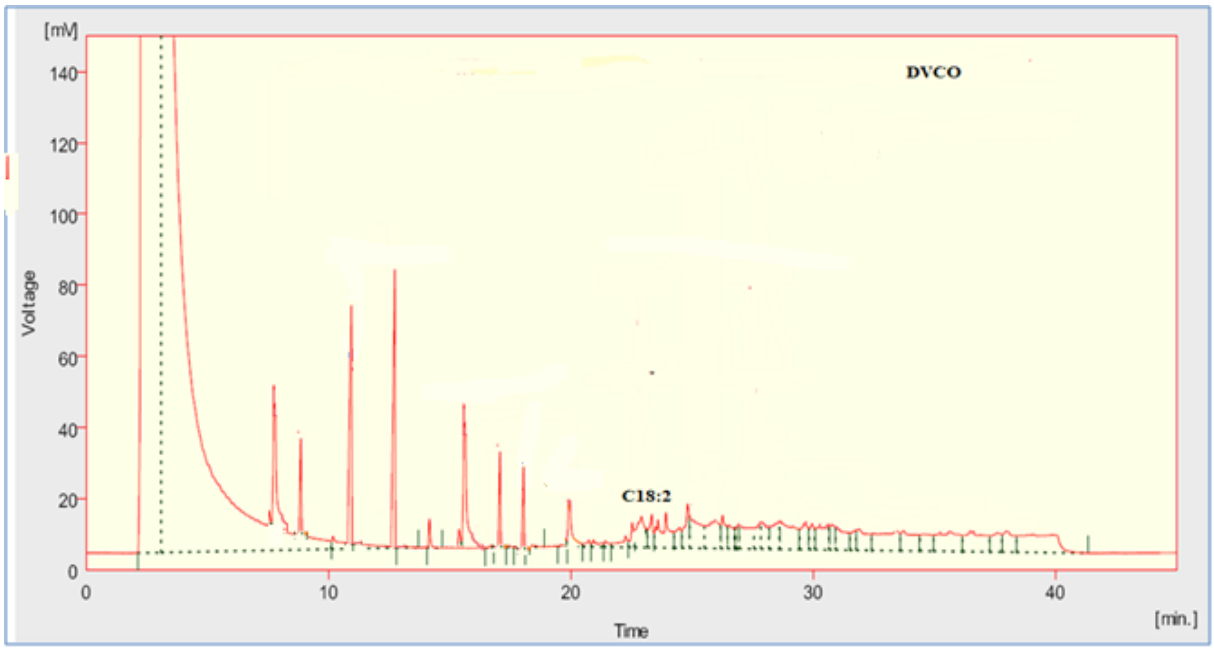


Figure 9b. FAME-GC of DVCO

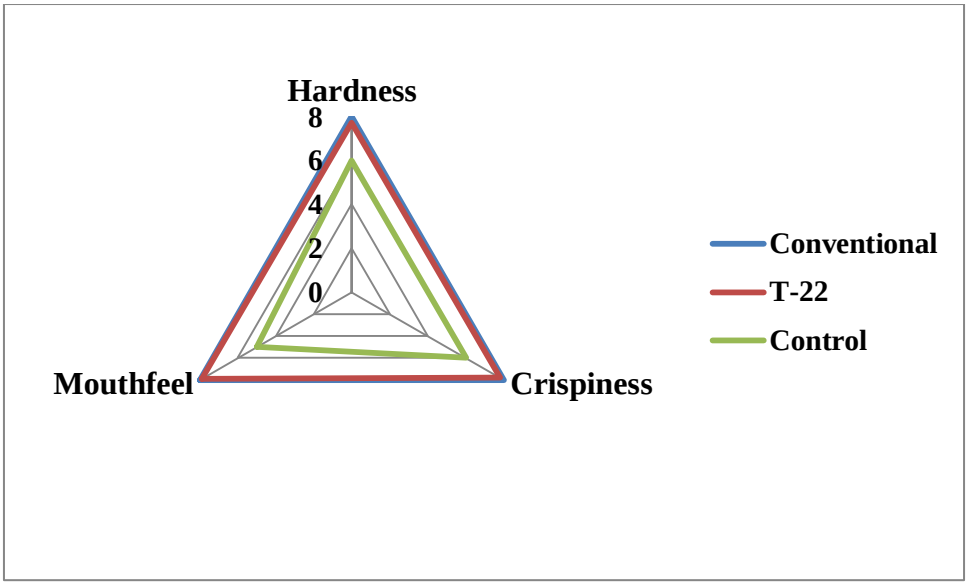


Figure 10. Objective sensory evaluation of three textural parameters of conventional, T-22 and control sets

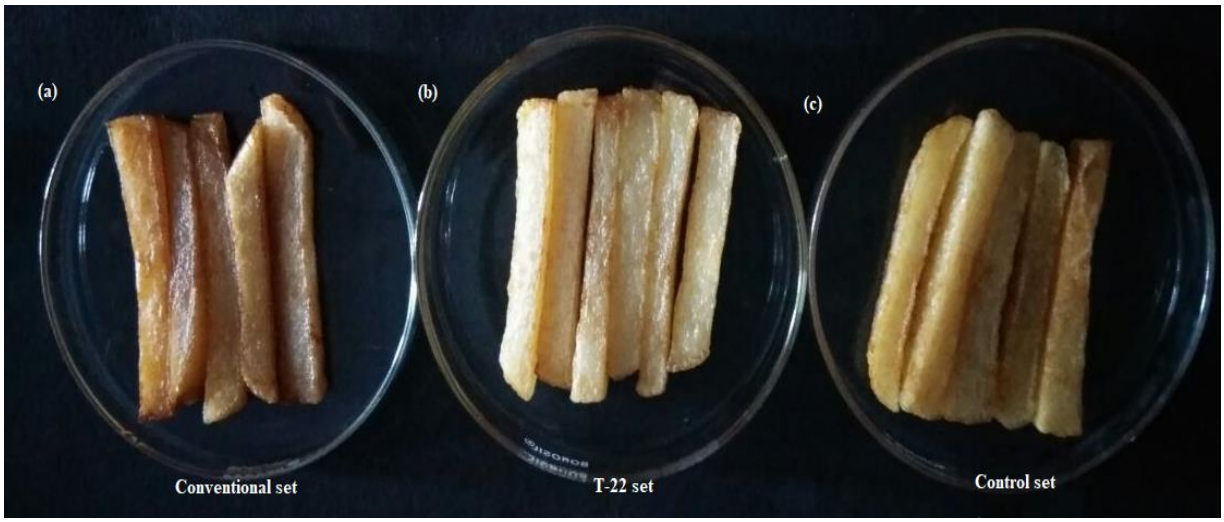


Figure 11. Fried potato crisps of (a) conventional set (b) T-22 set (c) control set

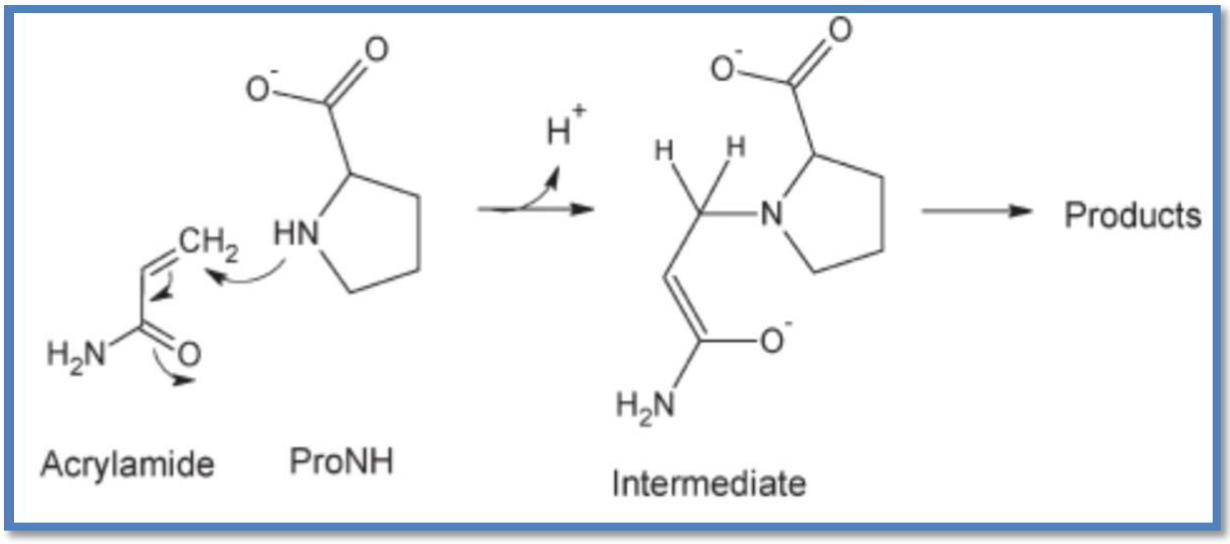


Figure 12. Michael addition reaction between L-proline and acrylamide

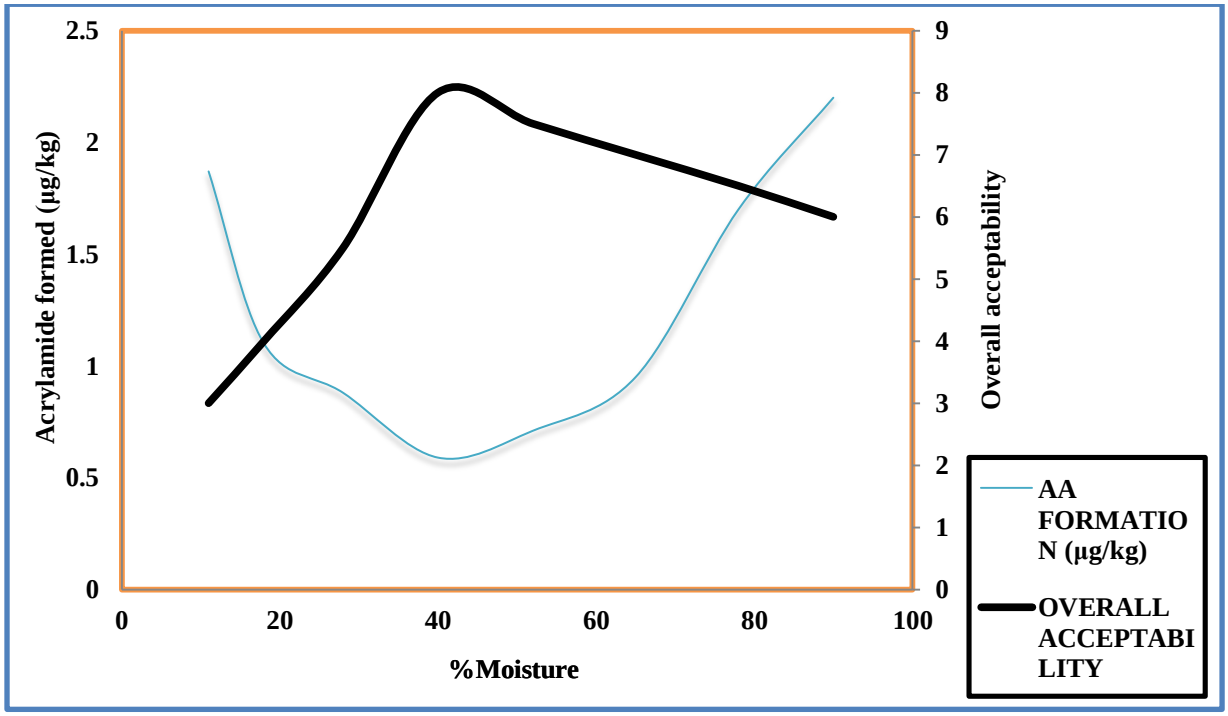
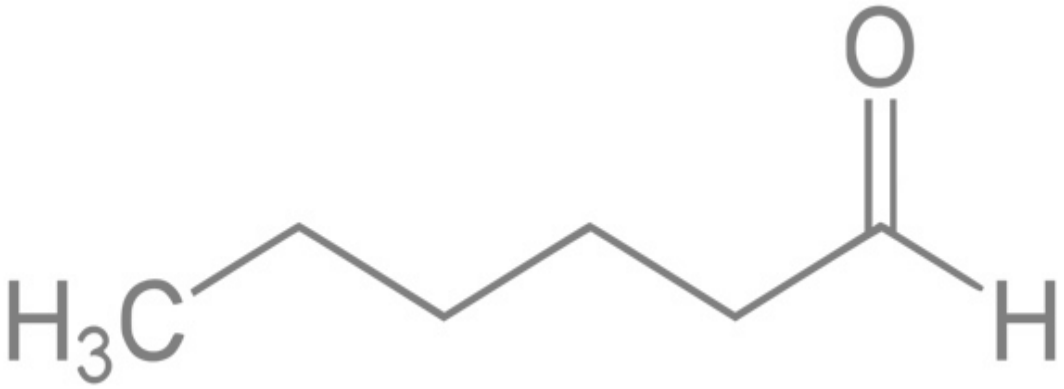


Figure 13. Graphical presentation of % final moisture content of potato slices of T-22 (prior to frying) in relation to acrylamide formation and overall acceptability of the fried product keeping other treatment conditions (aqueous pre-treatment, L-proline, frying condition) constant



CHAPTER 4:

DVCO as healthy frying oil: assessment of rancid compounds by GC-FID and electronic nose using scalar machine learning classified sensors

1. Introduction

Fried potato crisps are globally popular as deep fried snacks owing to their crisp texture, flavor, color and mouthfeel (Loon et al., 2007). However, the major problem associated with the same is storage instability owing to their propensity to deteriorate consequent to lipid oxidations (rancidity) and microbial infestation. Oxidative deterioration of lipids in fried products form hydroperoxides, which further break down into volatile aldehydes, esters, alcohols, ketones, and hydrocarbons. Some of these compounds impart disagreeable odors and render the fried products sensorially unacceptable for consumption as well as are responsible for harmful effects on human health. Among all the degenerated compounds, hexanal is considered to be the most important and prominent molecular marker of rancidity in oil-fried products (Grebenteuch et al., 2021). The proposed pathway of hexanal formation in these products have been already discussed in Chapter-1.

Detection of onset of rancidity is highly important for assuring the safety of the fried products prior to consumption. Fradulent practices at commercial production levels such as repeat use of frying oil and merchandise of rancid fried products to consumers are rampant. Sensorial perception of onset of rancidity in fried products is obsured not only due to presence of low concentration of hexanal but also by the food matrix (namely starch). Therefore, hexanal-free fried potato crisps is crucial to deliver safe-cum-healthy fried products to consumers.

Additionally, industries demand a faster and simpler method to assess the onset and progression of rancidity (rancidity status) for large number of samples. Fried potato-based products are considered to be rancid when hexanal is detected at a concentration greater than 0.03 ppm. Analytical gas chromatography (GC)-based method is commonly used for analysis of molecular markers of rancidity, chiefly the aldehydes. However, detection of onset of rancidity in fat-rich products is not possible by GC-flame ionization detector (FID) analysis since the concentration of hexanal produced just at onset of rancidity is below the LOD (0.89 ppm) and LOQ (2.97 ppm) of GC-FID. This study therefore endeavored to develop an alternate methodology for detection and quantification of remarkably low hexanal concentrations for assessment of onset of rancidity in fat-rich food products, using e-nose technology.

The rancidity status of two categories of fried potato crisps was investigated during storage. One set was fried in linoleic acid-lean DVCO under modified deep frying conditions designated as T-22 set (discussed in Chapter-2) and another set was fried in linoleic acid-rich

soybean oil under deep frying conditions, designated as EC set. These oils were so chosen that degree of rancidity in them would be widely different, low in T-22 and high in EC.

Prior to rancidity detection by e-nose, the MOS sensors that responded well to hexanal would be first identified by screening them using linear support vector machine (SVM) learning tool, followed by calibrating the selected sensors using standard hexanal. The progression of rancidity in T-22 and EC sets during storage under ambient conditions would be assessed by determination of their 'spoilage indices' and correlated with concentration of hexanal (by GC-FID analysis) by regression modeling. The best fit model equations would allow direct and accurate assessment of the prominent molecular marker of rancidity in the fried potato crisps, forgoing GC-FID analysis.

2. Material and Methods

2.1. Materials

Materials required for this study were the same as described in Chapter-2 (section 2.1). Speciality chemicals such as hexanal (~99% pure) and *n*-hexane were procured from M/s Sigma-Aldrich, Bengaluru, India; whereas dichloromethane (DCM) was purchased from M/s Merck, Mumbai, India.

2.2. Preparation of T-22 and EC sets

The processes for preparation of T-22 and EC sets of potato crisps have been described in Chapter-3.

2.3. Storage studies of T-22 and EC sets for shelf life assessment

2.3.1. Storage

The T-22 and EC sets post frying were wrapped in food grade Al foil and packaged in ziploc LDPE (linear density polyethylene) pouches. Nitrogen was flushed into them before sealing. Thereafter, the pouches were kept at 23±2 °C for a total period of 6 days (duration of storage was assessed after performing microbiological assays) and the samples were withdrawn for analyses at an interval of one day.

Preparation of deliberately-rancid or training sets

Sets of deliberately-rancid samples were prepared from freshly prepared T-22 and EC sets. These were subjected to rapid rancidity development by storing them in a chamber for 1, 2, 3 and 4 days, wherein temperature of 40±2 °C and UV light of wavelength 254 nm accelerated lipid oxidation in them. On completion of the rancidity (ageing) treatment, the samples were removed from the accelerated rancidity chamber, flushed with nitrogen and stored in a conservation chamber (23±2 °C, 75-80% RH) to arrest the final rancidity stage in accordance with the method reported by Chatterjee et al. (2014). The deliberately-made rancid fried potato crisp sets withdrawn from the conservation chamber and coded as TT22 and TEC (labelled apposite to the T-22 and EC sets), which comprised of 180 fried potato crisps of which 60 fried potato crisps were subjected to chemical analyses and the remaining 180 fried potato crisps to chromatographic and e-nose analyses. Labelling of samples from the said sets were performed in accordance with their respective day of analysis, *viz*. sample labels corresponded to their storage time in days (mentioned below).

Storage period (in day)	**Code**			
	EC	**T-22**	**TEC**	**TT-22**
0	EC:0	T-22:0	TEC-0	TT22-0
1	EC:1	T-22:1	TEC-1	TT22-1
2	EC:2	T-22:2	TEC-2	TT22-2
3	EC:3	T-22:3	TEC-3	TT22-3
4	EC:4	T-22:4	TEC-4	TT22-4
5	EC:5	T-22:5	–	–
6	EC:6	T-22:6	–	–

2.3.2. Microbiological analyses of T-22 and EC sets

The standard plate count (SPC), and yeast and mold counts of T-22 and EC sets on days 0, 1, 2, 3, 4, 5 and 6 (storage period) were conducted as per standard methods described in IS5402:2012 and IS5403:1999, RA 2018 (Table 1). The counts have been expressed in cfu/g fried potato crisps.

2.3.3. Sensory evaluation

Objective sensory evaluations of T-22 and EC sets during storage was performed by semi-trained panelists as per the process, described in section 2.11 in Chapter-3. Total six quality attributes such as overall appearance, color, aroma, mouthfeel, taste and after-taste of the

aforementioned sample sets were adjudged on the basis of the standard 9-point hedonic scale (Stone and Sidel, 2012). Since the panel members were semi-trained, variation in their sensory-perceptual for rancidity might influence the decision during sensory evaluation. Therefore, fuzzy logic analysis was performed to mitigate these variabilities and arrive at an unbiased evaluation of sensory parameters of the fried potato crisps.

2.3.4. Fuzzy logic analysis

Thirty panelists (15 men and 15 women) belonging to faculty, research scholars and staff of the department of FTBE, Jadavpur University willingly participated in subjective (linguistic) sensory evaluation of T-22 and EC sets. Quality parameters considered for this evaluation were: overall appearance, color, aroma, mouthfeel, taste and after-taste. The entire process of sensory evaluation has been discussed in Chapter-2. All sensory data were fed to fuzzy models which produced defuzzified scores following the steps discussed in Chapter-2.

2.3.5. Chemical assays of T-22 and EC sets

Standard chemical assays (PVs, AEF and MDA contents) were performed with T-22 and EC sets on days 0, 1, 2, 3, 4, 5 and 6 and their deliberately-rancid counterparts on days 1, 2, 3 and 4 for analyzing their rancidity status during storage. Mashed fried potato crisps (5 g) using a Soxhlet extraction assembly in accordance with standard AOAC method i.e., AOAC 963.15 (2000) using *n*-hexane as solvent and the extracted fat was subjected to chemical assays. PVs and AVs were determined in accordance with standard methods (Dutta et al., 2017; Bialek et al., 2017) and expressed in terms of meq. O_2/ kg of sample and mg of KOH/g of sample as % oleic acid, respectively. To determine the secondary oxidation products in the fried potato crisps, the MDA contents (mmol/g fried potato crisops) were quantified (from its standard graph prepared using a pure standard of the said chemical) by thiobarbituric acid-reactive substances (TBARS) assay, in accordance with the method reported by Che Man et al. (1999).

Determination of PV

The PVs of T-22 and EC sets of fried potato crisps were determined using standard procedures (AOCS, Cd 8-53, 1996).

Determination of AV

AV contents of T-22 and EC sets were analyzed in accordance with accordance with Method, described by Chatterjee et al. (2014).

Determination of MDA by TBARS assay

The secondary lipid oxidation in T-22 and EC sets was assessed by quantifying MDA following TBARS analysis. The procedure for determination of malondialdehyde was in accordance with the method reported by Che Man et al. (1999).

2.3.6. Extraction of fried potato crisps volatiles by Likens-Nickerson's steam-distillation-cum-solvent extraction method

Likens–Nickerson (L-N) concurrent steam-distillation-solvent extraction was conducted for extraction of VOCs from T-22 and EC sets (during storage), followed by concentration of the extracts using vigreaux fractional distillation column in accordance with method described by Bhattacharjee et al. (2005), with necessary modifications (details provided in Appendix 2D.1). The VOC concentrates were then subjected to GC-FID cum e-nose analyses for qunatificaton of the rancidity molecular marker, viz. hexanal.

2.3.7. GC analysis of hexanal of T-22 and EC sets

Hexanal analysis (Table 4) of extracted VOCs concerntrate of T-22 and EC sets of fried potato crisps (during storage) were performed on GC (Trace GC 700; Thermofischer Scientific) equipped with with TR-1 capillary column (30 m × 0.32 mm, i.d. 0.25 μm) and flame ionization detector (FID). The injector and detector temperatures were set at 250 ℃ and 260 ℃, respectively. N_2 was used as the carrier gas at a flow rate of 1 ml min^{-1}. The oven temperature was programed as follows: 70 ℃ (2 min hold), 70-120 ℃ at 5 ℃ min^{-1}, then increasing at 10 ℃ min^{-1} to 260 ℃ and final hold at 260 ℃ for 7 min. Extracted aroma compounds (dissolved in HPLC-grade DCM) were injected into GC in split-less mode for analysis. Identification of hexanal was performed using the standard of the same (Figure 1). Prior to sample analyses, a standard curve of hexanal (50-75 mg/ml) was prepared by dissolving a pure standard of hexanal in HPLC grade DCM. Dilutions were made immediately before injection into GC. The hexanal content was expressed as mg/kg (ppm) of crisps.

2.3.8. Analysis of fried potato crisps by ENOVISION during storage

Detection of rancidity in fried potato crisps using ENOVISION

Prior to performing the e-nose analyses of samples, several preliminary trials on the operating parameters such as sample size; time needed for saturation of headspace of sample vial by VOCs generated from sample, sampling and purging time were performed. The maximum response received from each sensor under the following operating conditions: sample size- 100 μL of extracted VOCs concerntrate; acquisition rate- 600 ppb; headspace generation time- 30 s; sampling time- 50 s; purging time-450 s. The e-nose system was trained with the corresponding training sets i.e., deliberately-rancid sets (*vide infra*) in accordance with the method reported by Chatterjee et al. (2014). Potato fries could be directly subjected to e-nose analysis for ascertaining their VOC profiles. However, in the current investigation, L-N extracts of VOCs were used for e-nose analysis to categorically quantify the chief rancidity marker molecule, viz. hexanal among other aldehydes and ketones.

Selection of appropriate sensors of ENOVISION

ENOVISION (Ver.1.Q) employed in this current study was equipped with eight MOS (mentioned in Chapter-1). Prior to rancid-acid odor analyses of T-22 and EC sets, the sensors were screened by training with deliberately-made rancid fried potato crisps viz. by TT22 and TEC sets in accordance with the procedures described by Chatterjee et al. (2014) and Dutta et al. (2017), followed by calibration with standard hexanal (rancidity marker) to enable reliable discrimination between rancid (just at onset) and non-rancid fried potato crisps. Sensor screening was performed using SMLC (Matlab® R2020a; Mathworks, Inc. Natick, MA, USA).The data generated from sensor array for each type of fried potato crisps was fed to different models of SMLC such as decision tree, SVM (support vector machines), KNN (k-nearest neighbors), neural network, ensemble classifiers and naïve base (Table 5). Different classifiers or models of SMLC were run using 5-fold cross-validation method with distribution of data set in 80:20 (training data set : test data set) for evaluating model's performance on the basis of root mean square error (RMSE) values. The optimum model was selected based on % accuracy and prediction speed of each classifier (coding presented in Appendices 2B.2). Responsible sensors were recognized using the best fit model (*vide infra*). Sensor classification was accomplished with the help of hyperplane (Figure 4). In this test frame-work, e-nose system with selected sensors was employed to evaluate the rancidity

profile of T-22 and traditional sets during storage (Figure 5) by computing sensor responses using eq. (2).

$$(|\Delta R|/R)_{hexanal} = |(R_{TS} - R_{hexanal})|/R_{hexanal} \quad (1)$$

$$(|\Delta R|/R)_{hexanal} = |(R_{S} - R_{hexanal})|/R_{hexanal} \quad (2)$$

where, R_{TS} = resistance of sensor towards the VOCs of training sets (*vide supra*); R_S = resistance of sensor towards the VOCs of experimental samples on day 0, 1, 2, 3, 4, 5 and 6, $R_{hexanal}$ = resistance of sensor towards standard hexanal. The volume of hexanal to be added was optimized to be 0.03 ml since volumes above this oversaturated the sensors and baseline correction was rendered impossible even after purging with air several times.

Hierarchical cluster analysis (HCA) analysis of rancidity status of T-22 and EC sets

In this study, HCA was employed as an 'unsupervised classification' method to assess the association of % similarity in selected sensor responses towards VOCs of rancid-acid odor of T-22 and EC sets during storage. The results of HCA are presented in a dendogram (Figure 6).

2.3.9. Determination of "spoilage index" for fried potato crisps

A scalar value was generated from the screened e-nose data of fried potato crisps (Table 6) using Mahalanobis distance (Chatterjee et al., 2014) which is effectively a multivariate equivalent of the Euclidean distance. It measures the distance between a point and a distribution (Anderson, 1958). This scalar value was nomenclatured as 'spoilage index' for fried potato crisps, which is proportional to the e-nose sensor signal responses. In the current investigation, the Mahalanobis distance (d^2) of T-22 and EC sets was determined using matrix operation, as described by Chatterjee et al. (2014). The data set i.e., N (811×2) obtained from screened sensors (selected from linear-SVM) of freshly prepaed sets was considered as the baseline vector. On each day of storage period, matrix i.e., M_i (811×2) was prepared from the data of fried potato crisp samples obtained from the screened sensors. Mahalanobis distance (eq. 3) was calculated between the matrix (M_i) and matrix (N) which generated 'd^2'.

$$d^2 = (x\text{-}m)^T . V^{-1} . (x\text{-}m) \quad (3)$$

where $x^T = \{x_1, x_2, \ldots, x_n\}$ vector for a single multivariate observation; $m_t = \{\mu_1, \mu_2 \ldots, \mu_n\}$ vector representing the population mean and V is co-variance matrix. Greater distance (Table 6) from the baseline matrix implied more rancidity. The 'spoilage index' values were plotted against the hexanal concentrations (during storage) of T-22 and EC sets. The regression equations thus developed would allow unanbiguous detemination of concentration (> 0.03 ppm) of hexanal generated consequent to rancidity.

2.4. Statistical analysis

All the experiments were conducted in triplicates and results were reported as mean ±SD of three independent experiments. The hierarchical cluster analysis was created using SPSS 20.0 software (IBM). A $p \leq 0.05$ was used to verify the significance of the tests.

3. Results and Discussion

3.1. Microbiological analyses of T-22 and EC sets during storage

According to WHO guidelines (1994), the acceptable limits of SPC and TFC were $\leq 2.0\times10^5$ cfu/g and $\leq 1.0\times10^5$ cfu/g, respectively. For, T-22 and EC sets, visible fungal growth was found on day 6 and day 4, respectively, corresponding to their SPC and TFC values (Table 1) exceeding the afore-mentioned limits. Therefore, T-22 and EC sets can be stored up to day 5 and day 3, respectively. These findings could be well justified by the antimicrobial activities of the medium chain fatty acids (MUFA) present in DVCO (Ghosh et al., 2016; Huang et al., 2011).

3.2. Fuzzy logic analysis of T-22 and EC sets during storage

Panelists showed increasingly low preference for T-22 and EC sets with time as reflected by their defuzzified scores (Table 2a and 2b). Thus the grade of T-22 shifted from category "good" to "moderate" on day 4 and to "not significant" on day 5. Whereas, the grade of EC set underwent a shifting from category "good" to "moderate" on day 2 and to "not significant" on day 3. Therefore, the sensorial acceptabilities of T-22 and EC sets were up to day 4 and day 2, respectively.

3.3. Chemical assays of T-22 and EC sets

3.3.1. Change in PVs of fried potato crisps during storage

PV is a measure of the primary oxidation of the triglycerides, and any value less than 6 ppm (miliequivalent of O_2/kg of fried potato crisps) is considered as non-rancid as per the literature review (Feiner, 2016). Although PVs of T-22 and traditional sets (Table 3) showed a increase with storage time, having lower ($p < 0.05$) value for T-22 set compared to that of EC set on each day of analysis (as DVCO is PUFA-lean and antioxidant-rich oil vis-à-vis soybean oil). PVs of T-22 set on day 5 and EC set on day 3 exceeded the limit of rancidity and were found similar ($p > 0.05$) to that of their respective 1-day stored deliberately-rancid fried potato crisps. From these findings, it is thus evident that rancidity of T-22 and traditional sets of fried potato crisps occured from day 5 and 3, respectively.

3.3.2. Change in AEFs of fried potato crisps during storage

As per FSSAI guidelines (FSSAI, 2018), AEF content in fried potato crisps more than 1.5 % was considered as the limit for rancidity. AEF values of T-22 and EC sets increased with storage time. However, it showed lower ($p < 0.05$) value for T-22 set compared to that of EC set on each day of analysis owing to antioxidant property of DVCO. The AEF values of T-22 set on day 5 and EC sets on day 3 exceeded the FSSAI limit and were similar ($p > 0.05$) to their respective of 1-day stored deliberately-rancid fried potato crisps. From these findings, it was evident that rancidity of T-22 and EC sets of fried potato crisps commenced on day 5 and 3, respectively.

3.3.3. Change in MDA values of fried potato crisps during storage

The MDA values of T-22 and traditional sets increased with storage time. However, it showed lower ($p < 0.05$) value for T-22 set compared to that of EC set on each day of analysis owing to antioxidant property of DVCO. The MDA values of T-22 and traditional set of fried potato crisps on day 5 and day 3 were much closer ($p > 0.05$) to that of their respective 1-day stored deliberately-rancid fried potato crisps. To the best of our knowledge, there are no set standards and reports in literature that indicate the acceptable level of MDA in potato crisps to enable comparison of our experimental data. Hence, an unpleasant greasy odor was perceived in T-22 and in the traditional sets from day 5 and 3 onwards, respectively, which could be attributed to the presence of MDA in a very higher ($p < 0.05$) amount in fried potato crisps.

3.4. GC analalysis of hexanal of fried potato crisps during storage

GC-FID analyses of hexanal of T-22 and EC sets were presented in Figures 2-3. In this study, LOD and LOQ of hexanal, analysed by GC-FID were 0.89 ppm (mg/kg of sample) and 2.97 ppm (mg/kg of sample), respectively. The amount of hexanal in fried potato crisps beyond 30 µg/kg i.e., 0.03 ppm (mg/kg of samples) correlates with rancidity (Agarwal et al., 2018) of the product. Although the storage study was conducted for 5 days, to obtain good correlations with the spoilage indices, hexanal analysis was continued for 7 days for T-22 sets and EC sets. The hexanal content in T-22 set was first identified and quantified on day 5 (Figure 2c) and obtained as 2.98 ppm and for traditional set on day 3 (Figure 3c) was 3.82 ppm. Although the same increased linearly with time for both the sets (Table 4), there was a significant ($p < 0.05$) difference in hexanal content between T-22 and EC sets because DVCO (frying oil of T-22 set) is PUFA-lean oil, containing 1.8% C18:2 vis-à-vis PUFA-rich soybean oil, containing 50.9% C18:2. In view of above, hexanal content for T-22 and EC sets on day 5 and 3, respectively were greater ($p << 0.05$) than its limit i.e., 0.03 ppm. These findings imply that onset of rancidity occurred before day 5 and 3 for T-22 and EC sets, respectively, but the same could not be analyzed owing to high LOD and LOQ values of hexanal by GC-FID. Chemical and GC analyses predicted that rancidity of T-22 and EC sets occurred from day 4 and 2, respectively.

3.5. Screening of sensors of e-nose system

The responses of each sensor towards VOCs of deliberately-rancid or training sets (withdrawn on days 1, 2, 3, 4) were fed to each 26 SMLC models (Table 5). The best fit model was found to be SVM (linear) with an accuracy of 93.90%. and prediction speed of 4600s. SVM (linear) performs (coding 2D.2) classification by finding the hyperplane that maximizes the margin between the two classes containing lower and higher response values. A scatter plot obtained for $|\Delta R|/R_{hexanal}$ values of all eight MOS was segregated by a linear hyper-plane with maximal margin (MMH = $D^+ + D^-$) using the following formula:

$$w^T \cdot (x) + b = 0 \qquad (4)$$ (Chang et al., 2001)

where, b = intercept and bias term of the hyper-plane equation; w = classification vector/weightage of attributes.

It was evident from Figure 4 that TGS 2626 and TGS 832 sensors were classified with

the highest accuracy because their response values were appreciably in the higher range ($|\Delta R|/R_{hexanal} > 0.2$) and each vector (point) was discrete in nature. The set comprising of the remaining sensors was regarded as the 'opponent group' w.r.t the above-mentioned groups since the sensors in the former set displayed lower $|\Delta R|/R_{hexanal}$ values of overlapping nature, implying that the responses of the said sensors to the VOCs of fried potato crisps were indistinguishable from each other. Thus, the SVM analysis successfully allowed recognition of the best combination of pairs of sensors, *viz.* TGS 2626 and TGS 832 for assessment of rancidity status of T-22 and traditional sets during storage.

3.6. E-nose analysis of fried potato crisps during storage and its classification by HCA

The e-nose analysis of T-22 and EC sets was performed up to day 7 with screened sensors (TGS 2626 and TGS 832), presented in Figure 5. Hence, to build the dendogram and to discriminate the samples with respect to freshness, onset of rancidity and progression of rancidity, screened sensor responses of both sample sets up to day 5 were considered. From these data, HCA (Figure 6) was generated which was used for finding relatively homogeneous clusters of cases based on rancid-acid odor.

In a dendogram, T-22:0, T-22:1, T-22:2, T-22:3 and EC:0, EC:1 clustered into group A. The T-22:4 and EC:2 exhibited association with each other, formed group B. The TT22-1, TT22-2 TT22-3, TT22-4, TEC-1, TEC-2, TEC-3, TEC-4, T-22:5, EC:3, EC:4 and EC:5 formed group C. Among these clusters, T-22:5, TT22-1 and EC:3, TEC-1, formed subgroup of CI and CII within group C. TT22-2, TT22-3, TT22-4, and EC:4, EC:5, TEC-2, TEC-3 , TEC-4, formed a subgroup of CIII and CIV within group C, respectively. Good classification among different groups and subgroups was found on the basis of degree of rancidity. Rancid status of the samples was assessed based on the rancidity of the training set i.e., the deliberately rancid set. If the sensor responses of the sample set fall in the same quardant, in close vicinity to the training set, that implies that the samples are rancid.

from fresh to rancid: Group A (fresh) > Group B > Group CI (rancid) > Group CII > Group CIII > Group CIV (extreme rancid)

It is evident from the above results that the quality status of group 'B' is neither fresh

nor rancid which imply that the onset of rancidity in the T-22 and EC sets occurred on day 4 and 2, respectively. Therefore, e-nose analysis with screened sensors coupled to HCA was able to predict the onset of rancidity in both sets accurately vis-à-vis the conventional methods. E-nose with screened MOS could successfully predict both onset and progression of rancidity w.r.t hexanal in T-22 and EC sets. Thus it can be concluded that the safe period for consumption of T-22 and EC sets were day 3 and day 1, respectively.

3.7. Spoilage index for potato wedges

From the HCA plot, it was found that the e-nose system with screened sensors could effectively discriminate the VOCs obtained from fresh and rancid T-22 and EC sets. In order to quantify the extent of spoilage, 'spoilage index' was generated for each set (on basis of screened sensor responses for 7 days) using Mahalanobis distance method (Table 6). From ANOVA study, it was found that the 'spoilage index' for both sets increased significantly ($p < 0.05$) with storage. The 'spoilage index' of T-22 set with storage period of 4 days (1.56) was slightly lower ($p = 0.0481$) than that of TT22-1 (1.79); whereas the same of T-22:5 (1.76) was similar ($p > 0.05$) to that of TT22-1. The 'spoilage index' of EC:2 (1.54) was slightly lower ($p = 0.0484$) than that of TEC-1 (2.08); however, the same of EC:3 (2.06) was similar ($p > 0.05$) to that of TEC-1. This finding is in consonance with HCA plot, Since, this scalar value of 'spoilage index' could directly predict the rancidity status of T-22 and EC sets of fried potato crisps.

3.8. Correlation of 'spoilage index' of T-22 and EC sets with chemical parameters

From the experimental data obtained from e-nose and GC-FID analysis of T-22 and EC sets during 7 days, regression (linear) correlations were established between 'spoilage index' with hexanal to predict the rancidity status as well as determine the hexanal content of the same as a function of 'spoilage index', forgoing the GC analysis. In this study, two equations i.e., eq. (5) and (6) were generated by regression analysis wherein hexanal contents of T-22 and EC sets could be determined as a function of 'spoilage index'.

$$\text{Hexanal} = 1.5472\ (\text{Spoilage index}) + 0.2157 \qquad (5)$$

$$\text{Hexanal} = 2.2951\ e^{0.2232(\text{Spoilage index})} \qquad (6)$$

The fitted models were validated by p- and F-values. The p-values showing less than 0.05

i.e., 0.0000 and reasonably high F-values of 686.00 and 687.05 corresponding to eq. (5) and (6), respectively, indicated that regression models to be a good fit with good regression coefficients (R^2 values of eq. 5 and 6 were 0.99 and 0.98, respectively) between hexanal contents and screened sensors responses. These good model fit equations i.e., eq. (5) and (6) allowed direct and accurate assessment of the prominent molecular marker of rancidity in potato crisps (viz. hexanal) fried in linoleic acid-lean and linoleic acid-rich frying oils, respectively.

4. Conclusions

Potato crisps fried in DVCO had a shelf-life lead of two days over crisps fried in soybean oil. A new methodology based on e-nose technology was sucessfully developed for detection of onset of rancidity in the fried crisps and quantified in terms of the molecular marker compound viz. hexanal using selected sensors screened by linear SVM coupled with HCA. Additionally, 'spoilage indices' of the crisps were determined during ambient storage using Mahalanobis distance method. The correlations of the indices with concentration of hexanal by regression modeling yielded good model fit equation(s) which allowed direct and accurate assessment of the rancidity marker in the crisps. The final deliverable of this work was safe hexanal-free fried potato crisps for consumers.

The next chapter will present yet another endeavour on use of e-nose technology (coupled with ANN) for rapid and accurate assessment of freshness of bread forgoing GC-FID analysis.

Novelty

The finding of this investigation is novel since it reports for the first time on-

- a rapid, accurate and reliable methodology for detection of onset of rancidity in fried potato crisps using the state-of-the-art e-nose technology
- development of 'spoilage index' value for quantification of rancidity marker compound (hexanal) in fried potato crisps forgoing GC-FID analysis

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Table 1. Microbiological analyses of EC and T-22 sets during storage

Microbiological parameters	Set of fried potato crisps	Storage day						
		0	**1**	**2**	**3**	**4**	**5**	**6**
SPC (30 ℃, 72 hr.) ($\times 10^3$ cfu/g)	EC	NG	12.2^{a}	70.5^{b}	87.8^{c}	**167.7^{d}**	-	-
	T-22	NG	3.7^{a}	19.3^{b}	40.2^{c}	68.3^{d}	81.5^{e}	**161.9^{f}**
Yeast and mould count or TFC (25 ℃, 5 days) ($\times 10^3$ cfu/g)	EC	NG	22.6^{a}	44.1^{b}	75.3^{ab}	**106.6^{d}**	-	-
	T-22	NG	9.8^{a}	28.7^{b}	47.9^{ab}	68.7^{c}	89.7^{d}	**105.3^{e}**

Values in the same column with different superscript ($^{a-e}$) are significantly different ($p < 0.05$)
Mean in a row with similar superscripts are not significant different at $p > 0.05$
Yeast and mould count showing < 10 on day 0 imply that the count is below LOQ
NG: No growth

Table 2a. Fuzzy logic analysis of sensory scores of T-22 set during storage

Sample ID.	SO			Y_a'/Y_a	Remarks
	a	**b**	**c**		
T-22:0	91.68	13.87	9.93	90.37	Excellent
T-22:1	86.55	13.37	9.88	85.39	Good
T-22:2	63.34	14.58	14.02	63.15	Good
T-22:3	62.86	14.22	14.20	62.71	Good
T-22:4	59.34	15	13.88	58.97	Moderate
T-22:5	9.61	18.00	19.32	10.05	Not Significant

Table 2b. Fuzzy logic analyses of sensory scores EC set during storage

Sample ID.	SO			Y_a'/Y_a	Remarks
	a	**b**	**c**		
EC:0	88.5	13.11	9.22	87.20	Good
EC:1	66.77	13.58	13.58	66.77	Good
EC:2	41.8	15.25	16.00	42.05	Moderate
EC:3	11.6	16.44	17.65	12.00	Not Significant

Table 3. Peroxide value, TBARS, AEF content of T-22 and EC sets of fried potato crisps and those of deliberately rancid crisps during storage

Day	T-22 set			EC set		
	PV (meq O_2/kg of crisps)	**MDA (micromole/kg of fried potato)**	**AEF (% oleic acid)**	**PV (meq O_2/kg of crisps)**	**MDA (micromole/kg of fried potato)**	**AEF (% oleic acid)**
0	0.26±0.01[a]	-	0.17±0.005[a]	0.61±0.02[b]	-	0.39±0.006[b]
1	0.71±0.02[a]	15.90±0.44[a]	0.31±0.03[a]	2.78±0.02[b]	37.07±0.40[b]	0.84±0.02[b]
2	2.56±0.03[a]	27.89±0.90[a]	0.58±0.02[a]	5.00±0.04[b]	52.03±0.94[b]	1.46±0.01[b]
3	3.50±0.06[a]	40.09±0.88[a]	0.95±0.05[a]	6.92±0.10[b]	106.33±0.70[b]	1.83±0.06[b]
4	5.35±0.05[a]	52.89±3.50[a]	1.25±0.06[a]	-	-	-
5	6.07±0.09[a]	86.77±2.03[a]	1.52±0.03[a]	-	-	-
Deliberately rancid						
	TT22 set			**TEC set**		
Day	**PV (meq O_2/kg of crisps)**	**MDA (micromole/kg of fried potato)**	**AEF (% oleic acid)**	**PV (meq O_2/kg of crisps)**	**MDA (micromole/kg of fried potato)**	**AEF (% oleic acid)**
1	6.09±0.11[a]	87.02±4.03[a]	1.55±0.10[a]	6.95±0.10[b]	106.89±3.93[b]	1.86±0.05[b]
2	6.55±0.15[a]	136.90±2.05[a]	1.62±0.11[a]	7.09±0.13[b]	157.10±1.95[b]	1.93±0.08[b]
3	6.86±0.13[a]	167.55±3.00[a]	1.73±0.20[a]	7.37±0.10[b]	198.07±2.90[b]	2.30±0.17[b]
4	7.05±0.10[a]	180.90±1.83[a]	1.78±0.23[a]	7.81±0.09[b]	252.20±1.83[b]	3.05±0.19[b]

Mean ± S.D of three samples of one experimental set
[a,b] Different letters in a row indicates significant difference ($p<0.05$)

Table 4. Hexanal content of T-22 and EC sets of fried potato crisps during storage

Day	Hexanal content (ppm)	
	T-22	**EC set**
1	N.D	N.D
2	N.D	N.D
3	N.D	3.82±0.04
4	N.D	4.79±0.03
5	2.98±0.02[a]	6.33±0.04[b]
6	3.42±0.04[a]	8.96±0.05[b]
7*	4.66±0.03[a]	15.98±0.05[b]

Mean ± S.D of three samples of one experimental set
[a,b] Different letters in a row indicates significant difference ($p<0.05$)
7*: The study period was 5 days. However, to make correlation study with spoilage index, hexanal analysis was continued for 7 days in case of T-22 sets and EC sets.
N.D: Not detected as hexanal content was below LOD and LOQ

Table 5. Sensor screening by different classifier of SMLC

Classifier	Classifier type	Accuracy (%)	Prediction Speed (Objects/s)	Training Time (s)
Decision tree	Fine Tree	82.24	3900	6.44
	Medium Tree	83.33	4100	3.56
	Coarse Tree	79.00	3650	2.21
SVM	Linear SVM	93.90	4600	1.82
	Quadratic SVM	87.54	2400	2.65
	Cubic SVM	83.50	4600	3.16
	Fine Gaussian SVM	79.99	1900	5.18
	Medium Gaussian SVM	86.66	2000	3.19
	Coarse Gaussian SVM	85.20	1500	5.39
Ensemble classifiers	Boosted Trees	34.55	2400	2.76
	Bagged Trees	88.80	3700	4.89
	Subspace discriminate	84.50	3800	3.51
	Subspace KNN	62.11	2500	3.84
Naïve Bayes	Kernel	90.00	1800	3.17
Neural network	Narrow	82.39	2700	4.51
	Medium	83.00	3600	4.40
	Wide	86.50	3900	5.20

The best classification method was judged based on the highest prediction speed and highest accuracy.
Prediction speed: It involves checking the value of one predictor (variable) in unit time. It is the combination of computational speed and accuracy

Table 6. ‘‘Spoilage index’’ of T-22 and EC sets of fried potato crisps obtained during storage using Mahalanobis distance methodology.

Day	Spoilage index	
	T-22 set	**EC set**
1	0.58[a]	0.96[b]
2	0.73[a]	1.54[b]
3	1.27[a]	2.06[b]
4	1.56[a]	3.42[b]
5	1.76[a]	4.53[b]
6	2.11[a]	6.59[b]
7*	2.86[a]	8.32[b]
	TT-22 set	**TEC set**
1	1.79[a]	2.08[b]
2	2.05[a]	3.58[b]
3	2.29[a]	5.62[b]
4	2.59[a]	7.55[b]

[a-b] Different letters in a row indicates significant difference ($p<0.05$); **7*:** The study period was 5 days. However, to make correlation study with spoilage index, e-nose analysis was continued for 7 days in case of T-22 sets and EC sets.

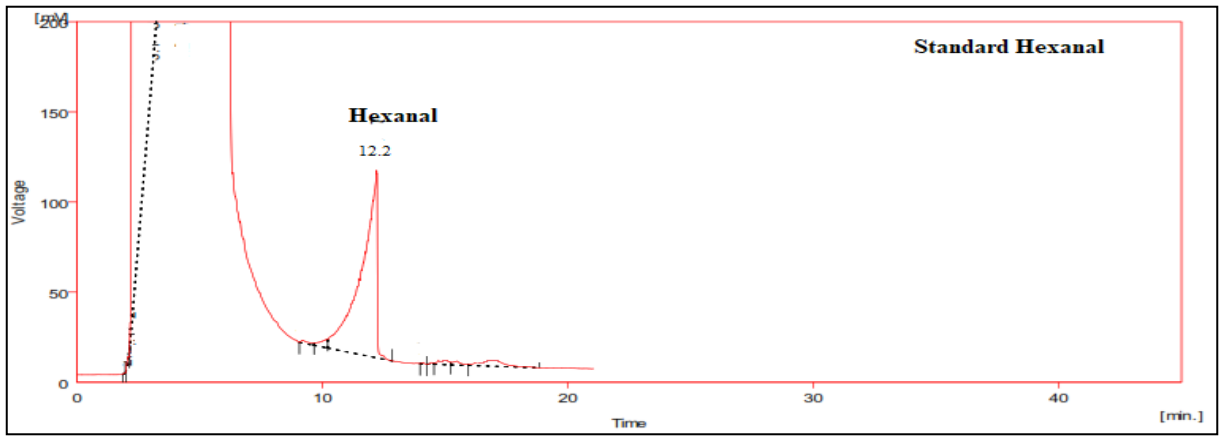


Figure 1. GC analyses of std. hexanal

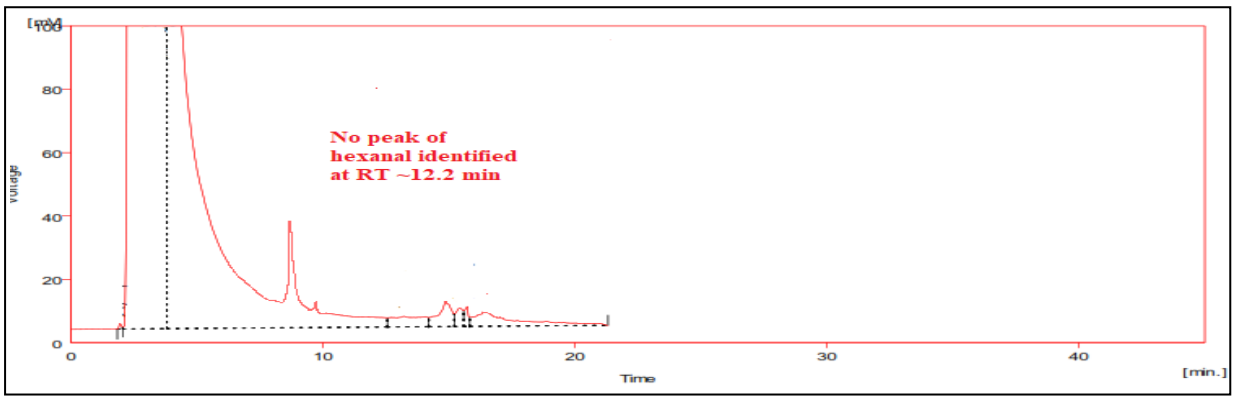


Figure 2a. GC analysis of hexanal of T-22 set on day 0

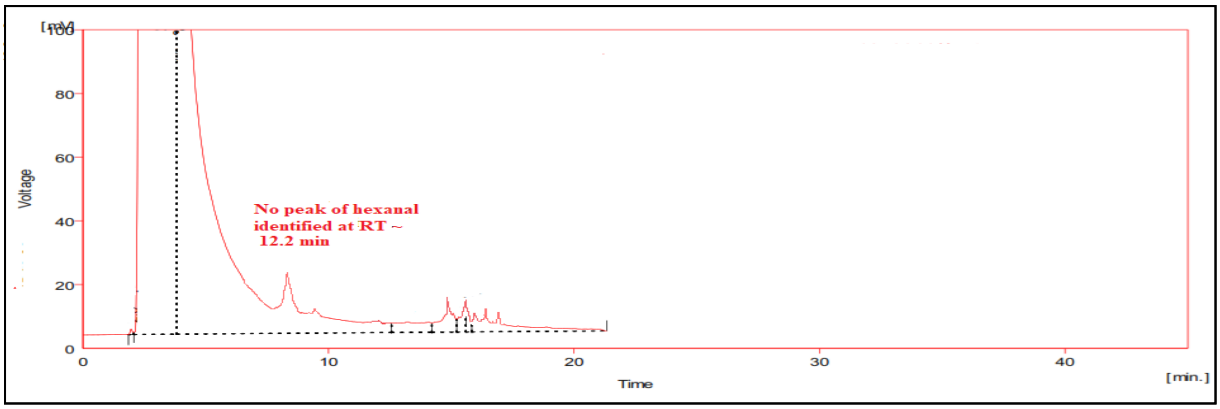


Figure 2b. GC analysis of hexanal of T-22 on day 4

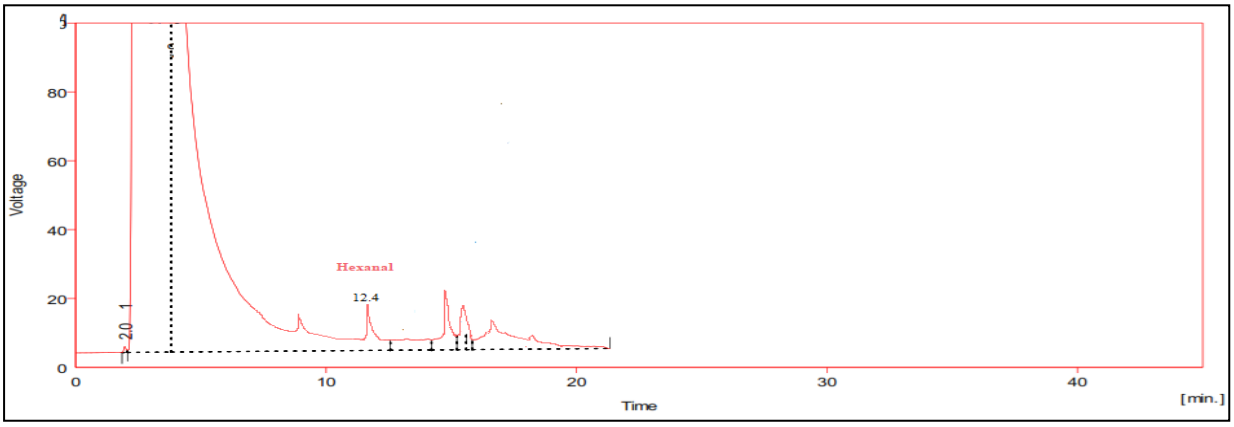


Figure 2c. GC analysis of hexanal of T-22 set on day 5

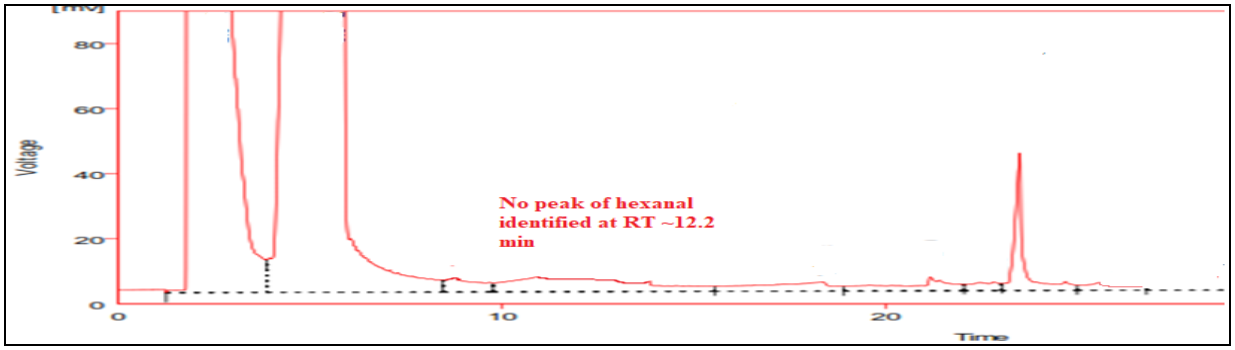


Figure 3a. GC analysis of hexanal of EC set on day 0

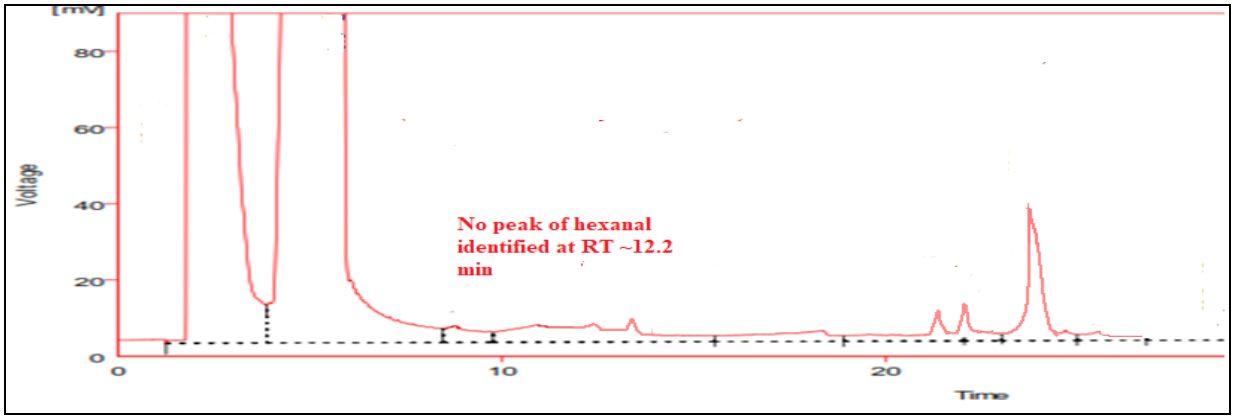


Figure 3b. GC analysis of hexanal of EC set on day 2

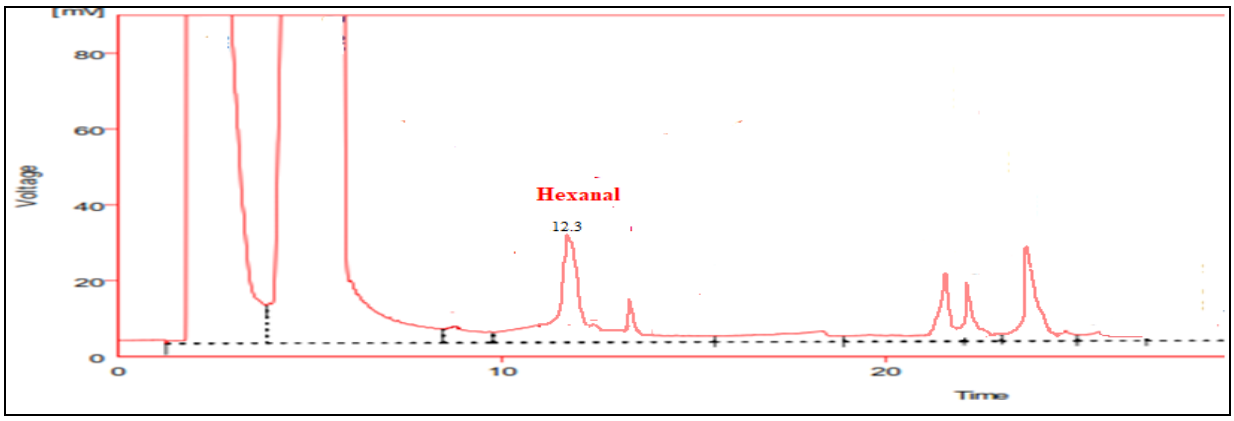


Figure 3c. GC analysis of hexanal of EC set on day 3

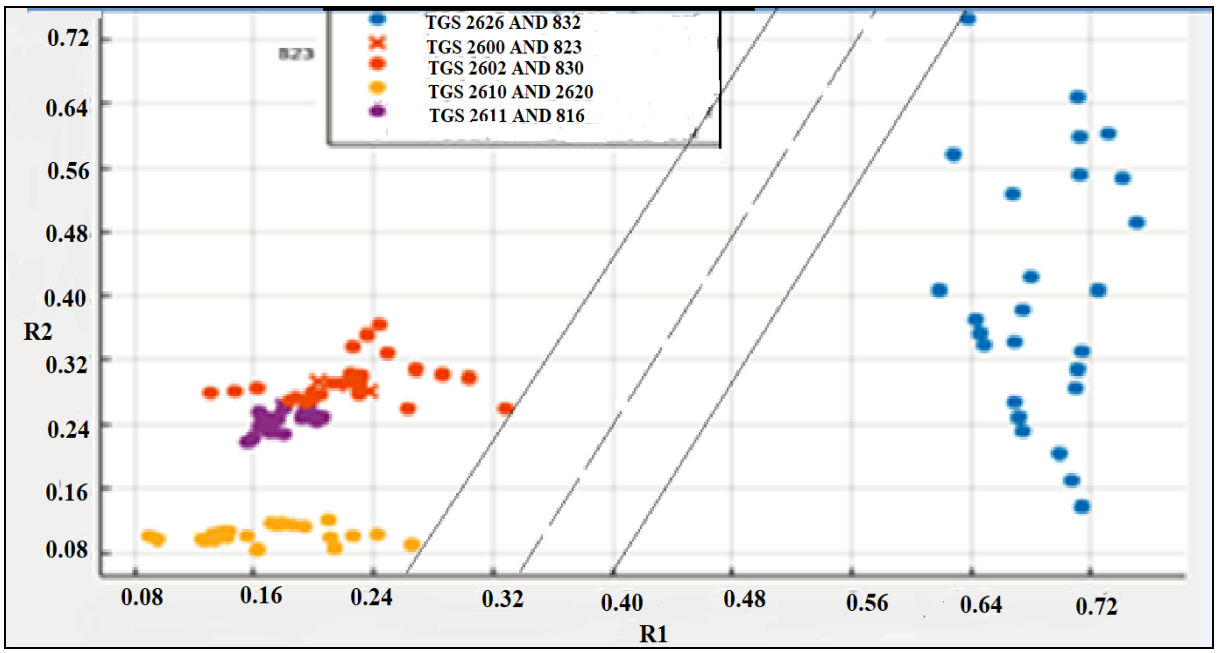


Figure 4. Storage analyses of samples performed with these screened sensors: TGS 2626 and TGS 832

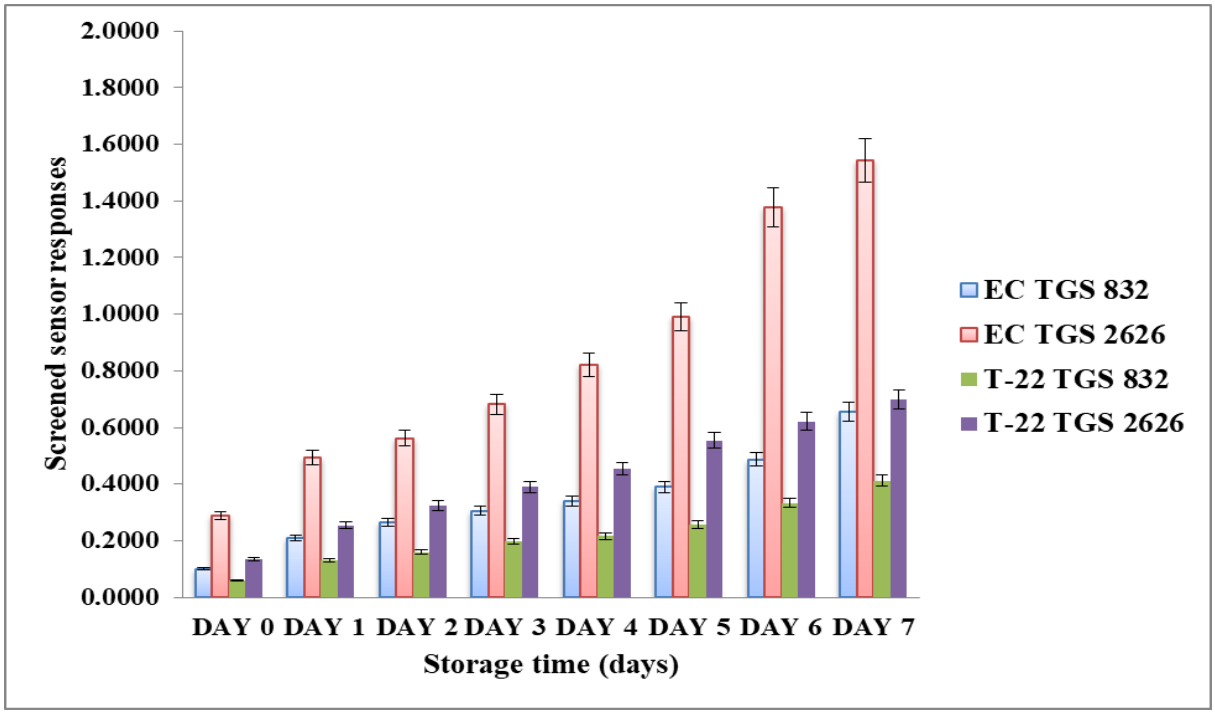


Figure 5. E-nose analyses of fried potato crisps with screened sensors during storage

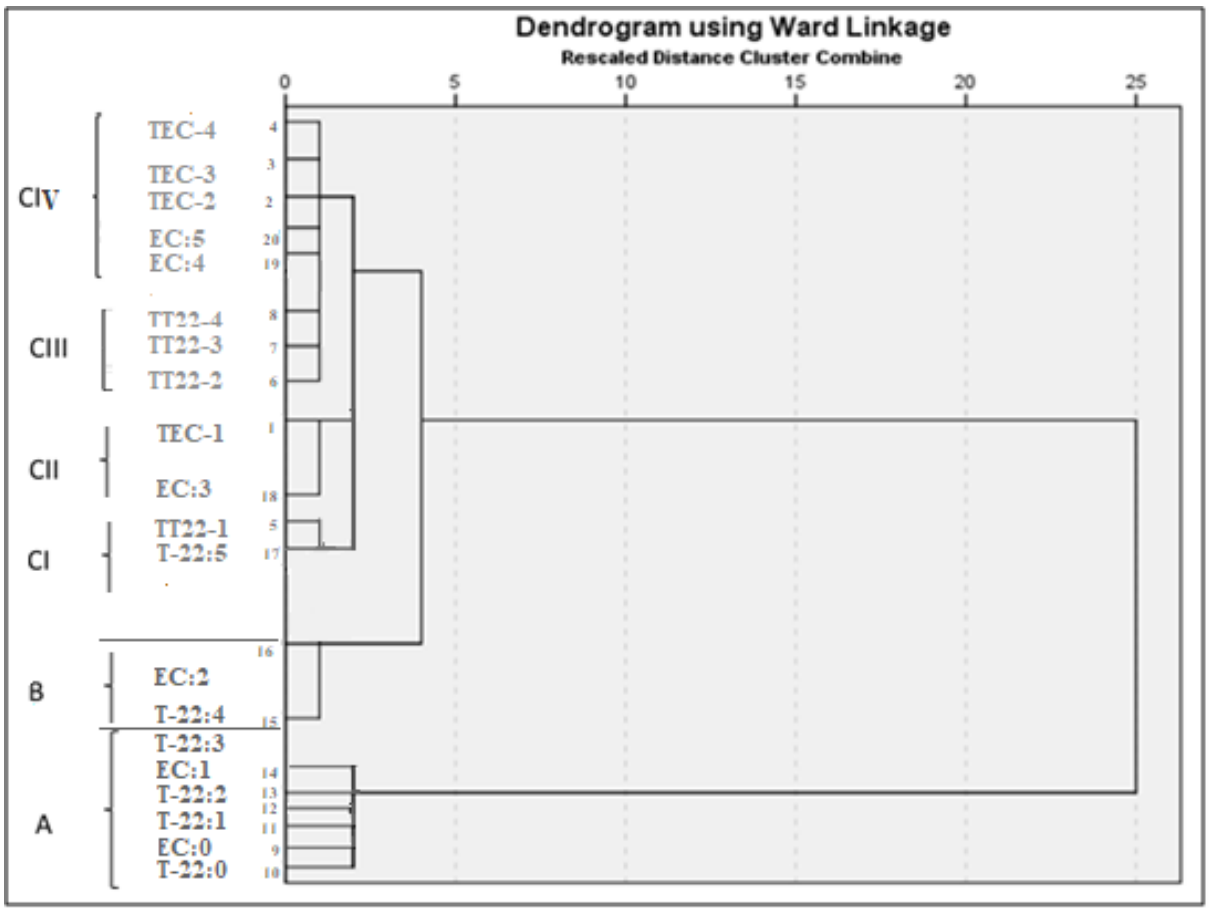
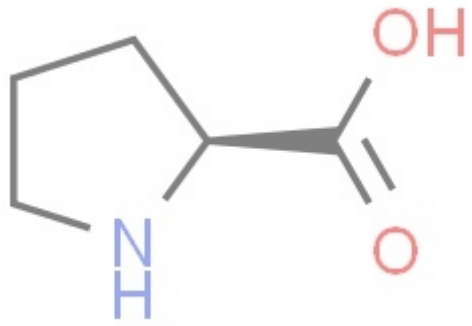
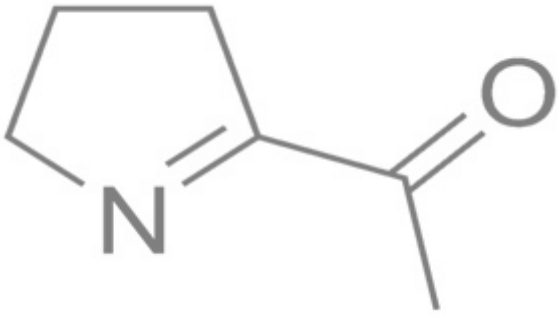


Figure 6. Hierarchical cluster analysis of T-22 and EC sets and their corresponding training sets based on screnned sensor responses towards VOCs (Dendogram) upto day 5





CHAPTER 5:

L-proline enrichment enhances KFO in bread: Assessment of freshness by electronic nose technology and BP-MLP

1. Introduction

Bread is the highest consumable bakery product worldwide. The flavor of bread depends on its ingredients and is mainly influenced by enzymatic reactions during dough fermentation and thermal reactions (Maillard reaction) during the baking process (Purlis, 2010). Generation of bread flavor and maintenance of its freshness is still a challenge faced by the bakery industries. The primary odorants that contribute to the flavor of wheat bread crust are 2-acetyl-1-pyrroline (2-AP), (E)-2-nonenal, 3-methylbutanal, 2,3-butanedione, methional, and (Z)-2-nonenal (Scieberle and Grosch, 1987; Cho and Peterson, 2010). Among them 2-AP has been designated to be the key food odorant (KFO) responsible for bread freshness (Scieberle and Grosch, 1987; Cho and Peterson, 2010; Dunkel et al., 2014). It imparts sweet "popcorn-like aroma" (Cho and Peterson, 2010). The two main precursors of 2-AP are- L-proline, an amino-acid which is naturally present in wheat flour in the range of 3.32-14.98 g/100 g of wheat flour protein (Siddiqi et al., 2012) and- reducing sugar, naturally present as 0.41 g/100 g of wheat flour (USDA Food Data Central, 2011). The instability of this compound causes a significant problem in commercial baking (Wei et al., 2017) since the same cannot be sensorially perceived 3-4 h post baking when stored at 23±2 ºC, 75-80% RH (Rychlik and Grosch, 1996). Therefore the challenge lies in retention of the freshness-aroma in bread until it reaches the consumer. Although considerable literature exist on isolation and identification of aroma and flavor compounds of bread, not much emphasis has been laid upon prolonged retention of the freshness-aroma to augment its marketability. In India and in other tropical countries, bread is especially prone to microbial (fungus) infestation, texture softening and loss of its freshness aroma in the hot-humid weather.

The main objective of the current investigation therefore was to develop a methodology for enhancement of freshness-aroma i.e., sweet "popcorn-like aroma" of 2-AP in bread by enriching the dough with its precursor molecule (viz. L-proline). The freshness aroma would be assessed by quantification of the KFO using GC-FID in tandem with fuzzy logic analysis of sensory scores. To circumvent the ambiguities of sensory assessment of the freshness aroma with the other quality attributes of bread (namely its crust color), i.e., to allow a holistic judgment of the sensory qualities of the bread, fuzzy logic analyses has been performed based on sensory data acquisition from panelists in linguistic forms. This endeavor would provide a rapid method for reliable and accurate assessment of freshness enhancement in bread.

Concomitantly, hurdle technologywould be appliedviz. combination of- addition of antimicrobial agents to the L-proline enriched bread dough, -choice of suitable flexible packaging laminate and -modified atmosphere placing (MAP) to prevent spoilage of bread and retain its freshness during storage. To augment rapid analysis of freshness during storage and for futuristic commercial applicationsinvolving large sample throughput in bakery industries, classical GC-FID analysis would be replaced with the state-of-the-art electronic nose (e-nose) technology. Freshness index obtained by e-nose data analysis would be correlated withthe 2-AP contentsand the regression models can be used for prediction of freshness status of bread, forging GC-FID analysis. Finally, ANN based deep learning tool *viz.* BP-MLP would be implemented to render the e-nose technology SMART (specific, measurable, achievable, realistic, time bound) to aid in automation of quality assurance of commercial bread.

2. Material and Methods

2.1. Materials

High gluten wheat flour having the following specifications: a. SV (sedimentation value), 28 ml; b. moisture, 13.5%; c. protein, 11.5%; d. water absorption power (WAP), 60% (M/s Shree Shyam Agro Pvt. Ltd., Asansol, West Bengal), skimmed milk powder (M/s Tasty Dairy Pvt. Ltd., Jainpur, India), sugar and salt (local super market of Jadavpur, Kolkata, India), instant dry yeast (*Saccharomyces cerevisiae*) (M/s Heilongjiang Juiding Yeast Co., Ltd., Heilongjiang, China), emulsifier (E 472e) (M/s Fine Organics Ltd., Mumbai, India), gluten powder (M/s Anhui International Trade Co. Ltd., Dehui Mansion, China), bread improver i.e., α-amylase (M/s Biocon Ltd., Bengaluru, India), acetic acid or vinegar (local super market of Jadavpur, Kolkata, India),); shortening i.e., hydrogenated vegetable oil (HVO i.e., hydrogenated palmolein oil of M/s Adani Wilmar Ltd., Haldia, India); butter (M/s Amul India Pvt. Ltd. Himatnagar, India)]; food grade L-proline (M/s Sha Narendra and Sons, George Town, Chennai, Tamil Nadu, India); and anti-microbial agents i.e., potassium sorbate and calcium propionate (M/s Titan Biotech Ltd., India) were the ingredients used in this study for bread formulation (Table 1). A commercial sample of bread was purchased from a local supermarket of Jadavpur, Kolkata, India for comparative analysis with the experimental samples. A food grade flexible packaging laminate (LDPE/MET-BOPP) was procured from M/s Fitrite Packers, Kanpur, India. Food grade L-proline was purchased from M/s ShaNarendra and Sons, George Town, Chennai, Tamil Nadu, India. Speciality chemicals

such as HPLC-grade acrylamide (~99% pure) and analytical reagent (AR)-grade L-proline were procured from M/s Merck, Mumbai, India, and HPLC-grade 2-AP (liquid) was purchased from M/s Clearsynth Labs Ltd., Mumbai, India. The ingredients used for bread preparation are enlisted in Table 1.

HPLC grade solvents namely *n*-hexane, acetonitrile, ethanol, deionized water and dichloromethane (DCM) for gas chromatography (ECD and FID SupraSolv®) were procured from M/s Merck, Mumbai, India. Plate count agar (for SPC analysis) and acidified potato dextrose agar (for yeast and mold count) were purchased from M/s HiMedia®, India. SPE-QuEChERS (Solid phase extraction-Quick, Easy, Cheap, Effective, Rugged, and Safe) was performed for extraction-cum-purification of acrylamide (AA) from bread samples using QuEChERS kit (p/n 5982-5850) of M/s Agilent Technologies, California, USA.

2.2. Preparation of bread

In the current investigation, bread-dough was prepared using straight dough method as reported by Cauvain and Stanley (2003). At first, instant dry yeast (3 g) was activated by dissolving in luke-warm water (38-40 °C) containing wheat flour and sugar in 1:1 ratio and then allowing it to stand undisturbed for 18 min. The dough for the experimental control sample of bread was prepared in a mixing bowl using all the dry ingredients mentioned in Table 1 except L-proline; along with anti-microbial agents such as potassium sorbate (0.1 %w/w), acetic acid (0.1% v/w) and calcium propionate (0.25 %w/w) on a d.w.b. Shortening (post melting), requisite amount of water and activated yeast slurry were added into the mixture which was then subjected to high speed mixing (Model no.: BLAM 1920 of M/s Bake Well Engineers) for 3 min. The dough (weight of dough ~ 120 g on w.w.b) was subjected to intermediate proofing (IP) at 28±2 °C, 75±2% RH for 10 min.Thereafter "moulding" of the dough was performed prior toplacing in mould of dimensions of 55×50×86.5 (L×B×H in mm) according to calculated weight of dough [= 0.4 g/cm^3 (bread density) × volume of mould (L×B×H in cm^3) = 0.4×238 = 95 g] as per IS 1483 (1988). Each weighed piece of dough was placed in mould and subjected to final proofing (FP) for 60 min at 40 °C, 95±2 %RH. Finally, baking was performed in a preheated rotary convection oven (Model no. CMH S108; M/s Chanmag Bakery Machine Co. Ltd.)in convection mode at 215 °C for 22 min. The experimental control set of bread was thus obtained.

For all test samples, *viz.* L-proline enriched bread (addition of varying concentrations of L-proline to the dough), the dough was prepared in a manner similar to that of the experimental set, except that L-proline additionally was added to the bread-dough while mixing the dry ingredients. After conducting several preliminary trials, concentration of L-proline (ranging from 0.6-1.4 % wt./dry wt. of flour) in bread-dough was varied at increments of 0.1 % wt./dry wt. of flour i.e., 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3% and 1.4% wt./dry wt. of flour; and the samples sets thus prepared were designated as E-1, E-2, E-3, E-4, E-5, E-6, E-7, E-8 and E-9, respectively. A commercial sample of bread purchased from a local store of Jadavpur area, Kolkata, India was used as the positive control (i.e., reference standard) for the work. In the current investigation, a laminate composed of low-density polyethylene (LDPE)/ metallized (MET) biaxially oriented polypropylene (BOPP) was used for packaging the test samples of bread for prolonged retention of their freshness (*vide infra*).

2.3. The experimental design for the entire study

The overall design of experiment (DOE) has been presented as a block diagram in Fig 1a and b. The following were performed with the test sample, experimental control sample and the positive control sample of bread: (1) extraction of 2-AP from bread samples by Likens-Nickerson's steam-distillation-cum-solvent extraction and its quantification by gas chromatography-flame ionization detector (GC-FID); (2) optimization of concentration of L-proline to be added to the bread-dough for maximum possible enhancement of freshness-aroma in bread (E-7) achievable in our laboratory conditions-based on defuzzified scores (*vide infra*) received by the baked bread samples and- their 2-AP contents obtained by GC-FID analysis; (3) the bread sample with enhanced freshness obtained in (2) was subjected to physical analyses to obtain its proximate composition; color and texture profiles; and its dough was analyzed for rheology; (4) e-nose analysis for assessment of degree of freshness and corroboration of the e-nose sensor responses with 2-AP contents obtained by GC-FID analysis; (5) evaluation of AA contents by reverse phase-high performance liquid chromatography (RP-HPLC) analysis; (6) shelf-life assessment of bread samples on the basis of retention of freshness in the same during storage; and microbiological (bacteria, yeast and mold); physical (texture and color profiles); chemical [namely, acidity of extracted fat (AEF), and GC-FID analysis of 2-AP]; sensory (followed by fuzzy logic analysis) and e-nose analyses of freshness odor (Figure 1b).

2.4. Quantification of 2-AP in Likens-Nickerson's steam-distillation-cum-solvent extract of bread volatiles by GC-FID (gas chromatography-flame ionization detector) analysis

2.4.1. Extraction of bread volatiles by Likens-Nickerson's steam-distillation-cum-solvent extraction method

Likens–Nickerson (L-N) concurrent steam-distillation-solvent extractionwas performed for extraction of 2-AP from bread crust of test, experimental control and positive control samples, following the method described by Bhattacharjee et al. (2005) with few modifications (provided in Appendix 2.E.1.

2.4.2. Analysis of aroma compounds (exclusively 2-AP) in extract by GC-FID

Trace GC 700 (Thermo Scientific, Waltham, MA, USA) equipped with a TR-1 capillary column (30 m× 0.32 mm, i.d. 0.25 μm) and flame ionization detector (FID) was used for analysis of 2-AP content in the bread samples (test, experimental control and positive control sets). The injector and detector temperatures were set at 250 °C and 260 °C, respectively. N_2 was used as the carrier gas at a flow rate of 1 ml min^{-1}. The oven temperature was programed as follows: 60 °C (2 min hold), 60-100 °C at 10 °C min^{-1}, then increased at 5 °C min^{-1} to 140 °C and finally held at 250 °C (10 min) at 20 °C min^{-1}. Extract dissolved in DCM was injected into GC column in split-less mode. A standard curve of 2-AP (1-5 mg/mL) was prepared by dissolving a pure standard of the same in HPLC grade DCM. Dilutions were made immediately before injection into GC-FID column. The variation of 2-AP contents among the bread samples have been depicted by the GC-FID chromatograms of the bread samples (*vide supra*) in Figure 2 and 3a-i. The LOD of 2-AP was determined to be 0.6 ng/g. Quantitative determination of 2-AP contents in the bread samples *viz.* in the extracts of test, experimental control and positive control (reference standard) sets was performed using the calibration graph.

2.5. Process of optimization of L-prolineconcentration in dough for freshness enhancement in bread

The addition of L-proline to the dry ingredients of the dough (section 2.2) prior to mixing was optimized on the basis of (a) amount of 2-AP formed during Maillard reaction (at the time of baking) in bread, obtained by GC-FID analysis and (b) sensory scores (*vide infra*) obtained from objective sensory evaluation of the test samples.

2.6. Sensory evaluation of the test samples

The process of sensory evaluation of test samples was performed in accordance with the method reported by Stone and Sidel (2012). The sensory evaluation of test samples was conductedin accordance with the procedure described in Chapter 4. Sensory attributes of both varieties of bread, including odor (freshness), color, texture, taste, after-tasteand overall acceptability were assessed using standard 9-point hedonic scale (Figure 4). However, the decision obtained from this objective sensory evaluation did not corroborate well with the concentrations of 2-AP in the bread samples obtained by GC-FID analysis, primarily because development of dark crust colorin the bread enriched with L-proline obscured sensorial perception of freshness odor (2-AP) in the same. To overcome this fuzziness in opinion, fuzzy logic analysis was performed wherein the hedonic rating was replaced with a linguistic scale.

2.7. Ranking of bread samples having different levels of freshness-aroma using fuzzy logic analysis

To conduct fuzzy logic analysis, subjective sensory evaluation of test samples was further performed with the same panel in accordance with the method described by Chakraborty et al. (2013). Panelists were asked to provide a tick (√) mark to an appropriate scale factor i.e., "not satisfactory," "fair," "medium," "good," and "excellent" for the quality attributes such as odor (freshness), color, texture, taste and after-taste, and also asked to rank the quality attributes of these samples by again giving a tick (√) mark to an appropriate scale factor, *viz*. "not at all important," "somewhat important," "important," "highly important," and "extremely important". Thedata (Table 3.1) thus obtained for each set was processed using fuzzy membership function to yield overall sensory scores for the same. Using centroid rule (based on triangular fuzzy number and fuzzy arithmetic operations), defuzzified scores (Table 3.2) for each set was calculated and ranking of samples E1-E9 was performed accordingly. Considering KFO (2-AP) content and defuzzified scores, E-7 was found to be the best.

2.8. Proximate composition analysis of E-7, experimental control and reference standard (positive control) bread samples

Proximate analyses of E-7, experimental control and positive control samples (Table 4) were conducted in accordance with AOAC (1990) methods which includes estimation of moisture (AOAC 930.15), protein (g/ 100 g d.w.) (AOAC 981.10), crude fat (AOAC 963.15), % ash (AOAC 900.02) crude fiber (AOAC 978.10), and % total carbohydrates (by difference) in

accordance with the methods described by AOAC (1990). The nitrogen conversion factor used for crude protein calculation was 5.70 (Mariotti et al., 2008). The findings of proximate analyses are expressed on a % d.w.b.

2.9. Physical characteristics of E-7, experimental control and positive control bread samples

The physical characteristics such as texture profile and color (Table 5) of optimized test, experimental control and positive control samples were conducted using the methods described by Makinde et al. (2014) and Chatterjee et al. (2014), respectively with few modifications.

To analyze texture profiles of E-7, experimental control and positive control bread samples (Table 6), two-bite compression test was conducted employing a TA.XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK), equipped with a cylinder shaped probe (Part code: P/36R). From the TPA graph, parameters such as hardness (g), adhesiveness (g.sec), springiness, cohesiveness, gumminess, chewiness and resilience of bread samples were ascertained with the following optimized TPA programming: pre-test speed: 1.00 $mm \cdot s^{-1}$, test speed: 5.0 $mm \cdot s^{-1}$, post-test speed: 5.0 $mm \cdot s^{-1}$, distance: 10 mm, time: 5.0 s, trigger force: 5.0 g and strain 40%. The bread sample sets of 35 mm thickness were subjected to texture profile analysis. The TPA graph (Figure 5a-c)was executed by software of Exponent Lite Express (version 6.1.4.0).

Color measurement of E-7, experimental control and positive control bread samples was carried out using the Minolta Chromameter (Model CR-300, Minolta Co., Ltd, Osaka, Japan) and reported as L*[L* = 0 (black), L* = 100 (white)], a*(−a* = greenness, +a* = redness) and b* (−b* = blueness, +b* = yellowness) values. The color co-ordinates of crumb and crust of E-7, experimental control and positive control bread samples were calibrated against a standard white plate. The browning (Ding and Ling, 2014) and whiteness indices (Joiner, 2010) of the above-mentioned bread samples for crust and crumb (Table 5) were determined according to equations (1) and (2), respectively. The color difference between experimental control and E-7 as well as positive control and E-7 sets of bread for crumb and crust were also evaluated employing color difference equation (ΔE*) as mentioned below (eq. 3). If ΔE* is more than one, it implies that color difference is perceived by naked human eyes and vice-versa.

$$\text{Browning indices (BI)} = \frac{100\ (x-0.31)}{0.17} \quad (1)$$

where x = $\frac{(a*+1.75L*)}{(5.645L*+a*-0.3012b*)}$

Whiteness indices (WI) =$100-[(100-L)^2+a^2+b^2]^{1/2}$ (2)

$\Delta E^* = [(L_c-L_e)^2 + (a_c-a_e)^2 + (b_c-b_e)^2]^{1/2}$ (3)

2.10. Determination of rheological-properties of dough

Rheological-properties of each set of dough [E-7 and experimental control] was measured employingextensograph [BrabenderExtensograph-E (Duisburg, Germany)] according to the AACC Method 54–21 (1995).The details of procedure was discussed in Appendix 2.E.2

2.11. Freshness (odor profile) analysis for E-7, experimental control and positive control sets of bread samples by e-nose technology

2.11.1. Optimum conditions of e-nose analysis

In our current investigation, e-nose system, ENOVISION Ver.1.Q (developed by M/s Centre for Development of Advanced Computing, Kolkata, India) equipped with eight metal oxide sensors i.e., MOSs (TGS 823, TGS 2620, TGS 816, TGS 830, TGS 2611, TGS 2610, TGS 832 and TGS 2600) was employed to assess the freshness of E-7, experimental control and positive control sets. After conducting several preliminary trials, the parameters such as sample size; time needed for saturation of headspace of sample vial by VOCs generated from sample; and sampling and purging time were optimized on the basis of the maximum response received from each sensor. The optimized parameters thus obtained were: sample size- 0.02 g of extracted VOCs; acquisition rate- 600 ppb; headspace generation time- 30 s; sampling time- 50 s; purging time-500 s.

Generally, there is no any sample preparation step prior to e-nose analysis. Usually ground samples are subjected to e-nose analysis. In this work, L-N extract bread samples was analysed by e-nose to avoid masking of sensors by undesirable odor notes of bread samples.

2.11.2. Screening of sensors of ENOVISION using SMLC and determination of sensor response on the basis of freshness-aroma

The sensors of ENOVISION are not specific to any particular VOC or KFO,therefore screening of sensors is required. First, sensors were trained by training-sets such as TB-1 (1.2 % L-proline added to the bread dough), TB-2 (.3% L-proline added to bread dough) and TB-3 (1.4% L-proline added to bread dough), followed by calibrationusing standard 2-AP. The

sensor responses towards the VOCs of training-sets (obtained by eq. 4) were fed to different models of supervised machine learning classifier (SMLC) such as decision tree, SVM (support vector machines), KNN (k-nearest neighbors), neural network, ensemble classifiers and naïve base (Table 7). Different classifiers or models of SMLC were run using 5-fold cross-validation method with distribution of data set in 80:20 (training data set : test data set) for evaluating model's performance on the basis of root mean square error (RMSE) values (Table 7). The optimum model was selected based on % accuracy and prediction speed of each classifier. Screened sensors classified with the help of the best fit model was depicted by parallel coordinates plot (Figure 7). In this test framework, e-nose system with selected sensors was employed to evaluate the degree of freshness of E-7, experimental control and positive control sets by computing sensor responses using eq. (5).

$$(|\Delta R|/R)_{2\text{-}AP} = |(R_{T.S} - R_{2\text{-}AP})|/R_{2\text{-}AP} \quad (4)$$

$$(|\Delta R|/R)_{2\text{-}AP} = |(R_{S} - R_{2\text{-}AP})|/R_{2\text{-}AP} \quad (5)$$

where, $R_{T.S}$ = resistance of sensor towards the VOCs of TB-1, TB-2, TB-3 and TB-4, R_{sample} = resistance of sensor towards the VOCs of bread samples on day 0, 1, 3, 5, 7, 9, 11, 13 and 15, $R_{2\text{-}AP}$ = resistance of sensor towards the VOCs of reference standard 2-AP at 0.05 ml (the said volume was thus chosen since concentration above this caused oversaturation of the sensors and rendered baseline correction impossible even after purging with fresh air several times).

2.12. Acrylamide (AA) extraction from E-7, experimental control and positive control sets using SPE-QuEChERS method and quantification by HPLC-method

The method for extraction of AA from E-7, experimental control and positive control bread samples (Figure 8a-c) was conducted usingSPE-QuEChERS kit (as explained in Chapter-3) and quantification of the same was performed by RP-HPLC analysis (explained in Chapter-3). The SPE-QuEChERS method involved two steps: a. extraction of AA from crushed bread sample using acetonitrile (ACN), followed by addition of NaCl and $MgSO_4$ for partitioning between the aqueous and organic layer b. clean up step by SPE sample-clean up tube containing primary secondary amine and C18 sorbent in order to remove interfering substances from AA.

2.13. Production cost analysis of E-7 and experimental control sets

Production cost is summation of direct and indirect costs. The principal cost would entail cost of ingredients needed for bread production. The analysis of production costs of experimental

control (Table 8) and E-7 (Table 9) sets were conducted to study the effect of the same by changes in formulation of bread.

2.14. Study for freshness-stability with time of bread samples

The freshness of bread is limited owing to the interplay of four factors: physiochemical changes in bread that are accentuated in the presence of oxygen, spoilage by aerobic microorganisms, the gas barrier properties of the packaging material and the environment (headspace) inside the bread package (Smith et al., 1990). Each of these factors alone or in combination results in loss of freshness-aroma and thus causes overall deterioration in bread quality.

2.14.1 Packaging of bread samples post manufacture: Characteristics of packaging materials used for packaging of bread samples

For packaging of bread samples, flexible laminate packaging was chosen over individual packaging owing to its better barrier properties to water vapor, oxygen and odor molecules (Robertson, 2009). Each bread loaf (approx. 95 g) from E-7 and experimental control sets was packaged in chemically inert 20 μ low-density polyethylene (LDPE)/25 μ metallized (MET- coated by aluminum) biaxial oriented polypropylene (BOPP) laminate under MAP conditions (using 30% CO_2 and 70% N_2 as filler gases) using gas flushing technique (M/s Inpak, Tamil Nadu, India; Model number IP-VT-1000). Thereafter, the package was heat sealed using heat sealer Delta Seal V2 (M/s Sevana Traders and Services Pvt. Ltd., Cochin, India). Flexibility and strength of laminate are characterized by its physical parameters such as GSM, breaking strength, bursting strength and tensile indices (Table 10). Packaged E-7 and experimental control samples were designated as E-7R and ECR and subjected to storage study.

2.14.2. Storage conditions

The packages were stored in an environmental chamber (23±2 °C, 75-80 %RH) along with a commercial sample for a total study period of 15 days. E-7R, ECR and commercial samples were withdrawn at an interval of 2 days (i.e., on days 0, 1, 3, 5, 7, 9, 11, 13 and 15) for microbiological, physical, acidity of extracted fat (AEF), 2-AP, fuzzy logic, and e-nose analyses.

2.14.3. Microbiological analyses of E-7R, ECR and positive control samples

The standard plate count (SPC) and total fungal count (TFC) of E-7R, ECR and positive control samples (Table 11) were estimated on days 0, 1, 3, 5, 7, 11, 13 and 15, as per standard methods described in IS5402:2012 and IS5403:1999.

2.14.4. Texture analysis of E-7R, ECR and positive control samples

The assessment of textural properties i.e., hardness (during storage period) of E-7R, ECR and positive control samples (Table 12) was performed by single compression test according to AACC standard 74-09 method. The optimized TA programming used in this study was: pre-test speed: 1.2 $mm \cdot s^{-1}$, test speed: 1.5 $mm \cdot s^{-1}$, post-test speed: 9.0 $mm \cdot s^{-1}$, distance: 5.0 mm, time: 9.0 s, trigger force: 5.0 g and strain 40%. The bread sample sets of 35 mm thickness were subjected to texture analysis. The TA graph was executed by software of Exponent Lite Express (version 6.1.4.0). Parameter such as hardness (g) was ascertained by this software during the storage period.

2.14.5. AEFanalysis of E-7R, ECR and positive control samples

AEF analysis(in terms of % oleic acid) of E-7R, ECR and positive control samples (Table 13) was conducted according to standard established protocols (IS 1011: 1992).

2.14.6. Fuzzy logic analyses of E-7R, ECR and positive control samples

Fuzzy logic analysis of E-7R, ECR and positive control samples were performed on the basis of quality attributes such as freshness-aroma (odor), texture, taste and after-taste in accordance with the procedure, described in section 2.7. The maximum storage time of E-7R, ECR and positive control samples was predictedin accordance to defuzzified score (Table 14) received by each set of samples on each day of storage.

2.14.7.2-AP analysis of E-7R, ECR and positive control samples

2-AP analysis of E-7R,ECR and positive control samples (Table 15) was carried out using GC-FID during storage to assess their freshness levels as described in section 2.4.2

2.14.8. Electronic nose (e-nose) analysis of E-7R, ECR and positive control samples using screened sensors

The e-nose with screened MOS was employed to predict the fresh-stability of E-7R, ECR and positive control samples (Figure 9) in the same process as described in sections 2.11.1 and 2.11.2.

2.14.9. Determination of freshness indices of E-7R, ECR and positive control samples

A scalar value was generated from the e-nose data of bread samples using Mahalanobis distance (Chatterjee et al., 2014) which is effectively a multivariate equivalent of the Euclidean distance (d). It measures the distance between a point and a distribution(Bedrick et al., 2000). This scalar value was named as freshness index (FI) for bread samples, which is proportional to the e-nose sensor signal responses. Following the method developed by Chatterjee et al. (2014), the data set (matrix) of e-nose sensor resistances i.e., N (800×2) obtained from screened sensors that responded well to the freshly prepared bread samples was considered as the baseline vector (applicable for both E-7R and CR sample sets). On each day of storage (i), the matrix M_i (800×2) was prepared from the sensor resistance data (concerning only the screened sensors) apposite to each set of bread samples. Following standard matrix operation, the Mahalanobis distance (d^2) was calculated by squaring the distance between the matrix M_i and matrix N. Greater distance from the baseline matrix indicated lesser freshness. The FI values were plotted against the changes in 2-AP contents (quantified by GC-FID and expressed in mg/100 g of bread) during storage to obtain a regression model which could directly predict the 2-AP contents in bread samples forgoing the need to perform GC-FID analysis.

2.14.10. Data analysis by back-propagation multilayer perceptron (BP-MLP) for large sample throughput

For prediction of freshness of bread samples in an industrial manufacture scale, the current investigation also endeavored designing a BP-MLP (a tool of ANN) model (codes in supplementary file, S1). This model encompassed sensor responses of all 8 sensors forgoing sensor screening so as to deliver an unbiased and robust model for practical applications. Moreover, the sensory scores of panellists were considered and not defuzzified values to obtain overall aroma scores (*vide infra*).

The BP-MLP model topology was designed as follows. The input layer in BP-MLP model was fed with data (of E-7R) generated from 8 sensors and training was accomplished by automatically adjusting the connection weights in order to reduce the error level. Therefore, this model had 1 input layer with 8 nodes (neurons). To fix the number of nodes in the output layer, overall aroma score (OAS) was obtained by arithmetic mean of the hedonic scores given by the panelists for 'odor' attribute in bread samples. The output layer was thus configured considering the variations in OAS (ranging from 9 to 4 i.e., 9,8,7,6,4) wherein the minimum OAS for acceptability of the product sensorially was pre-decided to be 6.0, in consonance with the findings reported by Muñoz et al. (1992). The output layer of the network was assigned five nodes. Optimization of the model topology was performed to assess number of neurons needed in the hidden layer. Similar 3-layer design of BP-MLP model for shelf-life analysis of banana samples, and for prediction of flavor intensities in different blackcurrant concentrates (variations on the basis of seasons, geographical origin, post-harvest storage and concentration methods used) have been reported by Sanaeifar et al. (2014) and Boccorh and Paterson (2002), respectively. Although characterization of aroma compounds in white bread by aroma-extraction-dilution analysis, aroma recombination and omission tests have been reported (Pu et al., 2019), applications of ANN in predicting the freshness-aroma intensity of bread and its changes with storage time have not explored earlier.

Training of network was performed by fixing the learning rate to 0.1. The data set i.e., 400 was divided into training and validation sets in 80:20 ratio. After performing several trial runs, the number of iterations for this ANN study was varied from 100-100000 epochs. Trial and error approach was pursued to decide the optimum topology on the basis of minimum root mean square error (RMSE) and the maximum R^2 (correlation coefficient) values obtained. The efficiency in freshness-stability prediction (in terms of OAS) by ANN model increased with the increase in proximity of R^2 to 1 and RMSE to 0. The number of neurons in the hidden layer was varied from 1 to 20. The single output for each hidden layer was produced using sigmoid transfer function. This ANN regression model was architectured using Matlab® R2021a.

2.15. Statistical analysis

The results were statistically analyzed using SPSS software (version 20) and consequent test of Duncan were realized at $p \leq 0.05$. In the current study, interrelationship among data sets,

acquired from sensor responses of different sets of bread samples (using the e-noses) were investigated by two dimensional PCA. Screening of sensors by SMLC was performed using Matlab® R2021a (Mathworks, Inc. Natick, MA, USA). Based on e-nose responses of screened sensors, Mahalanobis distance (Table 9) was computed and regression equations were developed accordingly to predict 2-AP content (freshness indicator) of both varieties of bread as a function of 'freshness indices'.

3. Results and Discussion

3.1. Optimization of L-proline concentration in bread dough

The concentration of 2-AP i.e., KFO in experimental control and positive control samples was 8.61 and 8.44 mg/100 g of bread, respectively. Significant ($p < 0.05$) enhancement of 2-AP content (12.90 - 65.79 mg/100 g of bread) of bread (Table 2) was evident by increasing the amount of L-proline (0.6% to 1.4%) in dough (Figure 2). Under our laboratory experimental conditions, the highest achievable concentration of 2-AP in bread was 65.78 mg/100 g of bread (E-7 set) when the bread-dough was enriched with 1.2% (wt./dry wt. of flour) of L-proline. A concentration greater than 1.2% did not contribute to significant enhancement in the 2-AP content of bread. This concentration of L-proline was thus considered to be the best for enhancing the KFO (2-AP) of bread.

3.2. Ranking of bread samples having different levels of freshness-aroma using fuzzy logic analysis

Enrichment of dough by L-proline significantly ($p < 0.05$) influenced the crust color of the bread and its after-taste by increasing browning index and by imparting mild bitterness (the latter being difficult to perceive sensorially). During evaluation, few panelists showed biasness on crust color and after-taste attributes of bread and thus their focus on these attributes dominated over their assessment of freshness-aroma. This inadvertently caused incorrect scoring of the test samples; more precisely, the 2-AP contents in the same analyzed by GC-FID were found not be in consonance with the overall hedonic scores given by the panelists. In view of above, sensory-based fuzzy logic analysis was applied. Defuzzified scores (Table 3.2.) of test samples i.e., E1-E9 were obtained using the equationsmentioned in Chapter-2. Each sample was assigned a defuzzified score on the basis of their sensory parameters (*vide supra*). From the results it was evident that E-7 received the highest

defuzzified score in the category 'good'. The remaining samples were ranked in decreasing order on the basis of their defuzzified scores (mentioned below):

E7 (73.80; good) > E8 (69.48; good) > E9 (67.11; good) > E6 (65.42; good) > E5 (63.76; good) > E4 (59.85; medium) > E3 (58.74; medium) > E1 (57.89; medium) > E2 (57.49; medium).

Based on the defuzzified score and content of 2-AP in bread samples (E1-E9), E-7 was considered to be the best formulated bread *(vide supra)* with respect to enhanced freshness and overall quality.

3.3. Proximate composition analysis of E-7, experimental control and positive control samples

The proximate composition (Table 4) of E-7, experimental control and positive control samples exhibited no significant ($p < 0.05$) changes with respect to moisture, crude-fat, ash and crude fiber (g/100 g dry wt. basis of bread) contents. However, protein and carbohydrate contents of E-7 exhibited significant ($p < 0.05$) changes compared to those of experimental control and positive control samples. The enhanced protein content in E-7was attributed to the free amino group contributed by L-proline(Nehete et al., 2013).

3.4. Physical characteristics of E-7, experimental control and positive control samples

3.4.1. Physical and rheological characteristics

The specific loaf volume of bread (Table 5) was found to be higher ($p < 0.05$) for E-7 (4.30 cm^3/g) by ~ 9 % vis-à-vis the experimental control (4.08 cm^3/g) and positive control (4.11 cm^3/g) samples. Consequently, hardness and gumminess attributes of E-7 were lower ($p < 0.05$) vis-à-vis experimental control and positive control samples; whereas, springiness and resilience were higher ($p < 0.05$) for the same (Table 6). The analysis also revealed that physical characteristics of experimental control and positive control samples were similar ($p > 0.05$).

The extensograph exhibited higher ($p < 0.05$) R_m values (650 BU) and lower ($p < 0.05$) E values (160 mm) for E-7 bread-dough vis-à-vis the dough of the control set (R_m: 580 BU; E: 175 mm) which signifies relatively better oven-spring of the former during baking. This increase in elasticity of bread-dough (E-7) and higher ($p < 0.05$) specific loaf volume of E-7 are possibly due to addition of L-proline which is reportedly known to aid in formation of

extensive intermolecular and intramolecular H-bonding during dough mixing leading to formation of conformational bends (Nelson and Cocx, 2012).

3.4.2. Color analysis

The brown crust color (Table 5) of E-7 was well explained by its higher ($p < 0.05$) BI values ($p < 0.05$) compared to the experimental control and positive control sets. This finding is attributed to the formation of significant amount of melanoidin (an indicator of the browning intensity) in bread crust owing to addition of L-proline (Shen et al., 2018). The above findings were in consonance with the higher ($p < 0.05$) ΔE^* values for crust of E-7 i.e., 2.54 (>>1) with respect to experimental control and 2.37 (>>1) with respect to positive control, implying significant ($p < 0.05$) color variation of crust of E-7 vis-à-vis the experimental control and positive control sets. Addition of L-proline however, barely influenced the color (Table 4) of crumb since the WI values of crumb of either bread sets showed no significant difference ($p < 0.05$). Moreover, ΔE^* value of the crumb of E-7 (Figure 6a) with respect to experimental control (Figure 6b) and positive control samples were found to be 0.06 (<1) and 0.08 (<1), respectively, signifying that color difference between them could not be perceived.

3.5. E-nose analysis of E-7, experimental control and positive control samples

3.5.1. Sensor selectivity by means of support vector machines (SVM)

The best fit model was found to be SVM-Fine Gaussian with an accuracy of 93.90% (Table 7). In PCP (Figure 7) TGS 832 and 823 formed a single cluster of distinct lines, implying high sensitivity of these sensors in assessment of freshness (in terms of 2-AP) of bread.

3.5.2. Assessment of degree of freshness in E-7, experimental control and positive control samples *by e-nose analysis*

Electronic nose analysis of E-7, experimental control and positive control sampleswere carried out based on $(|\Delta R|/R)_{2\text{-}AP}$ values of two selective sensors i.e., TGS 832 and TGS 823. The results of screened sensor responses revealed that degree of freshness of E-7 set was significantly ($p > 0.05$) high vis-à-vis experimental control and positive control samplesand thus corroborated the findings of GC-FID analysis.

3.6. Acrylamide content analysis of E-7,experimental control and positive control samples

There was around 38% and 41% mitigation of acrylamide (Figure 8a-c) content (170 µg/kg of bread) in E-7 compared to the experimental control (272 µg/kg of bread) and positive control (288 µg/kg of bread) samples, respectively. This could be attributed to the function of L-proline which during baking promotes alkylation reaction through nucleophilicity of its secondary amine group and forms Michael adduct with acrylamide via alkylation of the same (Koutsidis et al., 2009), consequently reducing the availability of free acrylamide in E-7.

3.7. Production cost analysis of control and E-7

The production cost of E-7 (Table 9) of bread was found to be higher ($p < 0.05$) by Rs. 4/ 100 g of loaf compared to that of control (Table 8), implying a minor variation. However, this minor variation in production cost would be balanced by market retention and high sale volume.

3.8. Storage analysesof E-7R, ECR and positive control samples

3.8.1. Microbiological analyses

The analyses of SPC and yeast and mold counts during the entire storage showed no significant differences ($p > 0.05$) between E-7R and ECR vis-à-vis positive control samples (Table S4). According to WHO guidelines (1994), the acceptable limit of SPC and TFC were $\leq 2.0\times10^5$cfu/g and $\leq 1.0\times10^5$cfu/g, respectively. The SPC and TFC values (Table 11) of both sets (E-7R and ECR)and positive control exceeded their respective aforementioned limits after day 13 and 3, respectively. Therefore, both sets (E-7R and ECR) and positive control samples can be stored up to 13 and 3 days (based on the results of SPC and TFC) respectively from date of preparation.

3.8.2. 2-AP, AEF and texture analyses of bread samples

The contents of 2-AP (Table 15) in E-7R, ECR and positive control samples decreased with storage. 2-AP content of E-7R on day 13 (4.82 mg/100 g of loaf) was found to be similar ($p > 0.05$) to that of ECR on day 3 (4.78 mg/100 g of loaf) and positive control samples on day 1 (4.70 mg/100 g of loaf). Additionally, hardness of E-7R, ECR and positive control samples (Table S6) increased with time. It was evident from Table 12 that TA values of E-7R was found to be consistently lower ($p < 0.05$) vis-à-vis the ECR and positive control samples,

owing to higher ($p < 0.05$) specific volume of E-7R consequent to addition of L-proline (mentioned in section 3.4.1).

AEF of E-7R and ECR were found to be lower ($p = 0.0023$) than their respective FSSAI limits (1.5% as oleic acid) up to day 13; whereas the same of positive control samples remained within the FSSAI limit up to day 3 (Table 13).

In view of the above analyses, it is evident that freshness stability of E-7R was extended up to day 13 vis-à-vis the ECR and positive control samples.

3.8.3. Fuzzy logic analysis

The maximum storage time of E-7R, ECR and positive control samples were assessed (on the basis of overall quality)using the defuzzified scores (Table 14). The defuzzified score of E-7R was under the category 'good' up to day 11 and shifted to category 'medium' and 'not significant' on day 13 and 15,respectively. ECR samples were 'good' only up to day 1 and shifted to the category 'medium' on day 3. Thereafter, it was under the category 'fair' up to day 13 and 'not significant' on day 15; whereas, positive control sample was under the category 'medium' on day 1 and subsequently, followed the category 'fair' and 'not significant' on day 3 and 5, respectively. The loss of freshness aroma with increase in hardness was primarily responsible for the above findings. Thus, by using fuzzy modeling it was evident that E-7R was acceptable for 13 days, a lead of 10 days and 12 days, vis-à-vis the ECR and positive control samples, respectively.

3.8.4. E-nose analyses

Electronic nose analysis of E-7R, ECR and positive control samples (Figure 9) were carried out based on $(|\Delta R|/R)_{2\text{-}AP}$ values of two selective sensors i.e., TGS 832 and TGS 823. The PCA plot (Fig. 10) thus obtained classified the samples (E-7R, ECR and positive control) during storage (based on their freshness aroma) by clustering their e-nose sensor responses (to odor profiles i.e., VOCs generated by the samples) in different positions inside four different quadrants. The discrimination index value of the plot was 99.85%, indicating the clusters to be distinctly separated. E-7R with storage period of 0-3 days (E0, E1, E3) and 5-7 days (E5, E7) formed two different clusters in quadrants IV and I, respectively, owing to its higher degree of freshness vis-à-vis ECR and the reference standard sets on the same day of storage. E7R with storage period of 9-13 days (E9, E11 and E13), ECR with storage period of 0-3 days (C0, C1, C3) and positive control with storage day 1(PC1)formed a single cluster in

quadrant II which was separated completely from quadrant III comprising of a well-defined cluster of ECR with storage period of 5-15 days (C5, C7, C9, C11, C15) and the positive control with storage period of 3 days(PC3). The odor profile of positive control sets with storage period of 5-15 days (PC5, PC 7, PC 9, PC 11, PC 13 and PC 15) formed a separate cluster in the same quadrant (III), but was distinctly separated from the cluster comprising of C5-C15 and PC3. The odor profile of E-7R with storage period of 15 days (E 15) was also present in quadrant II; however,it was distinctly separated from the cluster comprising of E9 - E13, C0-C3 andPC1.

Thus, e-nose analyses in couple with PCA established that degree of freshness of E-7R was significantly higher ($p < 0.05$) and its freshness-stability remained for a long period of time i.e., up to day 13 vis-à-vis other two sets. This approach could successfully provide a 'SMART' (specific measurable achievable realistic and time bound), stable, robust methodology for prediction of degree of freshness in any bread samples.

3.9. FI for bread samples

FI of E-7R, ECR and positive control samples (Table 16) was evaluated by Mahalanobis distance from their corresponding samples on day 0. It was found that the freshness index for E-7R with storage of 13 days (2.15) is close to corresponding value for 3-day stored ECR (2.23) and 1-day stored positive control sample (2.26). This finding is in well agreement to that depicted by the PCA plot which distinctly separated the E-7R having higher degree of freshness from ECR and positive control sets.

From the above-mentioned analyses, the freshness-stability i.e., sweet freshness "popcorn-like aroma" of E-7R was retained up to day 13 (from the date of preparation) under the above-mentioned storage conditions, i.e., freshness stability of E-7R had a lead of 10 and 12 days with respect to ECR and the positive control samples, respectively.

3.10. Correlation of chemical and freshness parameters of bread with FI

Polynomial regression equations (eq. 6) were generated which represent 2-AP contents of breadas a function of FI.

$$\text{2-AP (mg/100g)} = 26.469\, e^{-0.573(2-FI)} \qquad (6)$$

The regression coefficient of eq. (6) is 0.90 and its F value is 61.05, indicating it to be a good fit model. This equation can be successfully employed for evaluation of freshness of

industrial- scale bread samplesw.r.t their 2-AP contents (by evaluating their FI values using e-nose) forgoing GC-FID analysis.

3.11. BP-MLPs analysis

The number of nodes in hidden layer was selected according to ANN performance. Training of network was executed at 10,000 epochs (Figure 11). The configuration for the best topology (Figure 12) included one hidden layers with 15 neurons with RMSE as 0.0857 and R^2 as 0.9782 (Table 17) to predict the shelf-life (in terms of OAS, mentioned in section 2.14.) of E-7. Therefore, optimum ANN topology was 8-15-5 which could correctly assess the freshness of bread samples. The weight matrix for the hidden layer was 15×8 and for the output layer was 5×15. The performance of the ANN model for the training and validation data sets is graphically represented in Figure 13a and 13b, respectively. The R^2 values of the ANN model were 0.9801 for the training set and 0.9765 for the validation set. It is evident from these results that the training procedure for shelf-life prediction was very successful. The percentage of accuracy for shelf-life prediction of known and unknown set of samples with this BP-MLP model was 91% and 89%, respectively with reference to the OAS. Therefore, the BP-MLP model developed based on responses received from the e-nose technology, could accurately predict the freshness-stability of E-7R to be 13 days. This three-layered model is particularly sensitive towards the prediction of onset of spoilage i.e. loss of freshness in bread which provides accuracy in estimation of shelf-life for any unknown bread sample. To conclude, the benefits this BP-MLP model can provide to the bread industry are: (a) a rapid and reliable method for prediction of freshness status of bread minimizing huge analyses tasks of chemists, (b) elimination of uncertainties associated with conventional analyses since use of e-nose technology can forgo chemical and chromatographic analyses. Thus, this model affirms marked improvements in quality control and assurance of bread.

3.12. Importance of hurdle technology on freshness-stability of bread

The hurdle technology implemented in this study improved the freshness-stability (considering bioload of bacteria and fungus) of E-7 by ~4 fold vis-à-vis the positive control sample having 3-day shelf-life (w.r.t microbiological aspect) from the date of manufacturing. Use of combination treatments- addition of chemical preservatives such as acetic acid (0.10 % w/v), calcium propionate (0.25% w/w) and potassium sorbate (0.10% w/w) coupled with

MAP (CO_2:N_2:: 30%:70%) packaging in LDPE/MET-BOPP laminate (with low OTR and WVTR values) prolonged the shelf-life of E-7 by retaining it's freshness and prevented microbial growth in the same up to day 13 from the date of preparation.

4. Conclusion

The freshness-aroma i.e., "popcorn-like aroma" of bread was enhanced ~8 fold by enriching the dough with the precursor molecule of its KFO (2-AP) i.e., L-proline. Around 38% and 41% mitigation of AA content occurred in E-7 compared to the experimental control and positive control samples, respectively. For prolonged retention of the freshness aroma, hurdle technology was applied *viz*. combination of- addition of antimicrobial agents to the L-proline enriched bread-dough, and use of MAP in LDPE/MET-BOPP laminate packaged bread. The freshness-stability of E-7 was up to 13 days, with a lead of 12 days and 10 days, vis-à-vis the experimental control and a commercial sample, respectively, quantitated by freshness indices obtained from e-nose data using Mahalanobis distance. Correlation of 2-AP contents with the said indices yielded robust regression models which could accurately predict freshness status of bread, forging GC-FID analysis. At the industrial scale for high sample throughput, a 'SMART' model was developed employing BP-MLP coupled with e-nose technology for rapid-cum-accurate detection of freshness status of bread forgoing biochemical analysis in quality control laboratories.

Novelty:

The present work is novel because it reports for the first time on:

- a methodology for enhancement of freshness aroma i.e., sweet "popcorn-like aroma" (KFO) in bread
- a strategy for prolonged retention of the said aroma using hurdle technology
- 'freshness index' for quantification of the KFO in bread forgoing futuristic GC analysis
- a 'SMART' methodology involving BP-MLP coupled to e-nose technology which can successfully predict freshness status of bread rapidly and accurately when industrial-scale large sample throughput is concerned

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Table 1. Formulation of experimental control and test sets

Raw material	Quantity (g)/100 g	
	Experimental control set	**Test set**
Flour	100	100
Sugar	8.0	8.0
Salt	2.25	2.25
Instant dry yeast	3.00	3.00
Shortening	16.00	16.00
Emulsifier (E 472e)	3.00	3.00
L-proline	**-**	*****
Skimmed milk powder	5	5
Gluten powder	0.14	0.14
Bread improver	1.00	1.00
Potassium sorbate	0.10	0.1
Ca-propionate	0.25	0.25
Acetic acid (v/w) (ml)	0.1	0.1
Water (ml)	As per requirement	As per requirement

N.B.: *The quantity of L-proline added is not mentioned in this table since it was subjected to optimization

Table 2. 2-AP analysis at different concentration of L-proline amino acid by GC-FID

Experiment no.	Added L-Proline quantity (% w/dry wt. of dough)	2-AP content in 100 g of bread (mg/100 g of bread)
E-1	0.6	12.90 ± 0.05^{a}
E-2	0.7	18.90 ± 0.06^{b}
E-3	0.8	21.40 ± 0.09^{ab}
E-4	0.9	33.84 ± 0.07^{c}
E-5	1.0	40.97 ± 0.08^{cd}
E-6	1.1	64.90 ± 1.02^{d}
E-7	1.2	$65.78\pm.0.09^{e}$
E-8	1.3	65.79 ± 1.00^{e}
E-9	1.4	65.79 ± 1.02^{e}

Mean ± S.D of three samples of each set

Table 3.1: Scale factor and relative importance of different attributes of test samples

Attribute	Scale factor					Relative importance				
Color	**N.S**	**Fair**	**Medium**	**Good**	**Excellent**	**Not at all imp.**	**Somewhat imp.**	**Imp.**	**Highly imp.**	**Extre - melyi mp.**
E1	0	0	8	22	0	0	0	0	30	0
E2	0	0	10	20	0	0	0	0	30	0
E3	0	0	10	20	0	0	0	0	30	0
E4	0	0	11	19	0	0	0	0	30	0
E5	0	0	12	18	0	0	0	0	30	0
E6	0	0	12	18	0	0	0	0	30	0
E7	0	0	13	17	0	0	0	0	30	0
E8	0	10	20	0	0	0	0	0	30	0
E9	0	21	9	0	0	0	0	0	30	0
Odor										
E1	0	0	13	17	0	0	0	0	0	30
E2	0	0	15	15	0	0	0	0	0	30
E3	0	0	12	18	0	0	0	0	0	30
E4	0	0	9	21	0	0	0	0	0	30
E5	0	0	5	25	0	0	0	0	0	30
E6	0	0	2	28	0	0	0	0	0	30
E7	0	0	0	0	30	0	0	0	0	30
E8	0	0	0	0	30	0	0	0	0	30
E9	0	0	0	0	30	0	0	0	0	30
Texture										
E1	0	8	22	0	0	0	0	0	30	0
E2	0	6	24	0	0	0	0	0	30	0
E3	0	3	27	0	0	0	0	0	30	0
E4	0	1	29	0	0	0	0	0	30	0
E5	0	0	14	16	0	0	0	0	30	0
E6	0	0	8	22	0	0	0	0	30	0
E7	0	0	0	10	20	0	0	0	30	0
E8	0	0	0	10	20	0	6	0	30	0
E9	0	0	0	10	20	0	0	0	30	0
Taste										
E1	0	0	10	20	0	0	30	0	0	0
E2	0	0	8	22	0	0	30	0	0	0
E3	0	0	11	19	0	0	30	0	0	0
E4	0	0	9	21	0	0	30	0	0	0
E5	0	0	10	20	0	0	30	0	0	0
E6	0	0	10	20	0	0	30	0	0	0
E7	0	0	13	17	0	0	30	0	0	0
E8	0	0	20	10	0	0	30	0	0	0
E9	0	0	25	5	0	0	30	0	0	0
After taste										
E1	0	0	7	23	0	0	0	30	0	0
E2	0	0	8	22	0	0	0	30	0	0
E3	0	0	11	19	0	0	0	30	0	0
E4	0	0	12	18	0	0	0	30	0	0
E5	0	0	12	18	0	0	0	30	0	0
E6	0	0	13	17	0	0	0	30	0	0
E7	0	0	13	17	0	0	0	30	0	0
E8	0	0	21	9	0	0	0	30	0	0
E9	0	0	26	4	0	0	0	30	0	0

Table 3.2. Defuzzified scores of nine different freshness enhanced bread samples (test samples) obtained from fuzzy logic analysis

Sample	Overall sensory score (SO)			(a+c)	Defuzzified scores
	a	b	c		
E1	59.85	47.05	41.21	101.06	57.89
E2	59.39	47.05	41.36	100.76	57.49
E3	60.76	47.27	41.36	102.12	58.74
E4	62.05	47.73	41.59	103.64	59.85
E5	66.82	49.17	42.73	109.55	63.76
E6	69.09	49.85	43.18	112.27	65.42
E7	84.70	54.09	31.36	116.06	73.80
E8	78.27	52.68	30.06	108.33	69.48
E9	75.15	50.30	27.58	102.73	67.11

Table 4. Proximate composition of experimental control, positive control and E-7 sets of bread

Nutritional parameters	Proximate composition (g)/100 g		
	Experimental control set	Positive control set	E-7 set
Moisture	44.01 ± 0.06^{a}	44.11 ± 0.06^{b}	43.97 ± 0.05^{a}
Crude-fat	4.11 ± 0.30^{a}	4.07 ± 0.30^{a}	4.14 ± 0.07^{a}
Protein	13.02 ± 0.40^{a}	12.95 ± 0.40^{a}	13.32 ± 0.09^{b}
Ash	1.69 ± 0.05^{a}	1.74 ± 0.05^{a}	1.73 ± 0.04^{a}
Fibre	1.77 ± 0.27^{a}	1.73 ± 0.27^{a}	1.75 ± 0.05^{a}
Total Carbohydrate (by diff.)	35.40 ± 0.30^{a}	35.40 ± 0.30^{a}	35.09 ± 0.15^{b}

Mean ± S.D of three samples of each set
Mean in the same row with different superscript are significantly different ($p < 0.05$)

Table 5. Physicochemical characteristics of experimental control, positive control and E-7 sets of bread

Color attributes	Crumb values			Crust values		
	Experimental control set	**E-7 set**	**Positive control set**	**Experimental control set**	**E-7 set**	**Positive control set**
L*	77.28±1.60	77.23±1.55	77.32±1.60	49.08±1.00	47.66±1.05	49.11±1.05
a*	0.13±0.005	0.16±0.003	0.11±0.005	14.43±0.04	15.53±0.05	14.40±0.05
b*	12.03±0.05	12.05±0.03	12.01±0.05	8.3±0.10	6.5±0.10	8.0±0.10
WI_{crumb}/BI_{crust}	74.29±1.60[a]	74.24±1.50[a]	74.34±1.55[a]	21.84±0.70[a]	23.57±0.60[b]	21.73±0.60[a]
ΔE*	0.06	0.08		2.54	2.37	

Physical characteristics	**Experimental control set**	**Positive control set**	**E-7 set**
Oven spring%	400[a]	404[a]	420[b]
Loaf weight (g)	100±1.50[a]	99.5±1.50[a]	98.8±1.40[b]
Loaf volume (cm^3)	408±10.50[a]	409±10.50[a]	425±11.30[b]
Specific loaf volume (cm^3/g)	4.08±0.03[a]	4.11±0.03[a]	4.30±0.02[b]

Values in the same row with different superscript are significantly different ($p < 0.05$)
Mean ± S.D of three samples of each set

Table 6.Texture profile analyses (TPA) of experimental control, positive control and E-7 sets of bread

Textural attributes	**TPA**		
	Experimental control set (day 0)	**Positive control set (day 1)**	**E-7 set (day 0)**
Hardness	202.581±1.06[a]	205.173±1.16[a]	176.250±1.05[b]
Adhesiveness	-1.250±0.30[a]	-0.885±0.30[a]	-0.404±0.007[b]
Springiness	0.545±0.007[a]	0.588±0.007[a]	0.952±0.009[b]
Cohesiveness	0.428±0.006[a]	0.482±0.006[a]	0.819±0.004[b]
Gumminess	186.667±3.20[a]	176.432±3.20[a]	144.364±3.05[b]
Resilience	0.218±0.006[a]	0.321±0.006[a]	0.404±0.005[b]

Values in the same row with different superscript are significantly different ($p < 0.05$)

Table 7. Classification results for each classifier without using the PCA

Classifier	**Classifier type**	**Accuracy (%)**	**Prediction Speed (Objects/s)**	**Training Time (s)**
Decision tree	Fine Tree	82.24	3900	6.44
	Medium Tree	83.33	4100	3.56
	Coarse Tree	79.00	3650	2.21
SVM	Linear SVM	79.99	1900	4.46
	Quadratic SVM	87.54	2400	2.65
	Cubic SVM	83.50	4600	3.16
	Fine Gaussian SVM	**93.90**	**4600**	**1.82**
	Medium Gaussian SVM	86.66	2000	3.19
	Coarse Gaussian SVM	85.20	1500	5.39
Ensemble classifiers	Boosted Trees	34.55	2400	2.76
	Bagged Trees	88.80	3700	4.89
	Subspace discriminet	84.50	3800	3.51
	Subspace KNN	62.11	2500	3.84
Naïve bays	Gaussian	90.00	1800	3.17
	Kernel	82.39	2700	4.51
Discrimant	Linear	83.00	3600	4.40
	Quadratic	86.50	3900	5.20
KNN	Fine	82.24	3900	6.44
	Medium	83.33	4100	3.56
	Coarse	79.00	3650	2.21
	Cosine	79.99	1900	4.46
	Cubic	87.54	2400	2.65
	Weighed	83.50	4600	3.16

Table 8. Analysis of production cost of bread (experimental control set)

Raw material	Addition in term of	Quantity (g)	Rate/kg (Rs.)	Cost (Rs.)	Handling loss (HL)* (%)	Cost due to HL(Rs.)	Net cost (= cost + HL×cost) (Rs.)
Flour	w/w	100	21	2.10	0.01	0.021	2.12
Sugar	w/w	8.0	35	0.28	0.005	0.0014	0.28
Salt	w/w	2.25	8.5	0.02	0.002	0.00004	0.02
Instant dry yeast	w/w	3.00	400	1.20	0	0	1.20
Shortening	w/w	16.00	227	3.63	0.001	0.0036	3.63
Emulsifier (E 472e)	w/w	3.00	800	2.40	0.001	0.0024	2.40
Skimmed milk powder	w/w	5.00	180	0.90	0.002	0.0018	0.90
Gluten powder	w/w	0.14	160	0.02	0	0	0.02
Bread improver (Tower-300)	w/w	1.00	280	0.28	0.001	0.00028	0.28
Potasiumsorbate	w/w	0.10	360	0.036	0.001	0.00036	0.04
Calcium propionate	w/w	0.25	175	0.044	0.001	0.00044	0.04
Acetic acid	(v/w) (ml)	0.10	600/L	0.06	0.001	0.00006	0.06
Water	L	60	0	0	0	0	0
Cost for power consumption during baking	KWh/g	0.0022	0.0022	0.0022	-	-	0.02
Total wt. (g)		198.84				Total cost	11.01
Dividing wt. (g)		1.72					
Total yield (g)		= 198.84/ 1.72 = 115.60	Wastage%	0.01%	Wastage qty. (g)		= 115.60*0.01% = 1.16 g
Net yield (g)		= 115.60-1.16 = 114.44					
RM cost (Rs.)		=11.01/114.44 = 0.10					
Packing Material cost (Rs.)		0.05					
Total cost (Rs.)/g of loaf		= Cost (RM+ PM) = 0.10+0.05 = 0.15 (Rs. 16/100 g of loaf)					

HL: Handling loss

Table 9. Analysis of production cost of bread (E-7 set)

Raw material	Addition in term of	Quantity (g)	Rate/kg (Rs.)	Cost (Rs.)	% HL	Cost due to HL (Rs.)	Net cost (= cost + HL×cost) (Rs.)
Flour	w/w	100	21	2.10	0.01	0.021	2.12
Sugar	w/w	8.0	35	0.28	0.005	0.0014	0.28
Salt	w/w	2.25	8.5	0.02	0.002	0.00004	0.02
Instant dry yeast	w/w	3.00	400	1.20	0	0	1.20
Shortening	w/w	16.00	227	3.63	0.001	0.0036	3.63
Emulsifier (E 472e)	w/w	3.00	800	2.40	0.001	0.0024	2.40
Skimmed milk powder	w/w	5	180	0.90	0.002	0.0018	0.90
L-proline	w/w	1.2	1000	1.20	0.002	0.0024	1.20
Gluten powder	w/w	0.14	160	0.02	0	0	0.02
Bread improver (Tower-300)	w/w	1.0	280	0.28	0.001	0.00028	0.28
Potassium sorbate	w/w	0.1					
Calcium propionate	w/w	0.25					
Acetic acid	(v/w) (ml)	0.1	600/L	0.06	0.001	0.00006	0.06
Water		60	0	0	0	0	0
Cost for power consumption during baking	KWh/g	0.0022	0.0022	0.0022	-	-	0.02
Total wt. (g)		199.69				Total cost	12.21
Dividing wt. (g)		1.72					
Total yield (g)		=199.69/1.72 = 116.09	Wastage%	0.01%	Wastage qty. (g)	= 116.09*0.01% = 1.17 g	
Net yield (g)		= 116.09-1.17 = 114.92					
RM cost (Rs.)		=12.21/114.92 = 0.11					
Packing Material cost (Rs.) (Total cost/pc.)		0.09					
Total cost (Rs.)/g of loaf		= 0.11+0.09 = 0.20 (Rs. 20/100 g of loaf)					

HL: Handling loss

Table10. Analysis of laminate packaging material

Test parameters	Unit	LDPE/MET-BOPP

GSM (gram per square meter)	g/m^2	54.88
Breaking strength in machine direction	m	breaking length, m: 2377 elongation at break, percent: 390
Breaking strength in cross direction	m	breaking length, m: 1637 elongation at break, percent: 610
Bursting strength	kg/cm^2	0.90
Tensile index in machine direction	N/mg	20.60
Tensile index in cross direction	N/mg	15.99
Water vapor transmission rate	$g/100\ inch^2\ 24\ h^{-1}$/1 atm at 25 °C	0.01
Oxygen transmission rate	$cc/100\ inch^2\ 24h^{-1}$/1 atm at 25 °C	1.30
Migration	mg/dm^2	0.43

Table 11. Microbiological analyses of experimental control, positivecontrol and E-7 sets of bread during storage

Storage day / Microbiological parameters		**0**	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**
SPC (30 °C, 72 hr.) ($\times 10^3$ cfu/g)	CR	NG	4.2^{a}	20.5^{ab}	48.2^{ab}	64.6^{c}	71.4^{cb}	93.6^{d}	150.5^{d}	222.8^{e}
	E-7R	NG	3.9^{a}	19.3^{ab}	45.9^{ab}	63.3^{c}	69.0^{cb}	92.3^{d}	158.4^{d}	223.1^{e}
	Positive control	-	63.3	145.3	230.4	-	-	-	-	-
Yeast and mould count (25 °C, 5 days) ($\times 10^3$cfu/g)	CR	NG	17^{a}	30^{b}	43^{ab}	54^{ab}	77^{c}	86^{d}	100^{e}	138^{f}
	E-7R	NG	15^{a}	34^{b}	40^{ab}	58^{ab}	76^{c}	84^{d}	97^{e}	135.6^{f}
	Positive control	-	21	52	109	-	-	-	-	-

Values in the same column with different superscript ($^{a-e}$) are significantly different ($p < 0.05$)
Mean in a row with similar superscript are not significant different at $p \leq 0.05$
Yeast and mould count showing < 10 on day 0 imply that the count is below LOQ
As per WHO(1994), TPC/SPC limit value of spoilage : 2.0×10^5cfu/g
As per WHO(1994), Total fungal count : 1.0×10^5cfu/g
As per IDF (1994), Effective level of toxin formation: 10^5-10^6cfu/g
NG: No growth

Table 12. Texture analysis of experimental control, positive control and E-7 sets of bread during storage

Storage period	Average values of hardness (g)		
	CR	**Positive control**	**E-7R**
0	202.58±1.06[a]	-	176.25±1.05[b]
1	210.34±2.00[a]	205.173±1.16[a]	186.09±1.55[b]
3	250.34±4.00[a]	255.01±3.00[a]	200.98±2.00[b]
5	398.66±5.00[c]	423.09±3.00[b]	315.42±2.05[a]
7	520.54±6.00[b]	612.55±4.00[c]	424.53±1.50[a]
9	620.20±5.20[b]	734.64±5.00[c]	519.08±2.50[a]
11	800.77±8.00[c]	865.44±4.00[b]	692.76±2.00[a]
13	905.65±8.00[b]	1003.31±6.00[c]	760.65±3.00[a]
15	1076.53±7.00[b]	1158.85±9.00[c]	853.76±6.00[a]

Values in the same row with different superscript are significantly different ($p < 0.05$)
Mean ± S.D of three samples of each set

Table 13.AEF analysis of experimental control, positive control and E-7 sets of bread during storage

Storage period	AEF (% oleic acid)		
	CR	**Positive control**	**E-7R**
0	0.06±0.0008[a]	-	0.07±0.0006[a]
1	0.12±0.009[a]	0.32±0.009a	0.13±0.006[a]
3	0.21±0.02[a]	0.61±0.02a	0.23±0.02[a]
5	0.45±0.01[a]	1.55±0.01a	0.47±0.01[a]
7	0.75±0.01[a]	-	0.78±0.01[a]
9	0.83±0.02[a]	-	0.86±0.02[a]
11	0.94±0.02[a]	-	0.99±0.02[a]
13	1.17±0.06[a]	-	1.21±0.06[a]
15	1.53±0.04[a]	-	1.58±0.04[a]

Values in the same column with different superscript are significantly different ($p < 0.05$)
Mean ± S.D of three samples of each set
Mean in a row with dissimilar superscript are significant different at $p \leq 0.05$
AEF analysis for positive control set not performedfurther after day 5 as sample underwent rancidity

Table 14. Defuzzified scores of experimental control, positive control and E-7 sets of bread during storage

Sample	Day	Overall sensory score (SO)			Defuzzified scores	Remarks
		a	b	c		
E-7R	0	84.70	54.09	31.36	73.80	Good
	1	82.73	54.62	32.50	72.70	Good
	3	80.15	53.71	34.09	71.35	Good
	5	77.88	52.88	35.38	70.11	Good
	7	74.85	51.21	34.92	68.31	Good
	9	72.27	50.15	35.76	66.72	Good
	11	71.97	50.00	35.61	66.49	Good
	13	61.06	46.67	40.45	58.96	Medium
	15	3.18	4.77	26.59	10.45	Not significant
CR	0	72.35	51.52	45.08	67.53	Good
	1	67.05	49.62	43.56	63.93	Good
	3	60.91	47.95	43.41	59.20	Medium
	5	36.74	36.21	36.59	36.87	Fair
	7	35.38	35.30	36.82	35.88	Fair
	9	33.41	33.41	37.95	34.92	Fair
	11	29.47	29.09	34.62	31.31	Fair
	13	24.47	23.11	33.33	27.88	Fair
	15	0.00	0.00	25.00	8.33	Not significant
Positive control	0	-	-	-	-	-
	1	63.33	49.17	44.39	61.15	Medium
	3	38.03	38.86	36.59	37.27	Fair
	5	0.00	0.00	25.00	8.33	Not significant
	7	-	-	-	-	-
	9	-	-	-	-	-
	11	-	-	-	-	-
	13	-	-	-	-	-
	15	-	-	-	-	-

Table 15.2-AP analysis of experimental control, positive control and E-7 sets of bread during storage

Storage period	Analyses					
	2-AP content (mg/ 100 g of E-7R)	Overall aroma scores (OAS) of E-7R	2-AP content (mg/ 100 g of CR)	Overall aroma scores (OAS) of CR	2-AP content (mg/ 100 g of positive control sample)	Overall aroma scores (OAS) of positive control sample
0	65.78±1.20[a]	9.0	8.61±0.25[b]	8.0	-	-
1	52.14±1.10	9.0	6.20±0.10	7.0	4.70±0.04[c]	4.0
3	40.75±0.57	8.0	4.78±0.06[a]	6.0	1.48±0.02[a]	2.0
5	32.12±0.55	8.0	0.99±0.03	4.0	0.32±0.03	1.0
7	19.46±0.16	7.0	0.36±0.02	2.0	ND	-
9	10.82±0.05	7.0	ND	-	ND	-
11	7.44±0.04	7.0	ND	-	ND	-
13	4.82±0.05[a]	6.0	ND	-	ND	-
15	2.76±0.04	4.0	ND	-	ND	-

ND: Not detected as it is beyond the LOD

Table 16. 'Freshness index' of experimental control, positive control and E-7 sets of bread obtained during storage period using Mahalanobis distance methodology.

Storage period	Mahalanobis distance		
	CR set	**Positive control set**	**E-7R set**
0	0.00^{a}	-	0.00^{a}
1	0.51^{b}	2.26^{a}	0.23^{b}
3	2.23^{c}	5.02^{b}	0.43^{c}
5	5.95^{cd}	7.90^{c}	0.80^{cd}
7	7.75^{d}	10.07^{d}	1.20^{d}
9	9.92^{e}	12.44^{cd}	1.42^{e}
11	12.04^{f}	15.07^{e}	1.65^{f}
13	14.18^{fg}	18.05^{f}	2.15^{fg}
15	17.77^{g}	21.11^{g}	3.95^{g}

Values in the same column with different superscript are significantly different ($p < 0.05$)

Table 17. Performance of ANN model for predicting the freshness of bread

No. of nodes in hidden layer	RMSE	R^2
1	0.2162	0.8074
2	0.1798	0.8657
3	0.1740	0.8740
4	0.1691	0.8808
5	0.1957	0.8415
6	0.2264	0.7892
7	0.1829	0.8612
8	0.2455	0.7527
9	0.1447	0.9118
10	0.1708	0.8784
11	0.2406	0.7624
12	0.2167	0.8065
13	0.1040	0.9440
14	0.1987	0.8203
15	**0.0857**	**0.9782**
16	0.1935	0.8450
17	0.2014	0.8313
18	0.1740	0.8740
19	0.1992	0.8375
20	0.1669	0.8845

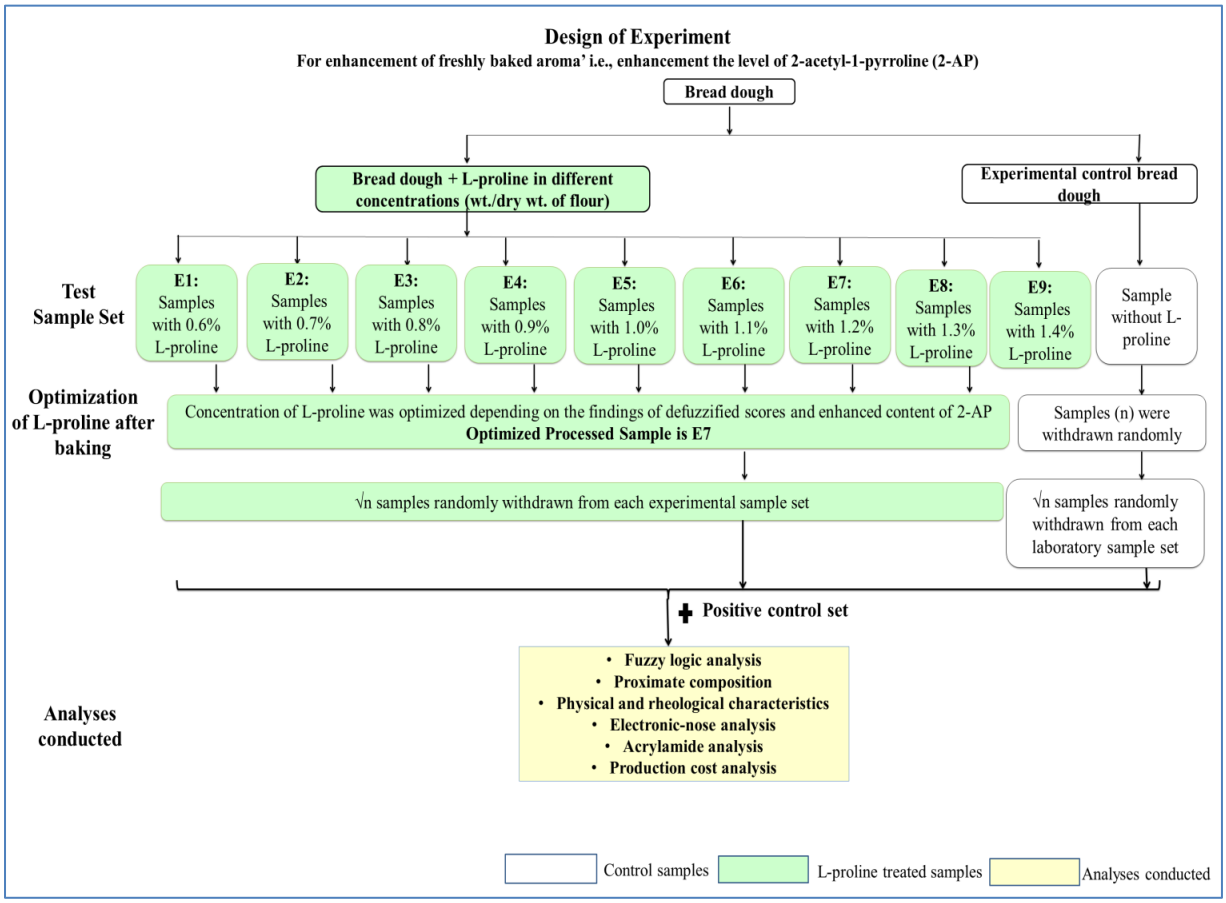


Figure 1a. Design of experiment for freshness enhancement of bread

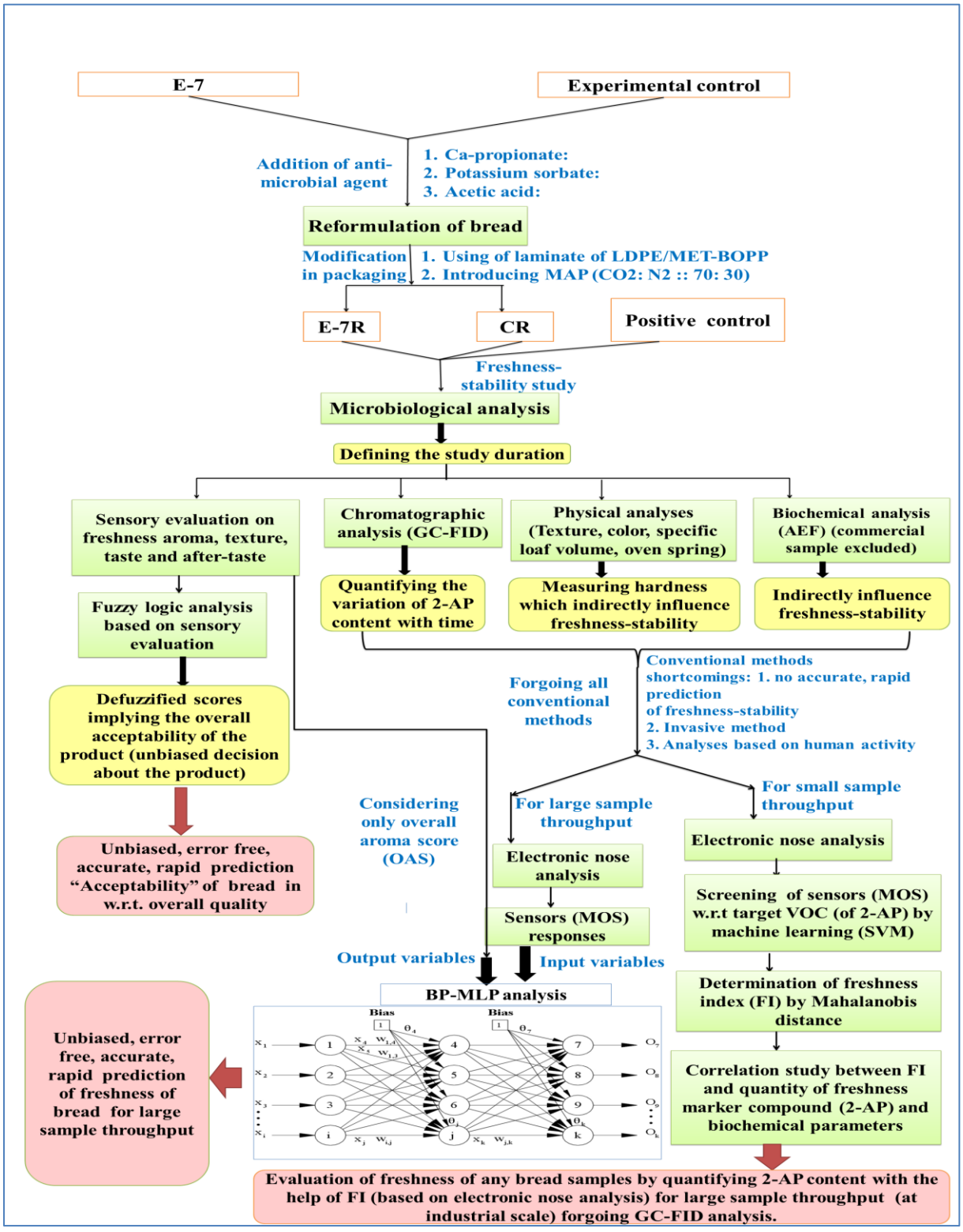


Figure 1b. Design of experiment for freshness-stability study of bread

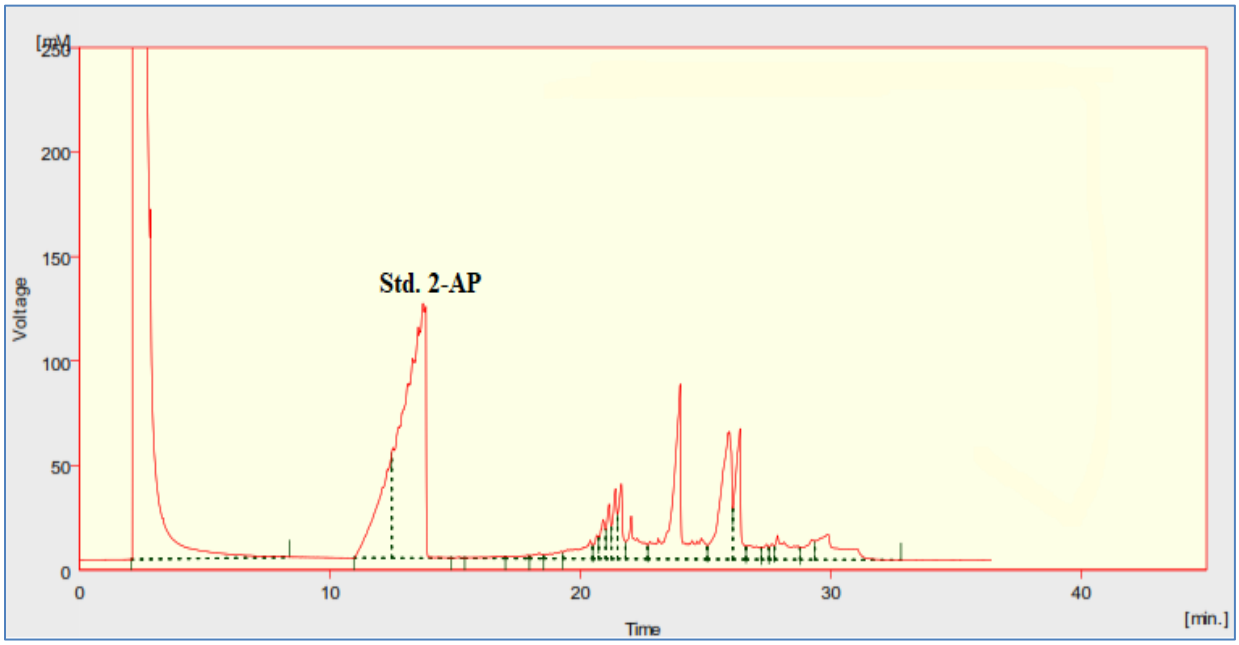


Figure 2. GC analyses of std. 2-AP

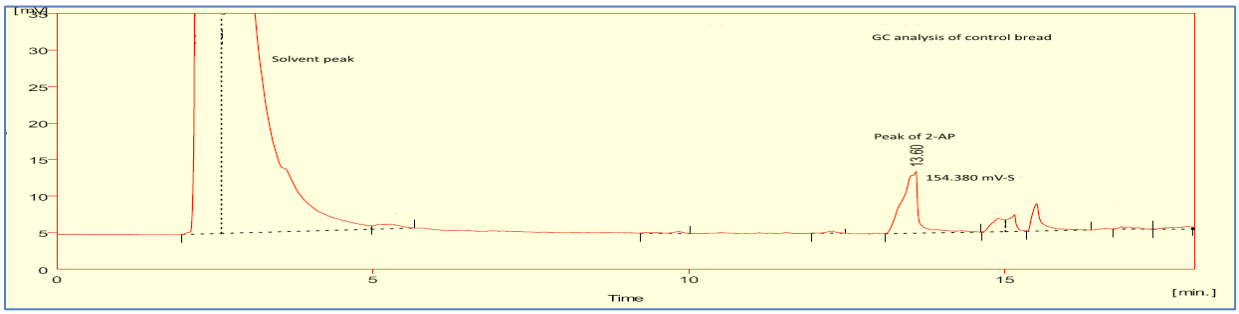


Figure3a. Analysis of 2-AP content of bread of experimental control set on day 0

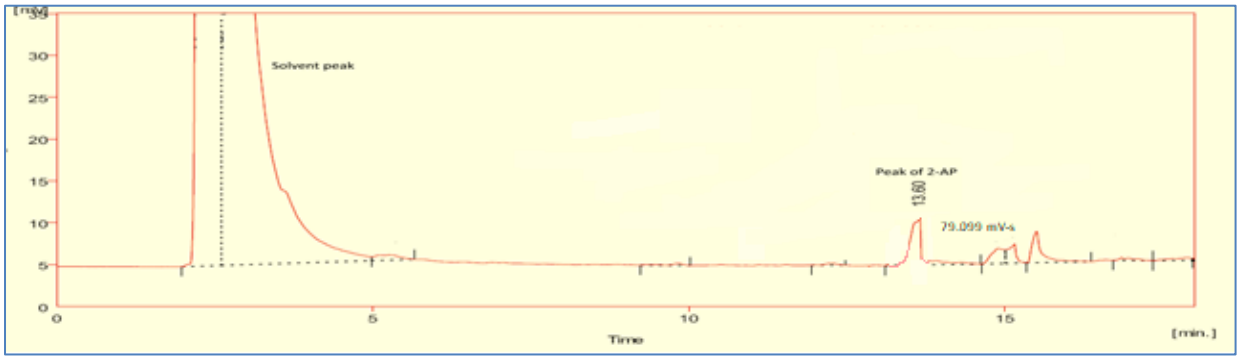


Figure 3b. Analysis of 2-AP content of bread of positive control set on day 1 (after the day of manufacturing)

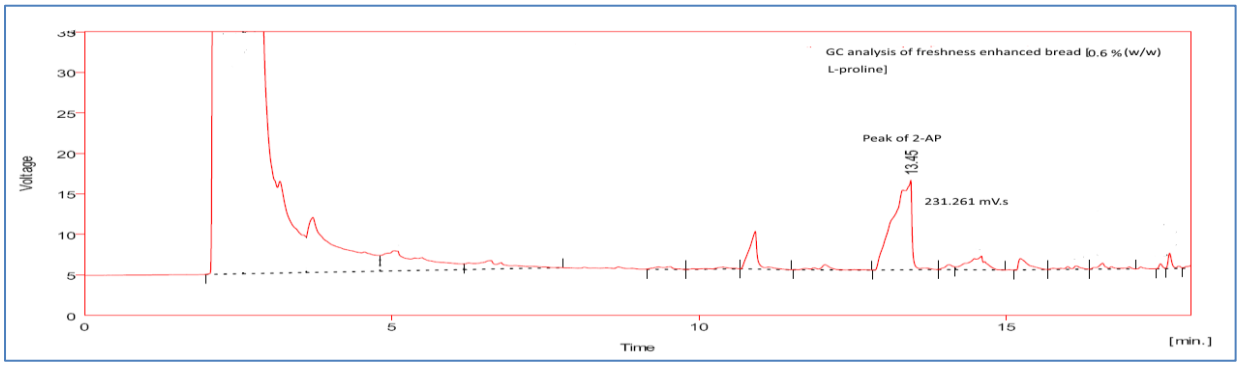


Figure 3c. Analysis of 2-AP content of bread, enriched with 0.6% (w/w) L-proline by GC-FID on day 0

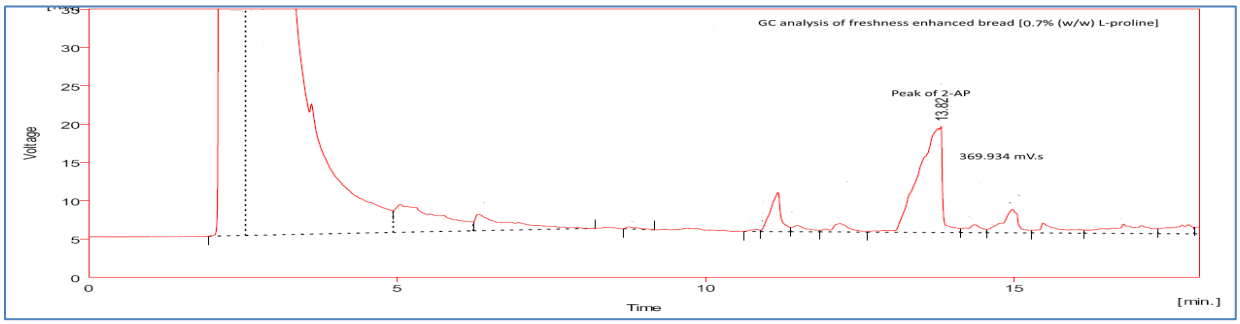


Figure 3d. Analysis of 2-AP content of bread, enriched with 0.7% (w/w) L-proline by GC-FID on day 0

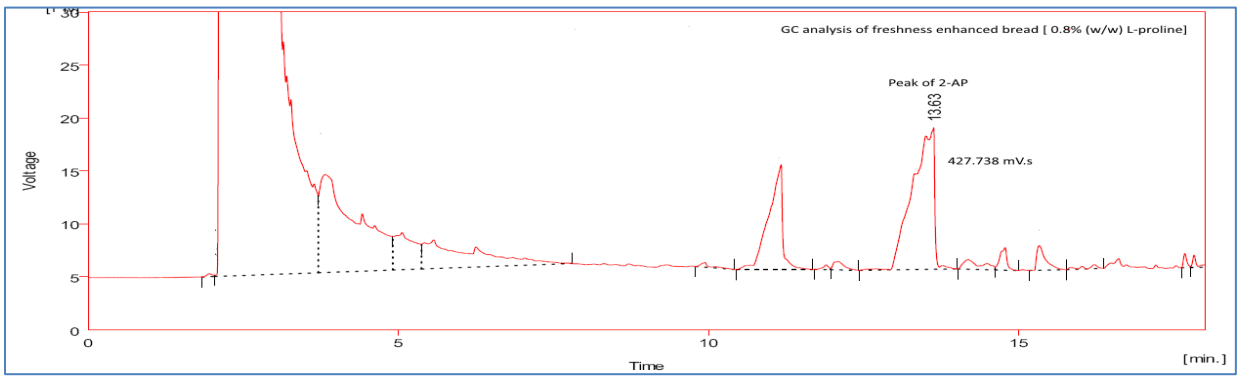


Figure 3e. Analysis of 2-AP content of bread, enriched with 0.8% (w/w) L-proline by GC-FID on day 0

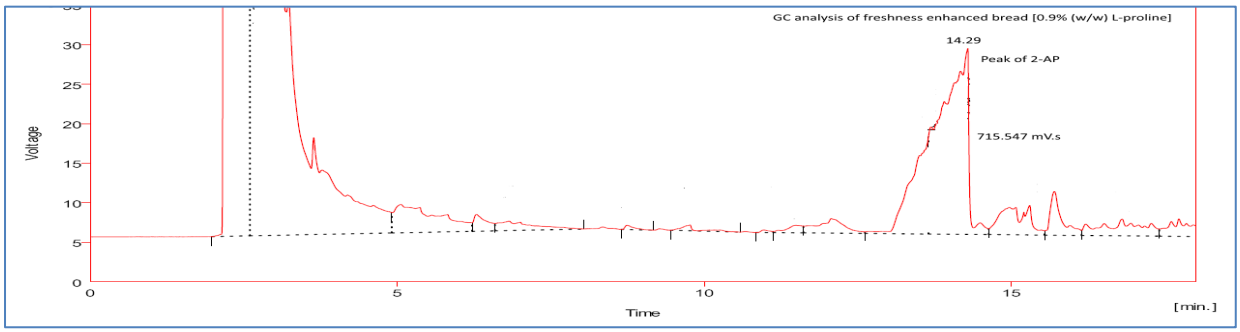


Figure 3f. Analysis of 2-AP content of bread, enriched with 0.9% (w/w) L-proline by GC-FID on day 0

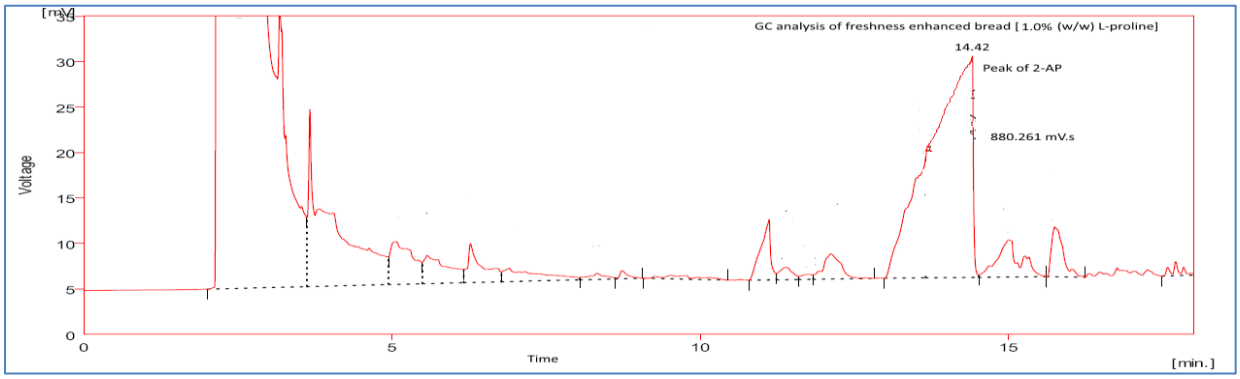


Figure 3g. Analysis of 2-AP content of bread, enriched with 1.0% (w/w) L-proline by GC-FID on day 0

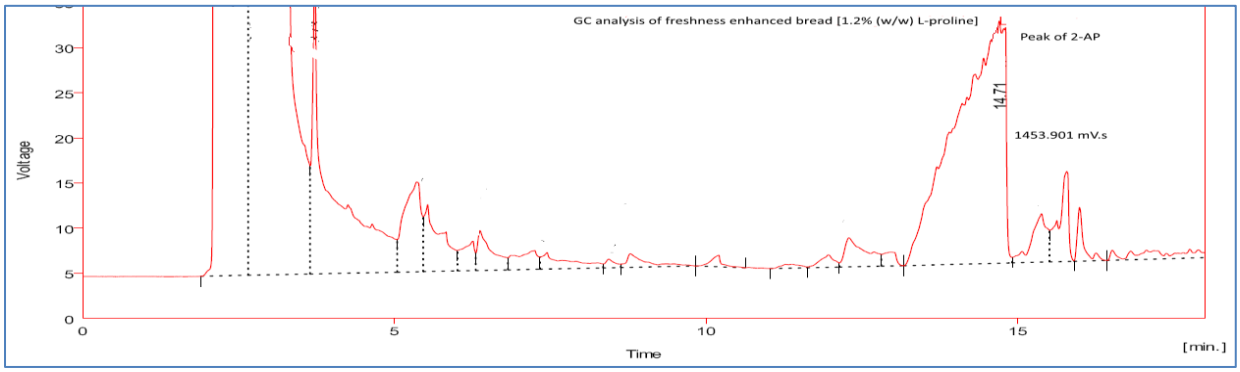


Figure 3h. Analysis of 2-AP content of bread, enriched with 1.2% (w/w) L-proline by GC-FID on day 0

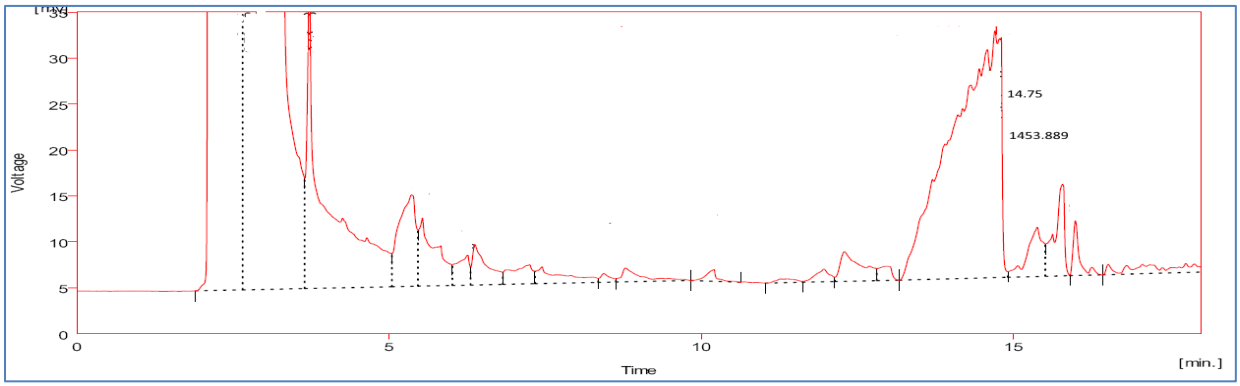


Figure 3i.Figure 4d. Analysis of 2-AP content of bread, enriched with 1.4% (w/w) L-proline by GC-FID on day 0

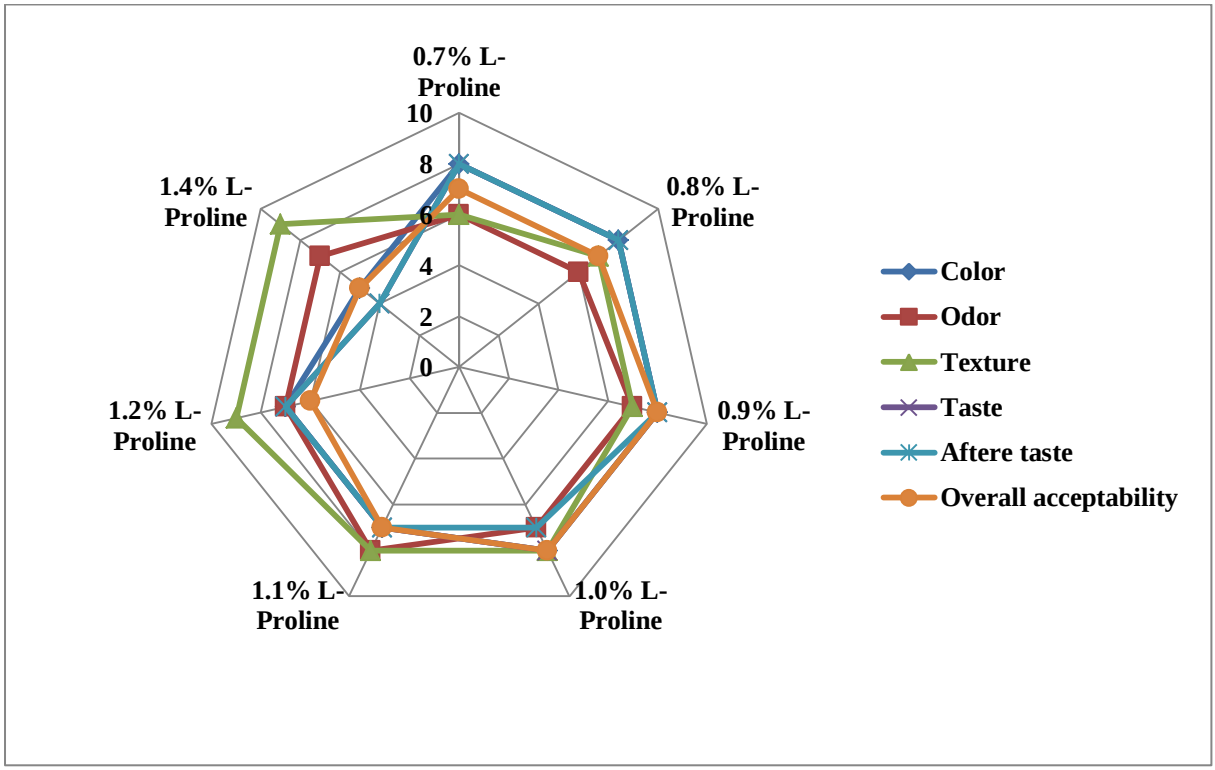


Figure 4. Radar diagram of sensory evaluation of freshness enhanced bread at different % of L-proline

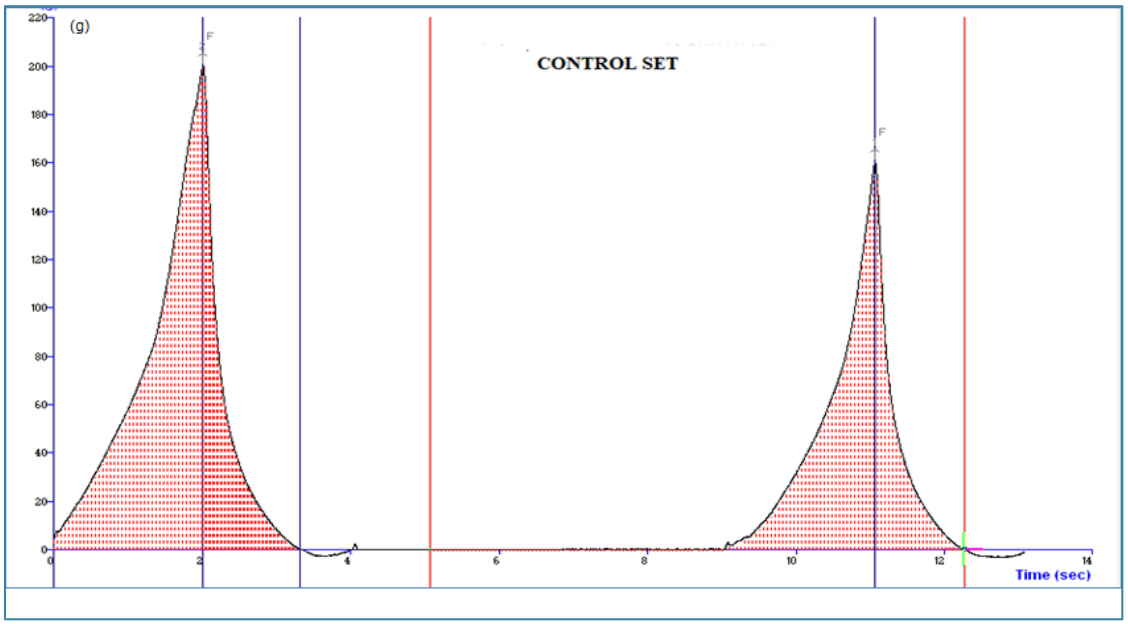


Figure 5a. Texture profile analysis of experimental control set of bread on day 0

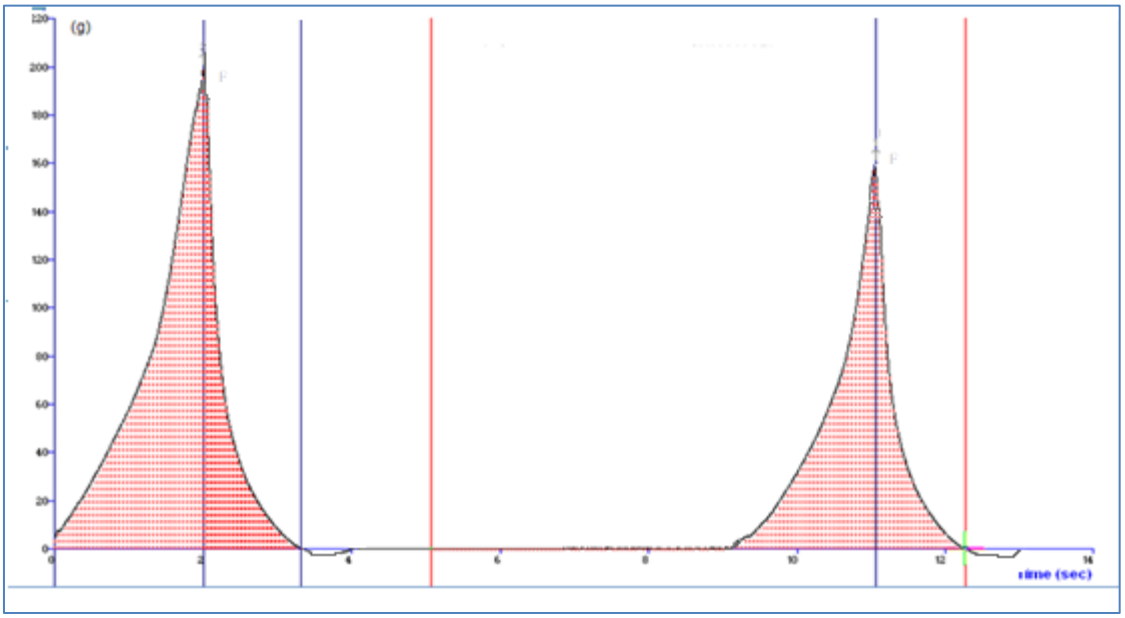


Figure 5b. Texture profile analysis of positive control set of breadon day 1 (after the day of manufacturing)

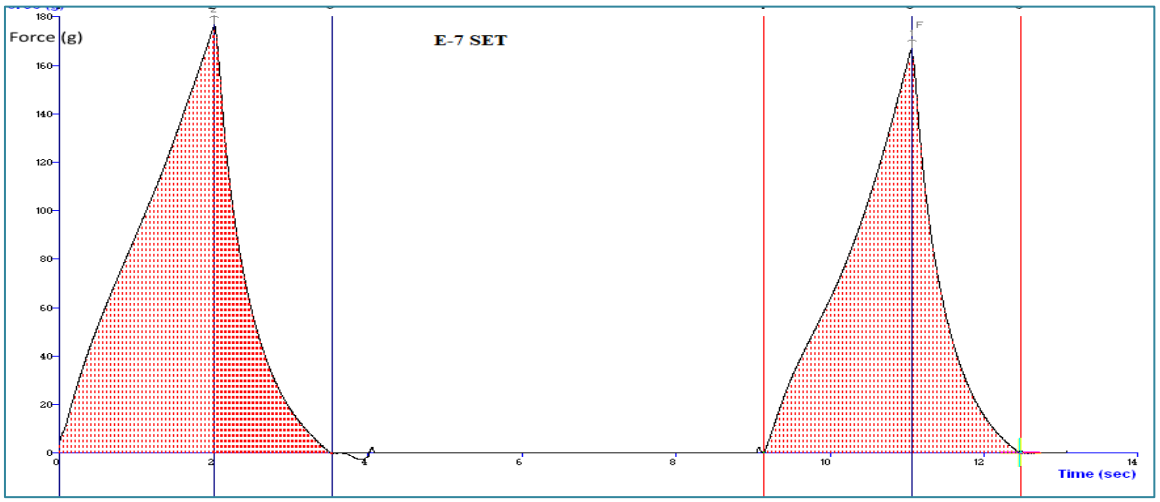


Figure 5c. Texture profile analysis of E-7 set of bread on day 0

a. b.

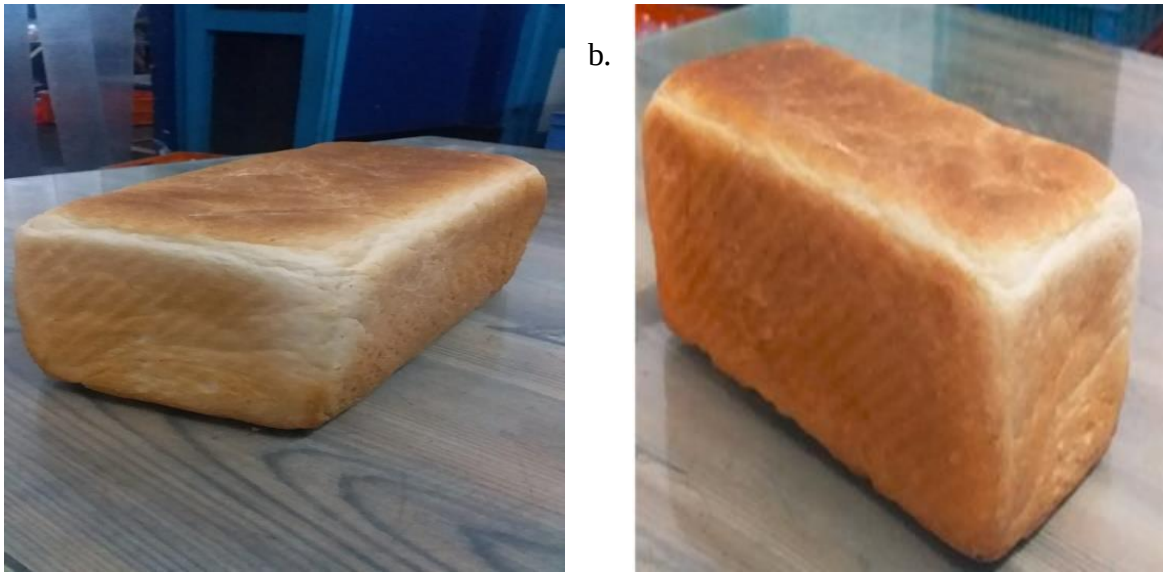


Figure 6a. Experimental control set of bread; **b**. E-7 set of bread

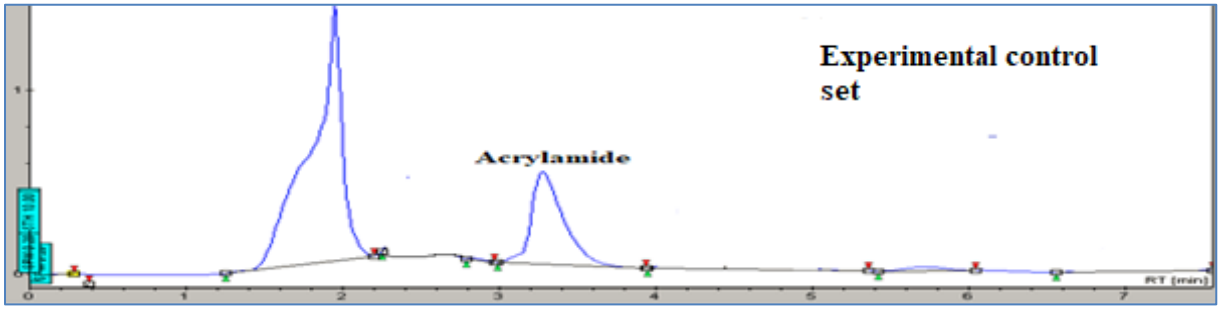


Figure 7a. Acrylamide analysis of experimental control set of bread

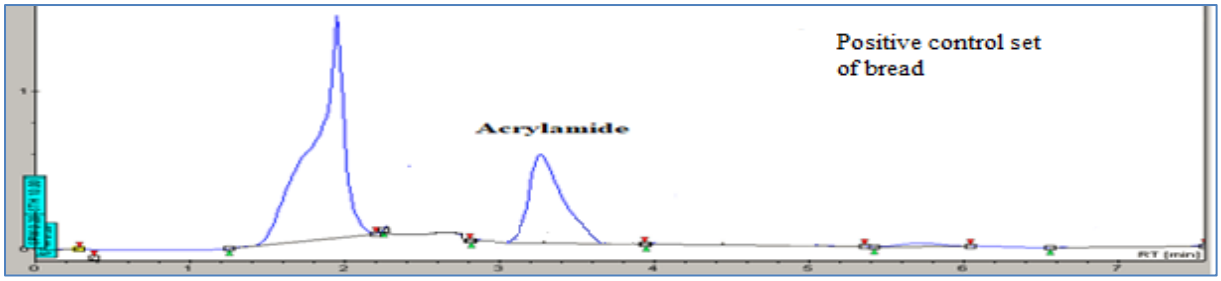


Figure 7b. Acrylamide analysis of positive control set of bread

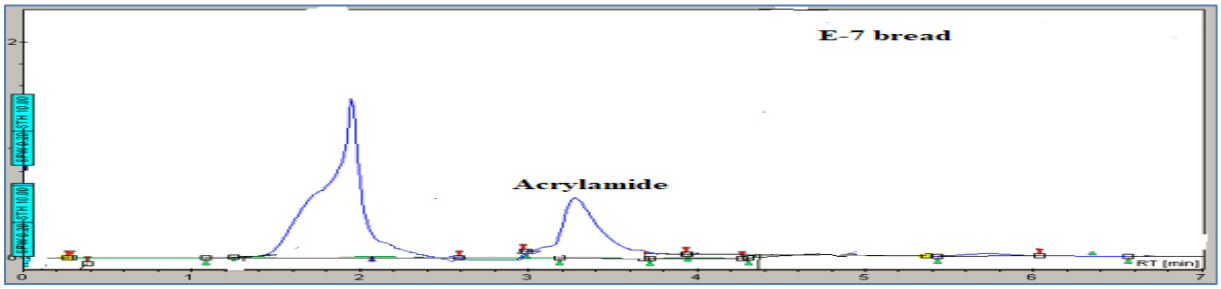


Figure 7c. Acrylamide analysis of E-7 set of bread

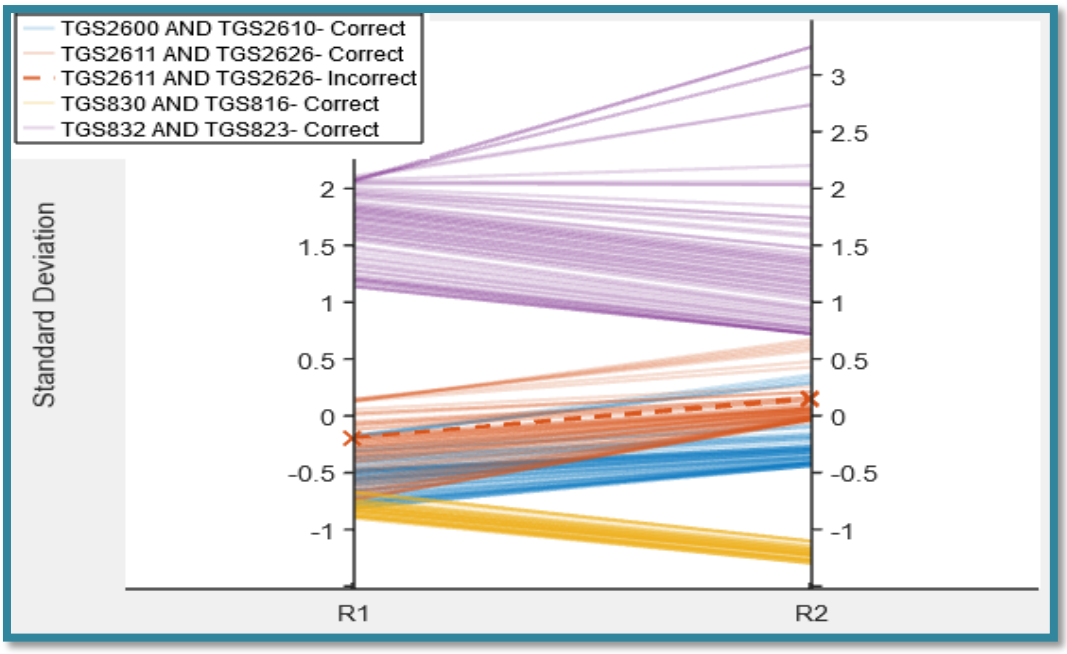


Figure 8. Metal oxide semiconductor sensor classification by SVM-Fine Gaussian method

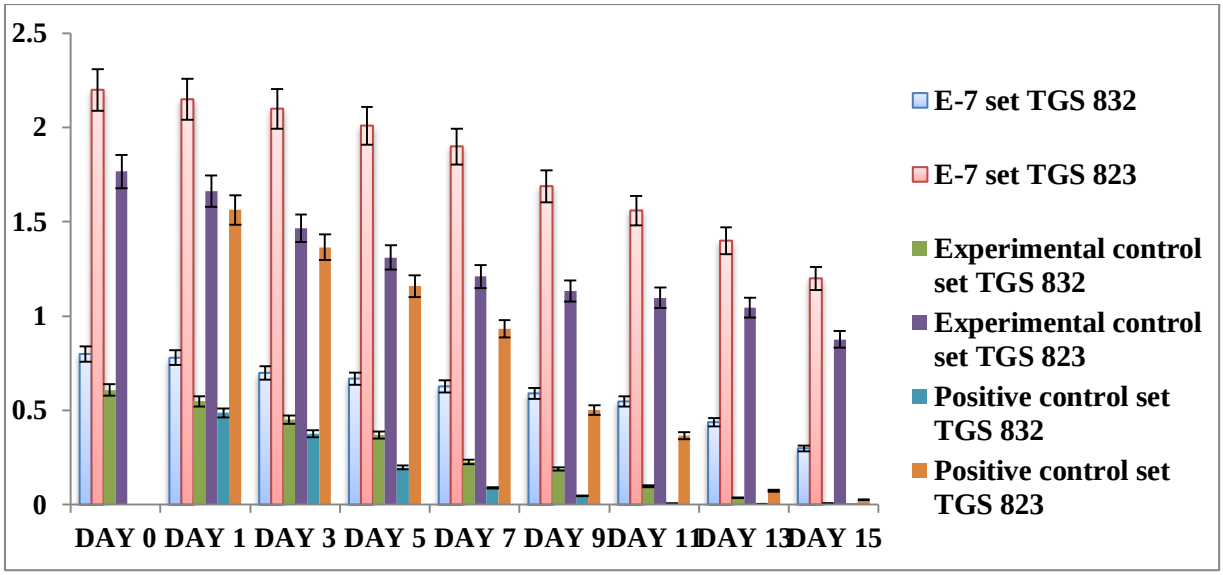


Figure 9.E-nose analyses of experimental control, positive control and E-7 sets of bread during storage with screened sensors

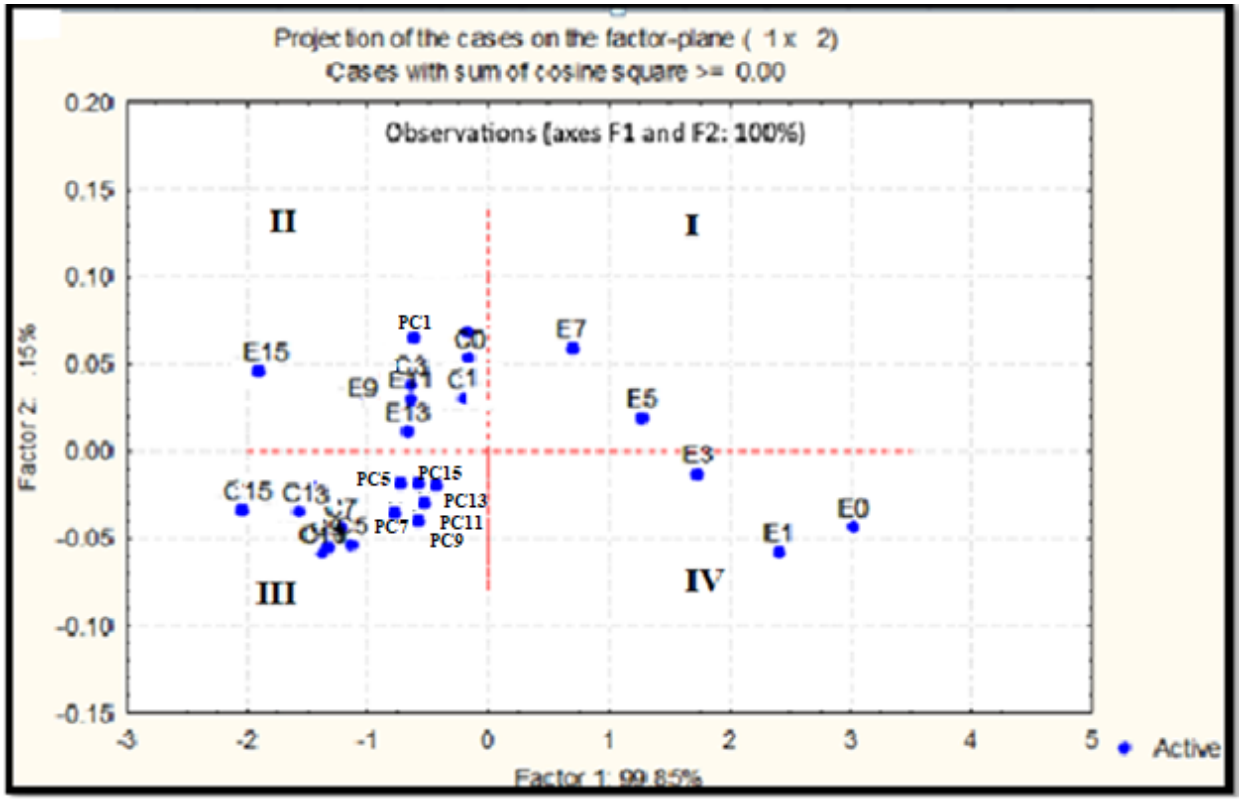


Figure 10. Odor map of experimental control, positive control and E-7 sets of bread on the basis of PCA using sensors TGS 832 andTGS 823 of C-DAC e-nose system.

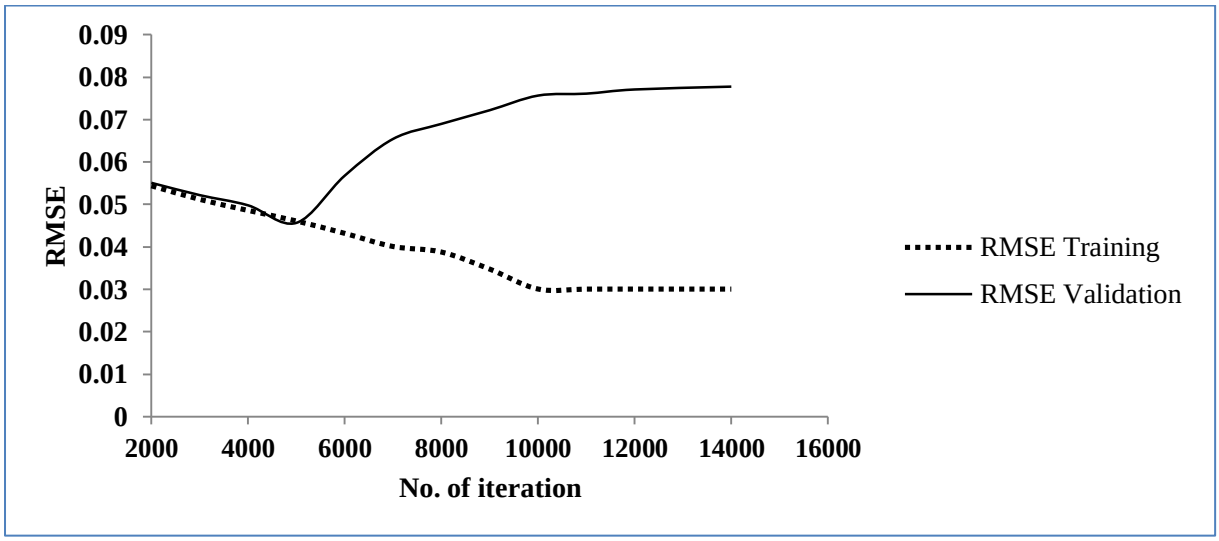
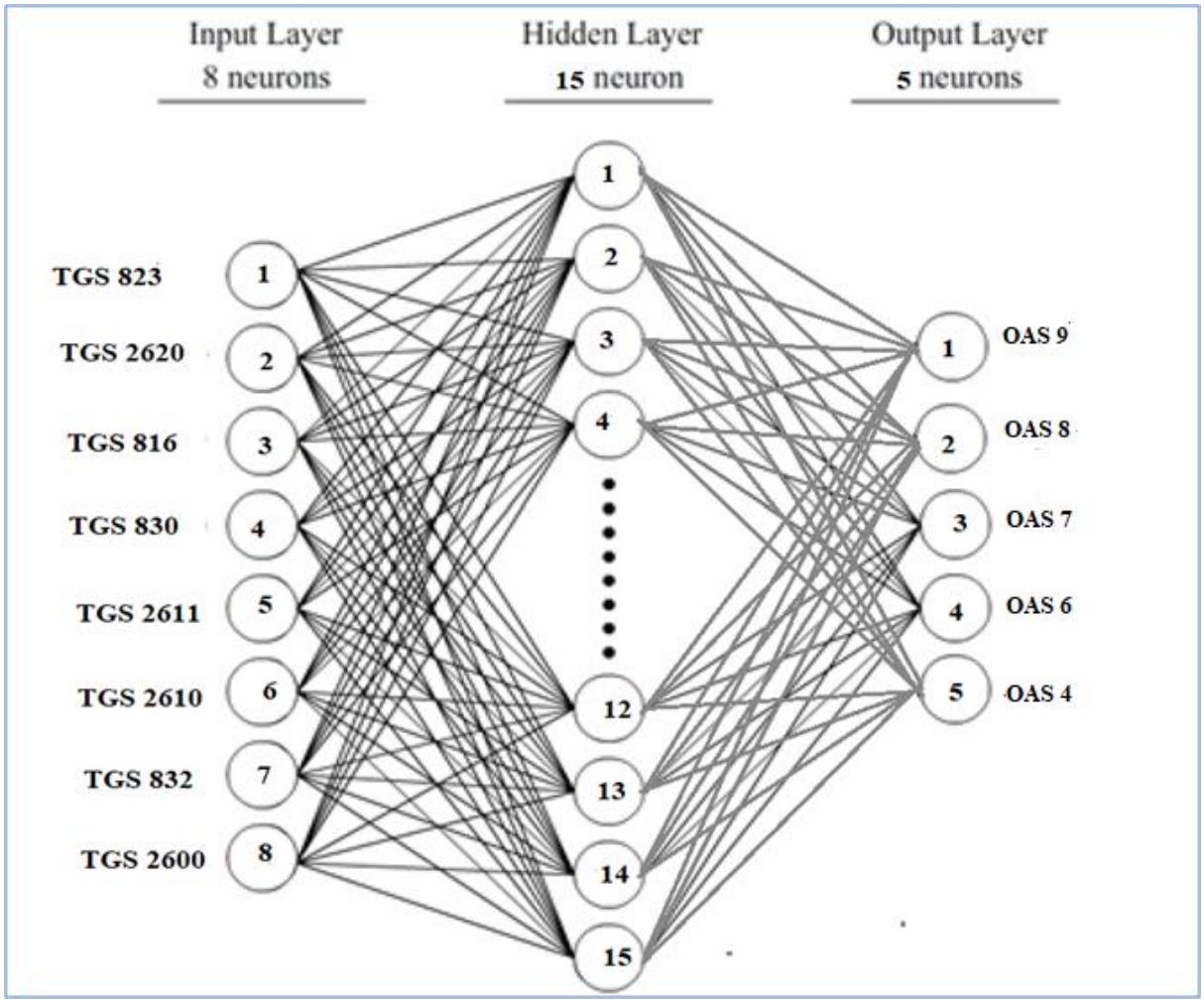


Figure 11. Determination of optimum no. of iterations



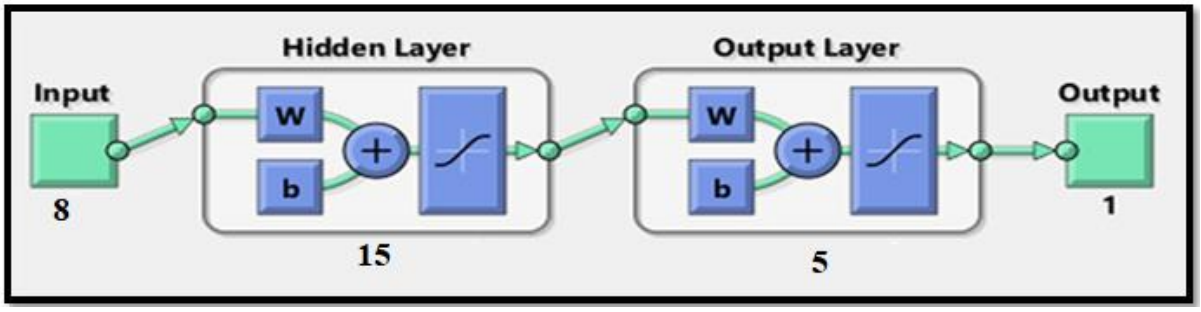


Figure 12. Architecture of BP-MLP network

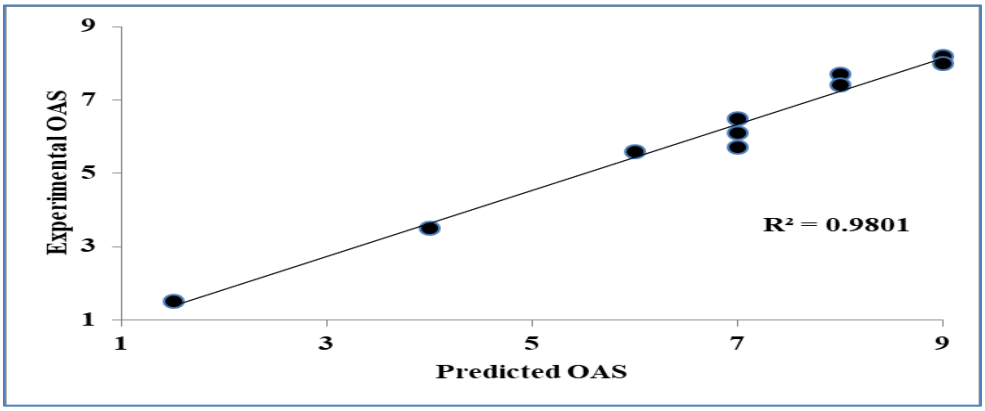


Figure 13a. Plot of predicted vs. experimental values by the selected ANN for training set

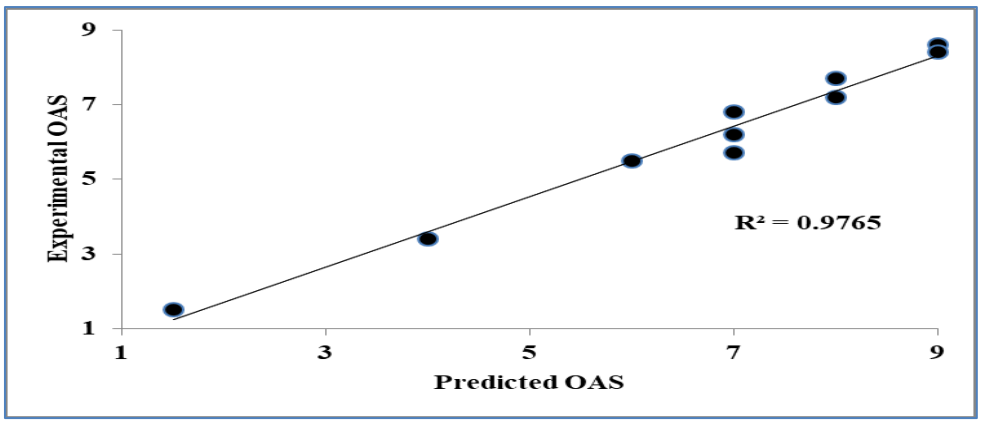
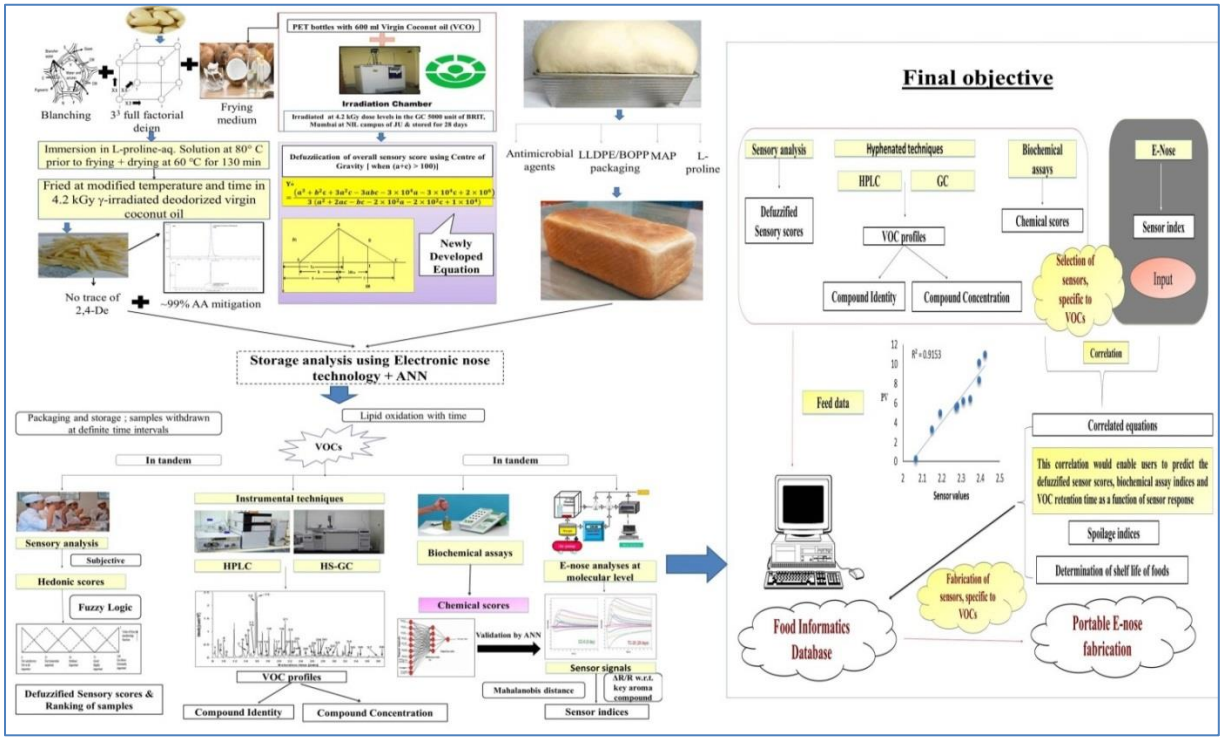


Figure 13b. Plot of predicted vs. experimental values by the selected ANN for validation set

Résumé

Résumé

The entire work of the thesis has been summarized and presented in the following figure.



The present investigation has focused on four aspects explained briefly as follows.

1. Sensory evaluation is a highly popular technique which provides the ultimate decision about products' acceptability. Mostly, semi-trained panellists are involved in this evaluation process. Hence, the correct decision about the products may be obscured by sensory-perceptual alterations and biasness in perceptions. Fuzzy logic analysis of sensory outcomes can successfully overcome these shortcomings of sensory evaluation and has therefore used in this work to re-affirm whether 4.2 kGy and 28 days storage were truly effective in removing obnoxious odor from VCO. Fuzzy logic analysis in this case necessitated development of a new equation which could successfully validate that DVCO was completely devoid of its obnoxious odor and thus could be a used as a new alternate frying oil.

2. A new and unique methodology was developed which delivered toxin-free, safe, organoleptically improved fried potato crisps to consumers and attested that DVCO is a healthy frying oil.

3. E-nose based artificial intelligence method was used for prediction of onset and progression of rancidity in fried potato crisps. This method was found to be a robust, fast, and accurate one in assuring safe quality of fried potato crisps.

4. A methodology was built which augmented the freshness aroma i.e., sweet "popcorn-like aroma" in bread significantly and the same could be retained for a prolonged period using hurdle technology. Also a 'SMART', intelligent, fast and automated model was developed by e-nose-based BP-MLP for prediction of freshness in production scale bread samples.

The present research work comprises of the following:

1. Development of a new equation in fuzzy logic analysis for ascertaining appropriate dose of gamma irradiation of virgin coconut oil

- A new equation was developed when (a+c) is more than 100 in case of overall sensory score.
- The defuzzified scores obtained from new developed equation validated that DVCO was completely devoid of its obnoxious odor and thus could be a used as new frying oil.

2. Acrylamide (CH_2=CH–CO–NH_2; 2-propenamide) mitigation and 2,4-decadienal elimination in potato-crisps using L-proline accompanied by modified processing conditions

- The optimized (employing a full factorial design of experiments and response surface methodology followed by ridge analysis) parameters for ~99% AA mitigation and prevention of 2,4-De formation in fried potato crisps were: blanching of potato slices: in water at 70 ℃ for 20 min; treatment by L-proline (%): immersion of blanched slices in 2.0% L-proline aqueous solution at 80 ℃ for 30 min; tray drying to 40% moisture: 60 ℃ for 130 min; frying conditions: 140 ℃ for 5 min (modified deep frying conditions); frying medium: 4.2 kGy γ-irradiated-28-days stored DVCO.
- This unique approach concomitantly improved organoleptic profile of the fried potato crisps with respect to their odor, color and taste.
- This work established superiority of DVCO over common frying oil such as soybean oil.

3. Development of methodology for assessment of rancidity status of conventional and acrylamide treated fried potato crisps using electronic noses: Sensor screening by Scalar machine learning classifier method

- DVCO as frying oil delayed the rancidity of fried potato crisps vis-à-vis soybean oil.
- Both onset and progression of rancidity w.r.t rancidity marker hexanal in fried potato crisps were rendered easy and accurate by e-nose analysis
- Determination of 'spoilage indices' of the same during storage yielded good model fit equation(s) which allowed direct and accurate assessment of the rancidity molecular marker in the crisps.

4. Improvement and extension of freshness of bread and validation by electronic nose technology in coupling with artificial neural network

- Enrichment of bread dough by 1.2% (wt./wt. of flour on d.w.b) L-proline produced freshness-enhanced bread by increasing its KFO i.e., 2-AP content by 8 fold without compromising other quality attributes of bread.
- This approach concomitantly reduced the AA content in bread by 38%.
- Hurdle technology (incorporation of antimicrobial agents such as acetic acid, potassium sorbate and calcium propionate accompanied by LDPE/MET-BOPP packaging of bread under MAP conditions) retained the freshness aroma i.e., sweet "popcorn-like aroma" in bread up to 13 days.
- Determination of 'freshness indices' of the same during storage yielded good model fit equation(s) which allowed direct and accurate assessment of KFO of bread.
- The e-nose technology was rendered SMART (specific, measurable, achievable, realistic, time bound) by application of a deep learning tool such as BP-MLP. The model thus developed can aid in automation of quality assurance of commercial scale bread samples. It comprised of 1 hidden layer consisting of 15 neurons which showed excellent fitting between predicted and experimental data. The optimum ANN topology obtained could accurately assess freshness of bread samples.

Suggestion for future work

Suggestions for Future Work

1. Further work can be conducted to design Smart/Active/Intelligent packaging for bread and potato fries for incorporation of screened MOS sensors (specific for the food odorants) therein for real time monitoring of the freshness status of the food products.

2. The data obtained from the correlation studies could serve as input to food informatics database which can be programmed into a microchip and used in the portable sensors for consumers who can judge the quality status of the baked-cum-fried products prior to consumption.

Anupama Bose

Appendix

APPENDIX 1

Symbols

r : Pearson correlation coefficient

t: Time (min)

T: Temperature (°C)

V: Volume (cm^3) (SI unit: 1 m^3= 1,000,000 cm^3)

ΔE*: Difference in color

L*: Lightness value

a*: Green–red opponent color

b*: blue–yellow opponent color

Greek Letters

ε: Molar absorptivity or molar extinction coefficient (L mol^{-1} cm^{-1})

μ: Micron (1μ = 1/1000 mm)

ρ: Density (kg/m^3)

List of Abbreviations

AA:	Acrylamide
2,4-De:	2,4-decadienal
ADI:	Acceptable daily intake
SC:	Safe concentration
VCO:	Virgin coconut oil
DVCO:	Deodorized virgin coconut oil
ANOVA:	One-way analysis of variance
RSM:	Response surface methodology
SPE-QuEChERS:	Solid phase extraction- Quick, Easy, Cheap, Effective, Rugged and Safe.
GC-FID:	Gas chromatography-flame ionization detector
HR-MS:	High resolution-mass spectrometry
LC-MS/MS:	Liquid chromatography-mass spectrometry/mass spectrometry
HPLC-PDA:	High performance liquid chromatography- Photometric diode array
kGy:	Kilo Gray
d.w.b.:	Dry weight basis
DCM:	Dichloromethane
SMLC:	Supervised machine learning classifier
SVM:	Support vector machine
C12:0:	Lauric acid
C18:2:	Linoleic acid

KJ:	Kilo Joule
KWh:	Kilo watt hour
AACC:	American Association for Clinical Chemistry.
FSSAI:	Food Safety and Standards Authority of India
WHO:	World health organization
AOAC:	Association of Official Analytical Collaboration
AACC:	American Association for Clinical Chemistry
AOCS:	American Oil Chemists' Society
FAO:	Food and Agriculture organization
TC:	Training set of control
EC:	Experimental control
PC:	Positive control
T-22:	Optimized treated set of fried potato crisps at 2% L-proline, 140 ℃ frying temperature, and 5 min frying time
TT-22: crisps	Training set of optimized treated set of fried potato
E-7:	Optimized treated set of bread at 1.2% L-proline
PV:	Peroxide value
TBARS:	Thiobarbituric acid reactive substances
AEF:	Acidity of extracted fat
$\lvert\Delta R\rvert/R$:	Sensor responses
R:	Resistance
e-nose:	Electronic nose

TPA:	Texture profile analysis
TA:	Texture analysis
TFC :	Total fungus count
SPC:	Standard plate count
s:v:	Surface to volume ratio
FP:	Final proofing
RH:	Relative humidity
R_m:	Resistance to extension
E:	Extensibility
SD:	Standard deviation
BU:	Brabender unit
RMSE:	Root mean square error
R^2:	correlation coefficient
BI:	Browning index
WI:	Whiteness index
S:	Triplet
SO:	Overall sensory score
Q_{REL}:	Relative weightage
Y_a:	Defuzzified score under condition of $(a+c) \leq 100$
Y_a':	Defuzzified score under condition of $(a+c) > 100$
B_x:	Overall membership function
S_m:	Similarity values
IVCO:	Irradiated Virgin Coconut Oil

LDPE:	Low density polyethylene
MET-BOPP:	Metallized-Biaxially oriented poly propylene

Glossary

Vide infra:	Explained later.
Vide supra:	Explained previously.
Scalping :	Scalping is a term used in the packaging industry to describe the loss of quality of a packaged item due to either its volatile flavors being absorbed by the packaging or the item absorbing undesirable flavors from its packaging.
i.e.:	That is

APPENDIX 2A

Let, the triplets of SO of any sample is obtained as (99.0 2.00 11.00)

(Steps involving to derive SO is explained in "**Chapter-2"**)

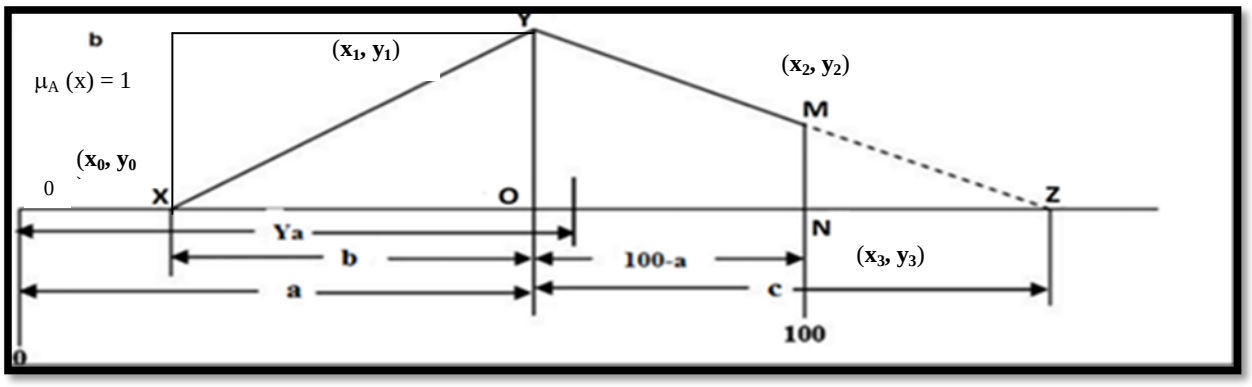
$\therefore$ (a + c) = (99.0 + 11.0) = 110 (> 100)

As per triangle theory, defuzzified score = 102.00

The limit of standard fuzzy scale is 0-100 (Das et al., 2011). But, defuzzified score (102.00) of sample exceeds the fuzzy scale limit and is beyond the judgment. This observation suggests to find new equation under the condition of (a+c) > 100 which is not reported yet. Therefore, first time a new equation was developed when (a+c) is more than 100 and it is an extended part of the work, done by Das (2005). With the help of new equation, the revalidation for deodorization of VCO was performed successfully and found in consonance with the findings of Ghosh et al. (2014). Thus, it ensured the using of DVCO as frying medium.

APPENDIX 2B

New developed equation in fuzzy logic:



N.B.: A : classical set
μ_A (x) is real valued function and real value (R) ➔[0,1]
Fuzzy set : $A^{\bullet}$
$A^{\bullet} = \{x, \mu_A (x)\}$; where, $x \in A$
$\mu_A (x) \in (0, 1)$

Centroid of XYMN (C_n) × Area of XYMN (A_n) = (Area of ΔXYO × Centre of gravity ΔXYO) + [(Area of Δ YOC × Centre of gravity of Δ YOC)–(Area of ΔMNZ × Centre of gravity of ΔMNZ)] = $\frac{1}{6}\sum_{i=0}^{n-1}(x_i + x_{i+1}).(x_i y_{i+1} - x_{i+1} y_i)$

Or, $C_n \times A_n = [1/2 \times AO \times BO \times \{(a-b) + \frac{2}{3} b\}] + [\frac{1}{2} \times OB \times OC \times (\frac{a+c}{3})]-[1/2 \times EC \times DE \times \frac{1}{3} \times \{(a+c)-100\}]$

Or, $C_n \times A_n = [\frac{1}{2} \times b \times 1 \times \frac{3a-b}{3}] + [(\frac{1}{2} \times 1 \times c \times \frac{3a+c}{3})-\{\frac{1}{2} \times [(a+c)-100] \times MN \times \frac{a+c-100}{3}\}]$

Or, $C_n \times A_n = \frac{3ab-b^2}{6} + [\frac{3ac+c^2}{6} - \frac{1}{6} \times \{(a+c)-100\} \times MN \times \{(a+c) -100\}]$

Or, $C_n \times A_n = \frac{1}{6} [3ab-b^2 + 3ac + c^2 – \{(a+c)^2-2 \times (a+c) \times 100 + 100^2\} \times MN]$

Or, $C_n \times A_n = \frac{1}{6} [3ab-b^2 + 3ac + c^2 – \{a^2+2ac + c^2-200a- 200c + 10000\} \times MN]$

Or, $C_n \times A_n = \frac{1}{6} [3ab-b^2 + 3ac + c^2 – a^2 MN - 2acMN - c^2MN + 200aMN + 200cMN + 10000MN]$

(1)

Now, $A_n = \frac{1}{2}\sum_{i=0}^{n-1}(x_i y_{i+1} - x_{i+1} y_i)$

$$= \frac{1}{2}\left[(x_0 y_1 - x_1 y_0) + (x_1 y_2 - x_2 y_1) + (x_2 y_3 - x_3 y_2)\right]$$

$$= \frac{1}{2}\left[\{(a+b) \times 1 - a \times 0\} + \{a \times MN - 100 \times 1\} + \{100 \times 0 - (a+c) \times MN\}\right]$$

$$= \frac{1}{2}\left(a - b - c \times MN - 100\right) \quad (2)$$

Now, to find the values of MN

Applying similarity principle between Δ YOZ and Δ MNZ

$$\frac{MN}{YO} = \frac{NC}{OZ}$$

$$or, \frac{MN}{1} = \frac{(a+c) - 100}{c}$$

$$or, MN = \frac{a}{c} + 1 - \frac{100}{c} \quad (3)$$

Now, putting eq. (2) and (3) in eq. (1), we get

$$C_n \times \left(2a - b + \frac{a^2}{c} - \frac{200a}{c} - 200 + \frac{10000}{c}\right) = \frac{1}{3}\left[2a^2 - 3ab + b^2 + 100a\left(\frac{a}{c} + 1 - \frac{100}{c}\right) - 10000 + a^2\left(\frac{a}{c} + 1 - \frac{100}{c}\right) - 100a - 20000\left(\frac{a}{c} + 1 - \frac{100}{c}\right)\right] \quad (4)$$

After going through a cascade of simplification steps, the final equation (eq. 5) was obtained as

$$\mathbf{C_n = Yá} = \frac{(a^3 + b^2 c + 3a^2 c - 3abc - 3\times10^4 a - 3\times10^4 c + 2\times10^6)}{3\,(a^2 + 2ac - bc - 2\times10^2 a - 2\times10^2 c + 1\times10^4)} \quad \mathbf{(5)}$$

APPENDIX 2 C

Table. Oil absorption by fried potato crisps

Parameters	Wt. of single potato tuber = 200 g (av.)		
	Conventional set	**Control set**	**T-22 set**
Moisture of potato tuber (g) **(A)**	=200*0.8 = 160	160	160
Dry matter in potato (g)	40	40	40
Fat content of potato tuber (g) **(B)**	=200*0.001 = 0.2	0.2	0.2
Mass of potato before frying (g)	200	200	(blanching+ aq. l-proline treatment+ drying) = 148.4
Mass of potato after frying (g)	138.4	182	136
Final fat content (g) **(C)** (DVCO density = 0.87 g/ml)	32	21	16.5
Oil absorption (g of fat absorbed/g dry weight of potato) = **(C-B)/A**	0.1987 (0.23 ml/g)	0.1300 (0.15 ml/g)	0.1018 (0.12ml/g)

Energy calculation for blanching at 70 ℃ for 20 min:

Total heat required by material in KJ = ($m \times c_p \times \Delta T$) = 2.0 * 4.18* (70-25) = 376.20 KJ

Here, m = 2.0 kg; c_p of water = 4.18 J/Kg-℃; ΔT = temperature difference in ℃

Now, 1 KWh = 3600 KJ

∴ **376.20 KJ = 0.11 KWh**

Energy calculation for conventional frying at 170 ℃:

Total heat required by material in KJ = ($m \times c_p \times \Delta T$) = 2.0 * 3.23* (170-25) = 936.70 KJ

Here, m = 2.0 kg; c_p of DVCO = 3.23 J/Kg-℃; ΔT = temperature difference in ℃

Now, 1 KWh = 3600 KJ

∴ **936.70 KJ = 0.26 KWh**

Energy calculation for L-proline treatment by immersion at 80 ℃:

Total heat required by material in KJ = ($m \times c_p \times \Delta T$) = 2.0 * 4.18* (80-25) = 459.80 KJ

Here, m = 2.0 kg; c_p of water = 4.18 J/Kg-℃; ΔT = temperature difference in ℃

Now, 1 KWh = 3600 KJ

∴ **459.80 KJ = 0.13 KWh**

Energy calculation for drying at 60 ℃:

m = 2 kg; initial moisture content (M_i) = 70%

∴ Moisture = (2000 × 70)/100 = 1400 g

∴ Dry matter = [2000 – 1400] = 600 g

Final moisture content (M_f) = 40%; 40 g moisture associated with 60 g dry matter

∴ (40 / 60) × 600 = 400 g moisture is associated with 600 g dry matter

∴ 2 kg original matter will lose = (1400-400) g = 1000 g = 1.00 kg of moisture

Initial temperature of potato slices = 25 ℃

∴Heat energy required for 1 kg original material = heat energy to raise temperature to 60 ℃ + latent heat to remove water = 0.6 x 3.48 x (60 - 25) + 1.0 x 4.18 x (60 - 25) + 1.00 x 2257 = 73.08 + 146.30 + 2257.00 = 2476.38 KJ

(Here, latent heat of vaporization of water at std. atmospheric pr. = 2257 KJ/kg; specific heat of water = 4.18 KJ/Kg-℃; specific heat of potato = 3.48 KJ/kg-℃)

Now, 1 KWh = 3600 KJ

∴ **2476.38 KJ = 0.69 KWh**

Energy calculation for frying at 140 ℃for 5 min:

Total heat required by material in KJ = ($m \times c_p \times \Delta T$) = 2.0 * 3.23* (140-25) = 742.90 KJ

Here, m = 2.0 kg; c_p of DVCO = 3.23 KJ/Kg-℃; ΔT = temperature difference in ℃

Now, 1 KWh = 3600 KJ

∴ **371.45 KJ = 0.21 KWh**

APPENDIX 2 D.1

Extraction of fried potato crisps volatiles by Likens-Nickerson's steam-distillation-cum-solvent extraction method

Likens–Nickerson (L-N) concurrent steam-distillation-solvent extraction was used to extract VOCs from T-22 and EC sets, individually. First, 3 L of distilled water and 6 ml of antifoaming agent (silicone oil) were placed in a 5 L round-bottom flask, and boiled to obtain a volatile-free mixture. The boiling was continued until the volume of the mixture was reduced by 250 ml. Now, 250 g of fried potato crisps were blended and mixed with 700 ml distilled water, and then filtered to remove residues. The filtrate was made up to 1000 ml with distilled water and the resulting solution was added gradually to the volatile-free mixture in the round-bottom flask. The mixture was steam-distilled and the flavour extract was collected in a 250 ml round-bottom flask containing 25 ml of distilled water, 1.5 ml of dilute sulphuric acid and 125 ml of DCM, maintained at 50 °C. After 5 hr. of continuous steam-distillation and solvent extraction in DCM, the solvent flask was removed and the sulphuric acid layer was transferred into a 250 ml Erlenmeyer flask, using a 250 ml separating funnel. The solution was neutralized by adding solid sodium bicarbonate, and then poured into a clean 250 ml separating funnel containing 100 ml of fresh DCM. The separating funnel was shaken vigorously until the DCM (upper layer) was clearly separated from the neutral solution (lower layer). The upper layer was poured into a clean 250 ml Erlenmeyer flask and the lower layer was discarded. Anhydrous sodium sulphate was added to the flask to remove dissolved water. Filtering through a nonabsorbent cotton bed of anhydrous sodium sulphate to dry the DCM extract further. The dry DCM extract was then filtered through a Whatman No. 1 filter paper into a 250 ml stoppered conical flask. It was concentrated using Vigreaux column under mild heating condition to 0.5 ml. This extracted fraction was subjected to gas chromatograohy (GC) and e-nose analyses.

APPENDIX 2 D.2

Coding for Support vector machine in MATLAB Matlab® R2020a

Step-1:

```
% % prepare data set

load enosessensordata

species_num = grp2idx(species);
```

Step-2:

```
%binary classification problem

X = random(100, 10);
```

Step-3:

```
X(:,[1,2,3,4,5,6,7,8]) = meas(1:100,:);

Y = species_num(1:100);

%80:20

rand_num = randperm(640);

X_train = X(rand_num(1:80),:);

Y_train = Y(rand_num(1:80),:);

X_test = X(rand_num(81:end),:);

Y_test = Y(rand_num(81:end),:);
```

Step-4:

```
% % CV partition

C = CYpartition(Y_train, ‘k’, 5);
```

Step-5:

```
% % feature selection
```

opts = statset(‘display’, ‘iter’);

fun = @(train_data, train_labels, test_data, test_labels)…

sum(predict(fitcsvm(train_data, train_labels, ‘KernelFunction’, ‘rbf’), test-data) ~ = test_label);

[fs, history] = sequentialfs(fun, X_train, Y_train, ‘CV’, C, ‘options’, opts, ‘nfeatures’, 2);

Step-6:

% % best hyperparameters

X_train_W_best_feature = X_train(:,fs);

Md1 = fitcsvm(X_train_W_best_features, Y_train, ‘KernelFunction’, ‘rbf’, ‘optimizeHyperparameters’, ‘auto’, HyperparameterOptimizationOptions’, Struct(AcquisitionFunctionNam’, ‘expected-improvement-plus’, ‘ShowPlots’, true));

Step-7:

% % test

X_test_W_best_feature = X_test (:, fs);

accuracy = sum (predict (Md1, X_test_W_best_feature) = = (Y_test)/length(Y_tyest)*640;

Step-8:

% % hyperplane

figure;

hgscatter = gsscatter(X_train_W_best_feature(:,1), X_train_W_best-feature(:,2), Y_train);

hold on;

h_SV = plot (Md1. SupportVector(:,1), Md1.SupportVectors(:, 2), ‘ko’, ‘markersize’, 8);

Step-9:

% % decision plane

XL1Ms = get (gca, ‘X1 im’);

```
YL1Ms = get (gca, 'Y1 im');

[Xi, Yi] = meshgrid ([XL1Ms (1):0.01: XL1Ms(2)], [YL1Ms (1):0.01:YL1Ms (2)]);

dd = [Xi (:), Yi(:)];

pred_mesh = predict(Md1, dd);

redcolor = [1, 0.8, 0.8];

bluecolor = [0.8, 0.8, 1];

pos = find(pred_mesh = = 1);

h1 = plot (dd(pos,1), dd(pos, 2), 's', 'color', redcolor, 'Markersize', 5, 'MarkerEdgeColor',
        redcolor, 'MarkerFac

pos = find (pred_mesh = = 2)

h2 = plot (dd(pos, 1), dd(pos, 2), 's', 'color', bluecolor, 'Markersize', 5, 'MarkerEdgeColor',
        bluecolor, 'MarkerFac

uistack (h1, 'bottom')'

uistack (h2, 'bottom');

legend ([hgscatter; h_SV], {'setosa', 'versicolor', 'support vectors'})
```

APPENDIX 2 E.1

Extraction of bread volatiles by Likens-Nickerson's steam-distillation-cum-solvent extraction method

Likens–Nickerson (L-N) concurrent steam-distillation-solvent extraction was used to extract 2-AP from bread crust. In this investigation DCM was used as the extraction solvent for 2-AP. First, 3 L distilled water and 6 ml antifoaming agent (silicone oil) were placed in a 5 L round-bottom flask, and boiled to obtain a volatile-free mixture. The boiling was continued until the volume of the mixture was reduced by 250 ml. 300 g fresh bread crust was mixed with 700 ml distilled water, and then filtered to remove residues. The filtrate was made up to 1000 ml with distilled water and the resulting bread crust solution was added gradually to the volatile-free mixture in the round-bottom flask. The mixture was steam-distilled and the flavour extract was collected in a 250 ml round-bottom flask (containing 60 ml distilled water, 1.5 ml dilute sulphuric acid and 150 ml DCM) maintained at 50 °C. After 4 h of continuous steam-distillation and solvent extraction in DCM, the solvent flask was removed and the sulphuric acid layer was transferred into a 250 ml Erlenmeyer flask using a 250 ml separating funnel. The solution was neutralized by adding solid $NaHCO_3$, and then poured into a clean 250 ml separating funnel containing 150 ml of fresh DCM. The separating funnel was shaken vigorously until the diethyl ether (upper layer) was clearly separated from the neutral solution (lower layer). The upper layer was poured into a clean 250 ml Erlenmeyer flask (and the lower layer was discarded) and dried by filtering through a non-absorbent cotton bed of anhydrous Na_2SO_4 further. The dry DCM extract was then filtered through a Whatman No. 1 filter paper into a 250 ml stoppered conical flask and concentrated to 1 ml using a Vigreux fractional distillation column. The extract was transferred to a screw-capped glass vial and concentrated further by slowly purging nitrogen so as to completely remove the solvent. The extract was stored at -20 °C for further analysis by GC and e-nose. The extract was diluted in DCM to an appropriate volume prior to GC analysis.

APPENDIX 2 E.2

Determination of rheological-properties of dough

The 150 g 'dough-wick' was allowed to stand for 45 min in temperature-controlled cabinet (known as thermostat period). Thereafter, the 'dough-wick' was stretched using stretching hook with the speed of 15.0 ± 0.5 mm/s until it got torn. The results were recorded on extensograms. The values of energy (area under the stretching curve) (A) (cm^2), resistance to extension (R) (BU), maximum resistance to extension (R_m) (BU), extensibility (E) (mm), R/E ($BU.mm^{-1}$) and R_m/E ($BU.mm^{-1}$) ratio of each set of control and experimental sets of dough (Table 4; Figure S1) were obtained by Brabender extensograph (version 4.3.2).

APPENDIX 2 E.3

Coding for BP-MLP in MATLAB Matlab® R2020a

Step-1: Import the data

```
data = readmatrix('shelflife.csv');
X=data (:, 1:8);
Y=data (:, 9);
m=length (Y);
```

Step 2: Normalization of the features and transferring the output (from 0 to 1)

```
Y2=log (1+y);
for i = 1:8
X2(:,i)=X(:,i) – min(X(:,i)))/(max(X(:, i)) – min(X(:,i)));
histogram (Y2,10);
end
histogram (X2(:,1), 10); ('1' can be changed depending on no. of input)
plot (X2 (:, 1), Y2, 'o'); ('1' can be changed depending on no. of input)
```

Step-3: Training of an artificial neural network

```
Xt = X2′;
Yt = Y2′;
hiddenLayerSize = 1; ('1' can be changed for optimization)
net = fitnet(hiddenLayerSize);
net.divideParam.trainRatio = 80/100;
net.divideParam.valRatio = 20/100;
net.divideParam.testRatio = 0/100;
[net,tr] = train(net, Xt, Yt);
```

Step-4: Performance of ANN

```
YTrain = exp(net(Xt(:, tr.trainInd)))-1;
```

```
YTrainTrue = exp(Yt(tr.trainInd))-1;

sqrt(mean(YTrain–YTrainTrue).^2)

Yval = exp (net(Xt(:, tr.valInd)))-1;

YvalTrue = exp (Yt(tr.valInd))-1;

sqrt (mean(Yval-YvalTrue).^2)
```

Step-5: optimization of the number of neurons in hidden layer

```
for i = 1:20

hiddenLayerSize = i;

net = fitnet(hiddenLayerSize);

net.divideParam.trainRatio = 80/100;

net.divideParam.valRatio = 20/100;

net.divideParam.testRatio = 0/100;

%training the ANN

[net, tr] = train(net, Xt, Yt);

%determine the error of the ANN

YTrainTrue = exp (Yt(tr.trialInd))-1;

YTrain = exp(net(Xt(:, tr.trainInd)))-1;

YvalTrue = exp (Yt(tr.valInd))-1;

Yval = exp (net(Xt(:, tr.valInd)))-1;

rmse_train(i) = sqrt(mean(YTrain-YTrainTrue).^2));

rmse_val(i) = sqrt(mean(YVal-YValTrue).^2));

end
```

Step-6: Selection of optimal number of neurons in hidden layer

```
plot(1:20, rmse_train); hold on;

plot(1:20, rmse_val); hold off;
```

(Now, by changing the no. of hidden layer under the section of "Training of an artificial neural network" , run the section repeatedly until lowest RMSE value is obtained. Now, again run the section , "Performance of ANN" from where RMSE values of validation and training set will be obtained for different hidden layer size)

Step-7: Prediction from ANN model

plot(YTrainTrue, YTrain, 'X'); hold on;

plot(0:100, 0:100), hold off;

plot(YValTrue, YVal, 'o'); hold on;

plot(0:100, 0:100), hold off;

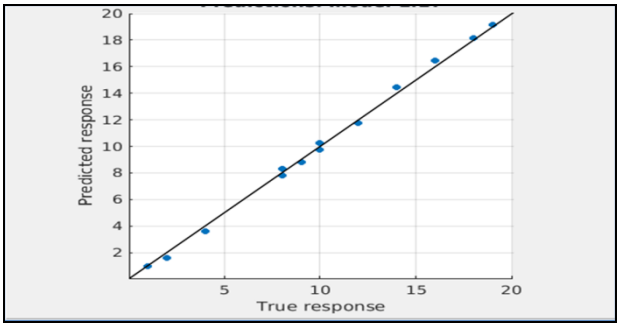


Fig. Plot of predicted vs. experimental values by the selected ANN for storage life stability in terms of freshness

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Method Article

Development of a new equation in fuzzy logic analysis for ascertaining appropriate dose of gamma irradiation of virgin coconut oil

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A B S T R A C T

Our previous investigation had established 4.2 kGy to be the appropriate dosage of gamma irradiation for removal of obnoxious rancid-acid-odor of virgin coconut oil (VCO) on the basis of sensory and electronic nose (e nose) studies. This study endeavored to revalidate the sensory data employing fuzzy logic analysis. An equation has been developed for the first time for deriving defuzzified scores, when the sum of the first and third coordinates of the triplet *(a b c)* of overall sensory score was greater than 100, i.e. (a + c) > 100. This study reaffirmed 4.2 kGy to be the most preferred dose for deodorization of VCO. Besides, ranking of the VCO samples were similar by either approach.

- According to the fuzzy logic method, overall sensory scores were assigned to the VCO samples under investigation, these sensory scores have been represented by a triangle and a polygon when (a+c) is less and more than100, respectively.
- The coordinates of the polygon were determined and a new equation has been developed for evaluating defuzzified scores, which has been validated by similarity value analysis.
- This new methodology of fuzzy logic analysis can be used to rank samples rapidly and reliably, without any complexity of conventional similarity value approach.

A R T I C L E I N F O

Keywords: Deodorized coconut oil, Shelf-life study, Sensory evaluation, Fuzzy logic, New developed equation, Similarity values

Article history: Received 24 November 2017; Accepted 29 January 2018; Available online 1 February 2018

Abbreviations: S, triplet; SO, overall sensory score; Q_{REL}, relative weightage; Ya, defuzzified score under condition of (a + c) ≤ 100; Ya´, defuzzified score under condition of (a + c) > 100; B_x, overall membership function; S_m, similarity values; IVCO, irradiated virgin coconut oil.

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Specifications Table

Subject Area	Agricultural and Biological Sciences
More specific subject area:	Food Technology
Method name:	Fuzzy logic analysis
Name and reference of original method	Fuzzy logic analysis H. Das, Sensory Evaluation Using Fuzzy Logic In Food Processing Operations Analysis, 2005, Asian Books, New Delhi, 383-402.
Resource availability	Hard copy of the above mentioned book

Method details

Background

Presently, quantification of food quality is being addressed in food research and business practices [1]. Consequently, data are essential to describe food qualities for product development, quality control and process control. Statistical techniques for sensory tests are routinely in use and are under continuous advancement. The data for statistical analysis are in linguistic expressions which must be converted by cumbersome processes to numerical values. This is circumvented by use of fuzzy logic theory, a mathematical technique which can directly quantify human linguistic expression and therefore enables quantification of primary and imprecise data obtained from sensory tests. This logical approach comprises of mainly three steps: i) an appropriate definition of how good a certain quality parameter level is, ii) a sound way to combine several quality parameters and iii) a way to express the overall quality based on all these individual parameters, considering their individual relative importance [2,5].

Studies from our previous investigation [3] have established 4.2 kGy to be the appropriate dosage of gamma irradiation for removal of obnoxious rancid-acid-odor of virgin coconut oil (VCO). This dosage was confirmed by sensory evaluation of irradiated oil samples and their odor profile analyses by e-nose. This study endeavors to establish concurrence of data obtained from e-nose analysis (of deodorized VCO conducted at a regular interval of seven days by thirty semi-trained panelists for a storage period of 40 days) and fuzzy logic analysis of sensory scores. The main objective of the current study was to develop an equation for deriving defuzzified scores when the sum of the first and third coordinates of the triplet (a b c) of overall sensory score (SO) is greater than 100, i.e. $(a + c) > 100$. The secondary objective of this work was to validate the new developed equation using the data obtained from similarity value and electronic nose(e-nose) analyses.

Procurement of raw material

A gift sample of a leading brand of expeller pressed Virgin coconut oil (VCO) (obtained from coconut copra of *West coast tall* variety coconuts) with no added antioxidant and preservative was used in this study, in accordance with our previous study [3].

Preparation of sample for irradiation

Thirty LDPE co-polymer screw capped bottles (500 mL) were sterilized under UV light in a laminar hood. Each bottle was filled with 500 mL VCO and was numbered serially. The bottled oil samples were then irradiated in a Co-60 γ irradiation chamber [(GC 5000; Serial No. GIC 038); source: Cobalt 60 solid (dose rate: 5.201 kGy h^{-1})] of BRIT, Mumbai in the National Instrument Laboratory (NIL) campus of Jadavpur University at 23 ± 2 °C at the pre-selected dose levels of 0.0 kGy (control set, IVCO 1), 4.0 kGy (IVCO 2), 4.2 kGy (IVCO 3), 4.5 kGy (IVCO 4) and 5.0 kGy (IVCO 5), in accordance with the procedure reported by [3]. Post irradiation, all samples were stored at room temperature (23 ± 2 °C) in these

airtight LDPE co-polymer screw capped bottles for 40 days. Sensory evaluation was conducted for the irradiated oil samples at an interval of seven days for a total period of forty days in accordance with our previous report [3].

Sensory evaluation of irradiated coconut oil samples

Sensory evaluation of the above irradiated coconut oil samples were conducted inside a university classroom at 24 ± 1 °C in bright light. The panel for sensory evaluation consisted of 30 members (in the age group of 21–45 years) including trained students and few semi-trained staff of the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, India. The 30-member panel was sufficient for our analysis since fuzzy logic reportedly minimizes all the biasing effect, generated due to different consumers' perception [5]. Panelists evaluated the samples in terms of aroma, color, homogeneity and flowability using the standard 5-point hedonic scale (1–5) [1 = not satisfactory, 2 = fair, 3 = medium, 4 = good, 5 = excellent]. During testing, the panelists were monitored by the authors. The panelists were served with coffee beans (for smelling) in between to enable them to correctly differentiate the aroma among the irradiated oil samples. The order of the samples served was same for all the panelists in a particular session, but varied between two sessions. Panelists were asked to provide numerical score against each sensory attribute of each sample and also give their preference against each quality attribute (aroma, color, homogeneity and flowability), following respective scale factors [not at all important (NI), somewhat important (SI), important (I), highly important (HI), extremely important (EI)] for each sample, relative to the entire sample set. The main steps of fuzzy modeling of sensory evaluation are: (1) calculation of triplet (S) corresponding to different quality attributes (aroma, color, homogeneity and flowability); (2) relative weightage (Q_{REL}) of particular quality attributes (aroma, color, homogeneity and flowability), (3) overall sensory score (SO); (4) calculation of defuzzified score (Y_a) while $(a + c) < 100$ and (Ya) when $(a + c) > 100$ and (5) ranking of quality attributes and grading of samples [5].

Triangular fuzzy number (TFN) and fuzzy arithmetic operations

Triangular membership function distribution pattern of sensory scale is shown in Fig. 1. This represents a 5-point sensory scale viz., poor/not at all important, fair/somewhat important, good/important, very good/highly important and excellent/extremely important. The triplet (a, b, c) associated with sensory scale is a triangular fuzzy number. Here, 'a' (first number of triplets) is called the mean value of the fuzzy number and it denotes the coordinate of abscissa at which the value of membership function is 1. The second and third numbers of the triplets 'b' and 'c' are called the left and right spreads respectively, whose membership functions are 0 [6].

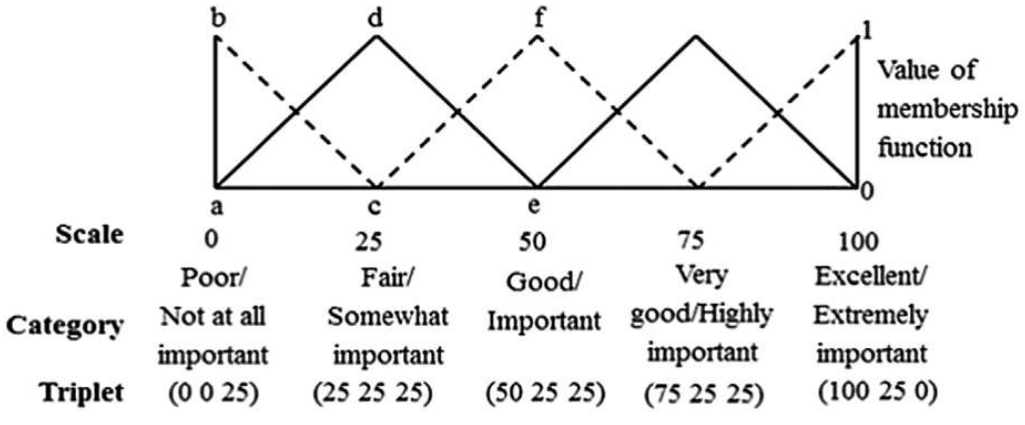

Fig. 1. Distribution pattern of five point sensory scale.

Triplets for sensory scores of irradiated oil samples and overall quality of samples

Triplets corresponding to specific quality attributes such as aroma, color, homogeneity and flowability were derived from the sum of sensory scores (Table 1) and the triplets associated with sensory scale and total number of panelists. For example, for IVCO 1 (0.0 kGy) on day 0: out of 30 panelists, 18 panelists agreed on a 'not significant' score, while 12 panelists provided the score 'fair' against the sensory attribute aroma (Table 1). Therefore, the triplets of the sensory scores for aroma of

Table 1
Panelists' preference for specific quality attributes of irradiated coconut oil samples and corresponding triplets.

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory score
Aroma							
IVCO 1	0	18	12	0	0	0	$S1A_0$ = (10.00 10.00 25.00)
	7	16	14	0	0	0	$S1A_7$ = (11.67 11.67 25.00)
	14	18	12	0	0	0	$S1A_{14}$ = (10.00 10.00 25.00)
	21	19	11	0	0	0	$S1A_{21}$ = (9.17 9.17 25)
	28	20	10	0	0	0	$S1A_{28}$ = (8.33 8.33 25.00)
	40	20	10	0	0	0	$S1A_{40}$ = (8.33 8.33 25.00)
IVCO 2	0	12	18	0	0	0	$S2A_0$ = (15.00 15.00 25.00)
	7	15	15	0	0	0	$S2A_7$ = (12.50 12.50 25.00)
	14	17	13	0	0	0	$S2A_{14}$ = (10.83 10.83 25.00)
	21	18	12	0	0	0	$S2A_{21}$ = (10.00 10.00 25.00)
	28	19	11	0	0	0	$S2A_{28}$ = (9.17 9.17 25.00)
	40	20	10	0	0	0	$S2A_{40}$ = (8.33 8.33 25.00)
IVCO 3	0	0	17	13	0	0	$S3A_0$ = (35.83 25.00 25.00)
	7	0	11	19	0	0	$S3A_7$ = (40.83 25.00 25.00)
	14	0	10	20	0	0	$S3A_{14}$ = (41.67 25.00 25.00)
	21	0	8	22	0	0	$S3A_{21}$ = (43.33 25.00 25.00)
	28	0	0	0	7	23	$S3A_{28}$ = (94.17 25.00 5.83)
	40	0	0	0	1	29	$S3A_{40}$ = (99.17 25.00 0.83)
IVCO 4	0	0	16	14	0	0	$S4A_0$ = (36.67 25.00 25.00)
	7	0	21	9	0	0	$S4A_7$ = (32.50 25.00 25.00)
	14	0	30	0	0	0	$S4A_{14}$ = (25.00 25.00 25.00)
	21	17	13	0	0	0	$S4A_{21}$ = (10.83 10.83 25.00)
	28	18	12	0	0	0	$S4A_{28}$ = (10.00 10.00 25.00)
	40	19	11	0	0	0	$S4A_{40}$ = (9.17 9.17 25.00)
IVCO 5	0	0	17	13	0	0	$S5A_0$ = (35.83 25.00 25.00)
	7	0	24	6	0	0	$S5A_7$ = (30.00 25.00 25.00)
	14	0	30	0	0	0	$S5A_{14}$ = (25.00 25.00 25.00)
	21	18	12	0	0	0	$S5A_{21}$ = (10.00 10.00 25.00)
	28	19	11	0	0	0	$S5A_{28}$ = (9.17 9.17 25.00)
	40	20	10	0	0	0	$S5A_{40}$ = (8.33 8.33 25.00)
Colour							
IVCO 1	0	0	17	13	0	0	$S1C_0$ = (35.83 25.00 25.00)
	7	0	18	12	0	0	$S1C_7$ = (35.00 25.00 25.00)
	14	0	17	13	0	0	$S1C_{14}$ = (35.83 25.00 25.00)
	21	18	12	0	0	0	$S1C_{21}$ = (10.00 10.00 25.00)
	28	19	11	0	0	0	$S1C_{28}$ = (9.17 9.17 25.00)
	40	19	11	0	0	0	$S1C_{40}$ = (9.17 9.17 25.00)
IVCO 2	0	0	19	11	0	0	$S2C_0$ = (34.17 25.00 25.00)
	7	0	19	11	0	0	$S2C_7$ = (34.17 25.00 25.00)
	14	0	18	12	0	0	$S2C_{14}$ = (35.00 25.00 25.00)
	21	11	19	0	0	0	$S2C_{21}$ = (15.83 15.83 25.00)

Table 1 (*Continued*)

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory score
	28	12	18	0	0	0	$S2C_{28}$ = (15.00 15.00 25.00)
	40	12	18	0	0	0	$S2C_{40}$ = (15.00 15.00 25.00)
IVCO 3	0	0	18	12	0	0	$S3C_0$ = (35.00 25.00 25.00)
	7	0	13	17	0	0	$S3C_7$ = (39.17 25.00 25.00)
	14	0	12	18	0	0	$S3C_{14}$ = (40.00 25.00 25.00)
	21	0	5	20	5	0	$S3C_{21}$ = (50.00 25.00 25.00)
	28	0	0	5	15	5	$S3C_{28}$ = (62.50 20.83 16.67)
	40	0	0	0	2	28	$S3C_{40}$ = (98.33 25.00 1.67)
IVCO 4	0	0	16	14	0	0	$S4C_0$ = (36.67 25.00 25.00)
	7	0	11	19	0	0	$S4C_7$ = (40.83 25.00 25.00)
	14	0	11	19	0	0	$S4C_{14}$ = (40.83 25.00 25.00)
	21	0	13	17	0	0	$S4C_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S4C_{28}$ = (38.33 25.00 25.00)
	40	0	15	15	0	0	$S4C_{40}$ = (37.50 25.00 25.00)
IVCO 5	0	0	17	13	0	0	$S5C_0$ = (35.83 25.00 25.00)
	7	0	10	20	0	0	$S5C_7$ = (41.67 25.00 25.00)
	14	0	15	5	0	0	$S5C_{14}$ = (20.83 16.67 16.67)
	21	0	13	17	0	0	$S5C_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S5C_{28}$ = (38.33 25.00 25.00)
	40	0	15	15	0	0	$S5C_{40}$ = (37.50 25.00 25.00)
Homogeneity							
IVCO 1	0	0	15	15	0	0	$S1H_0$ = (37.50 25.00 25.00)
	7	0	15	15	0	0	$S1H_7$ = (37.50 25.00 25.00)
	14	0	18	12	0	0	$S1H_{14}$ = (35.00 25.00 25.00)
	21	0	13	17	0	0	$S1H_{21}$ = (39.17 25.00 25.00)
	28	0	13	17	0	0	$S1H_{28}$ = (39.17 25.00 25.00)
	40	0	13	17	0	0	$S1H_{40}$ = (39.17 25.00 25.00)
IVCO 2	0	0	19	11	0	0	$S2H_0$ = (34.17 25.00 25.00)
	7	0	16	14	0	0	$S2H_7$ = (36.67 25.00 25.00)
	14	0	17	13	0	0	$S2H_{14}$ = (35.83 25.00 25.00)
	21	0	19	11	0	0	$S2H_{21}$ = (34.17 25.00 25.00)
	28	0	21	9	0	0	$S2H_{28}$ = (32.50 25.00 25.00)
	40	0	21	9	0	0	$S2H_{40}$ = (32.50 25.00 25.00)
IVCO 3	0	0	14	16	0	0	$S3H_0$ = (38.83 25.00 25.00)
	7	0	13	17	0	0	$S3H_7$ = (39.17 25.00 25.00)
	14	0	14	16	0	0	$S3H_{14}$ = (38.33 25.00 25.00)
	21	0	7	19	4	0	$S3H_{21}$ = (47.50 25.00 25.00)
	28	0	0	5	25	0	$S3H_{28}$ = (70.83 25.00 20.00)
	40	0	0	0	3	27	$S3H_{40}$ = (98.33 25.00 25.00)
IVCO 4	0	0	17	13	0	0	$S4H_0$ = (35.83 25.00 25.00)
	7	0	15	15	0	0	$S4H_7$ = (37.50 25.00 25.00)
	14	0	11	19	0	0	$S4H_{14}$ = (40.83 25.00 25.00)
	21	0	13	17	0	0	$S4H_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S4H_{28}$ = (38.33 25.00 20.00)
	40	0	15	15	0	0	$S4H_{40}$ = (37.50 25.00 25.00)
IVCO 5	0	0	16	14	0	0	$S5H_0$ = (36.67 25.00 25.00)
	7	0	16	14	0	0	$S5H_7$ = (36.67 25.00 25.00)
	14	0	14	16	0	0	$S5H_{14}$ = (38.33 25.00 25.00)
	21	0	13	17	0	0	$S5H_{21}$ = (39.17 25.00 25.00)
	28	0	14	16	0	0	$S5H_{28}$ = (38.33 25.00 20.00)
	40	0	15	15	0	0	$S5H_{40}$ = (37.50 25.00 25.00)

Table 1 (*Continued*)

Sensory quality attributes of irradiated coconut oil samples	Days	Not satisfactory	Fair	Medium	Good	Excellent	Triplets of sensory score
Flowability							
IVCO 1	0	0	12	18	0	0	$S1F_0$ = (40.00 25.00 25.00)
	7	0	17	13	0	0	$S1F_7$ = (35.83 25.00 25.00)
	14	0	16	14	0	0	$S1F_{14}$ = (36.67 25.00 25.00)
	21	0	14	16	0	0	$S1F_{21}$ = (38.33 25.00 25.00)
	28	0	14	16	0	0	$S1F_{28}$ = (38.33 25.00 25.00)
	40	0	14	16	0	0	$S1F_{40}$ = (38.33 25.00 25.00)
IVCO 2	0	0	15	15	0	0	$S2F_0$ = (37.50 25.00 25.00)
	7	0	18	12	0	0	$S2F_7$ = (35.00 25.00 25.00)
	14	0	15	15	0	0	$S2F_{14}$ = (37.50 25.00 25.00)
	21	0	14	16	0	0	$S2F_{21}$ = (38.33 25.00 25.00)
	28	0	15	15	0	0	$S2F_{28}$ = (37.50 25.00 25.00)
	40	0	15	15	0	0	$S2F_{40}$ = (37.50 25.00 25.00)
IVCO 3	0	0	13	17	0	0	$S3F_0$ = (39.17 25.00 25.00)
	7	0	15	15	0	0	$S3F_7$ = (37.50 25.00 25.00)
	14	0	14	16	0	0	$S3F_{14}$ = (38.33 25.00 25.00)
	21	0	7	20	3	0	$S3F_{21}$ = (46.67 25.00 25.00)
	28	0	0	16	14	0	$S3F_{28}$ = (61.67 25.00 25.00)
	40	0	0	0	10	20	$S3F_{40}$ = (91.67 25.00 8.33)
IVCO 4	0	0	18	12	0	0	$S4F_0$ = (35.00 25.00 25.00)
	7	0	18	12	0	0	$S4F_7$ = (35.00 25.00 25.00)
	14	0	18	12	0	0	$S4F_{14}$ = (35.00 25.00 25.00)
	21	0	14	16	0	0	$S4F_{21}$ = (38.33 25.00 25.00)
	28	0	15	15	0	0	$S4F_{28}$ = (37.50 25.00 25.00)
	40	0	16	14	0	0	$S4F_{40}$ = (36.67 25.00 25.00)
IVCO 5	0	0	20	10	0	0	$S5F_0$ = (33.33 25.00 25.00)
	7	0	18	12	0	0	$S5F_7$ = (35.00 25.00 25.00)
	14	0	19	11	0	0	$S5F_{14}$ = (34.17 25.00 25.00)
	21	0	15	15	0	0	$S5F_{21}$ = (37.50 25.00 25.00)
	28	0	14	16	0	0	$S5F_{28}$ = (38.33 25.00 25.00)
	40	0	15	15	0	0	$S5F_{40}$ = (37.50 25.00 25.00)

IVCO 1 (S1A) can be obtained as follows:

$$S1A_0 = \frac{18(0025) + 12(252525) + 0(502525) + 0(752525) + 0(100250)}{18 + 12 + 0 + 0 + 0}) \quad (1)$$

Similar values for other quality attributes of all samples (during the entire storage period) were obtained using the aforesaid equation (Eq. (1)). Relative weightage (Q_{REL}) for all sensory attributes (aroma, color, homogeneity and flowability) were calculated by dividing the triplets of each quality attribute with Q_{SUM} as has been described by Das [2] (Table 2). The triplets of overall sensory scores of all irradiated oil samples for each quality attribute were evaluated using Eq. (2). For example, the overall sensory score of IVCO 1 at day 0 (i.e., $SO1_0$) (Table 4) would be obtained as follows:

$$SO1_0 = S1A_0 \odot QA_{REL} \oplus S1C_0 \odot QC_{REL} \oplus S1H_0 \odot QH_{REL} \oplus S1F_0 \odot QF_{REL} \quad (2)$$

where, $S1A_0$, $S1C_0$, $S1H_0$ and $S1F_0$ represent the triplets corresponding to aroma, color, homogeneity and flowability of IVCO 1 at day 0, respectively. QA_{REL}, QC_{REL}, QH_{REL} and QF_{REL} signify the triplets corresponding to relative weightages of aroma, color, homogeneity and flowability, of irradiated oil samples, respectively. In this way, the overall sensory scores for all samples (during storage period) were calculated.

Table 2
Panelists' preference for quality attributes of irradiated coconut oil samples in general and corresponding triplets.

Quality attributes	NI	SI	I	HI	EI	Triplets of sensory score	triplets of relative weightage
Aroma	0	0	0	0	30	(75.00 25.00 25.00)	(0.50 0.17 0.17)
Colour	30	0	0	0	0	(0 0 25.00)	(0.00 0.00 0.17)
Homogeneity	0	0	30	0	0	(50.00 25.00 25.00)	(0.33 0.17 0.17)
Flowability	0	0	0	0	0	(25.00 25.00 25.00)	(0.17 0.17 0.17)

Triplets (a, b, c) representing the overall sensory score (SO) was denoted by triangle XYZ (Fig. 3a and b). When the value of $(a + c) \leq 100$, the triangle XYZ lies within the sensory scale interval [0,100]. This is represented by Fig. 3(a). Under this condition, the value of Y_a in terms of a, b and c can be expressed by Eq. (3) [7], where Y_a is the centroid as well as the defuzzified score of triangle XYZ (Fig. 3a).

$$Ya = \frac{(3a - b + c)}{3} \tag{3}$$

However, under the second condition, i.e. $(a + c) > 100$, polygon XYMN has to be considered instead of triangle XYZ, to maintain the standard sensory scale in the range 0–100 (Fig. 3b). This necessitated development of a new equation for deriving the defuzzified score of a sample when $(a + c) > 100$.

Development of new equation for obtaining defuzzified scores

Eq. (3) was not considered for finding the value of defuzzified scores of polygon XYMN [condition: $(a + c) > 100$]. It is well known that the centroid of the polygon represents the defuzzified score of the same. Here, centroid of polygon XYMN was derived using Eq. (4) which represents centroid of a polygon in general.

$$Ya = \frac{1}{6A}\sum_{i=0}^{n-1}(x_i + x_{i+1})(x_i y_{i+1} + x_{i+1} y_i) \tag{4}$$

As per Fig. 3(b), Eq. (4) can be written in the following way:

$$Ya = \frac{1}{6A}[(x_0 + x_1)(x_0 y_1 - x_1 y_0) + (x_1 + x_2)(x_1 y_2 - x_2 y_1) + (x_2 + x_3)(x_2 y_3 - x_3 y_2)] \tag{5}$$

where,

$$A = \frac{1}{2}[(x_0 y_1 - x_1 y_0) + (x_1 y_2 - x_2 y_1) + (x_2 y_3 - x_3 y_2)] \tag{6}$$

It therefore follows that

$$\therefore Ya = \frac{(x_0 + x_1)(x_0 y_1 - x_1 y_0) + (x_1 + x_2)(x_1 y_2 - x_2 y_1) + (x_2 + x_3)(x_2 y_3 - x_3 y_2)}{3(x_0 y_1 - x_1 y_0) + (x_1 y_2 - x_2 y_1) + (x_2 y_3 - x_3 y_2)} \tag{7}$$

Here, Ya and A are the centroid and area of polygon XYMN, respectively. In Fig. 3b, the coordinates of points X, Y, M, N are $(x_0\ y_0)$, $(x_1\ y_1)$ $(x_2\ y_2)$ $(x_3\ y_3)$, respectively. In accordance with Fig. 3b, the values of these coordinates are as follows: $x_0 = (a–b)$, $x_1 = a$, $x_2 = x_3 = 100$ and $y_0 = y_3 = 0$, $y_1 = 1$, $y_2 = MN$. The MN value was obtained from the rule of similarity of triangle, discussed below. Here, Δ YOZ and Δ MNZ are always right angled triangles. Therefore, the rule of similarity was employed here and values of MN were obtained (shown below). The centroid of polygon XYMN was found by putting all the values of coordinates in Eq. (7). Thus final equation of Ya of polygon XYMN has been derived and reported by us

for the first time and is represented by Eq. (8). This equation is not reported in literature.

$$\begin{aligned} \mathrm{MN/YO} &= \mathrm{NZ/OZ} \\ \therefore \mathrm{MN} &= \frac{a}{c} + 1 - \frac{100}{c} \\ \therefore \mathrm{A} &= \frac{2ac - bc + a^2 + 200a - 200c + 10000}{c} \\ \therefore Ya &= \frac{(a^3 + b^2c + 3a^2c - 3abc - 3 \times 10^4 a - 3 \times 10^4 c + 2 \times 10^6)}{3(a^2 + 2ac - bc - 2 \times 10^2 a - 2 \times 10^2 c + 1 \times 10^4)} \end{aligned} \tag{8}$$

Defuzzified values for ranking of samples and their quality attributes

The relative importance of four quality attributes (*viz.*, aroma, color, homogeneity and flowability) was obtained from their defuzzified scores. The defuzzified scores were compared with the six point sensory scales of linguistic parameters, i.e., not satisfactory, fair, satisfactory, good, very good and excellent (Fig. 2), which are classified by following number ranges: 10, 10–30, 30–50, 50–70, 70–90, 90–100, respectively. In order to judge all the samples on each day of storage with respect to all quality attributes (aroma, color, homogeneity and flowability), the defuzzified scores were considered in this comparative study, instead of S_m. The defuzzified scores of all samples were calculated using Eq. (3) or Eq. (8), depending on the value of (a + c).

It was observed that, (a + c) values of IVCO 3 on days 21, 28 and 40 were 101.53, 144.58 and 164.44, respectively [i.e. (a + c) > 100]. For these three cases, Eq. (8) was used instead of Eq. (3) for obtaining their respective defuzzified scores.

Shortcoming of the procedure for ranking of the samples by similarity principle

The overall sensory score (obtained as a single triplet) was fitted into the six point sensory scale (referred to as standard fuzzy scale) employing similarity analysis. Calculation of S_m is very time consuming owing to complexity of its calculation. S_m for the irradiated oil samples were calculated employing overall membership function values of sensory scores and values of membership functions of standard fuzzy scale. Standard fuzzy scale, viz. not satisfactory/not at all necessary, fair/somewhat necessary, medium/necessary, good/important, very good/highly important and excellent/extremely important was designated as F1, F2, F3, F4, F5 and F6, respectively (Fig. 3). As per Fig. 3, the values of membership functions are defined by a set of 10 numbers, elaborated in (9).

$$\begin{aligned} F1 &= (1, 0.5, 0, 0, 0, 0, 0, 0, 0, 0) \\ F2 &= (1, 0.5, 0, 0, 0, 0, 0, 0, 0, 0) \\ F3 &= (0, 0, 0.5, 1, 1, 0.5, 0, 0, 0, 0) \\ F4 &= (0, 0, 0, 0, 0.5, 1, 1, 0.5, 0, 0) \\ F5 &= (0, 0, 0, 0, 0, 0, 0.5, 1, 1, 0.5) \\ F6 &= (0, 0, 0, 0, 0, 0, 0, 0, 0.5, 1) \end{aligned} \tag{9}$$

The membership function of a triplet (a b c) has been presented graphically in Fig. 3. According to Fig. 3, for the triplet (a b c), the value of membership function is 1 when the value of abscissa is 'a' and

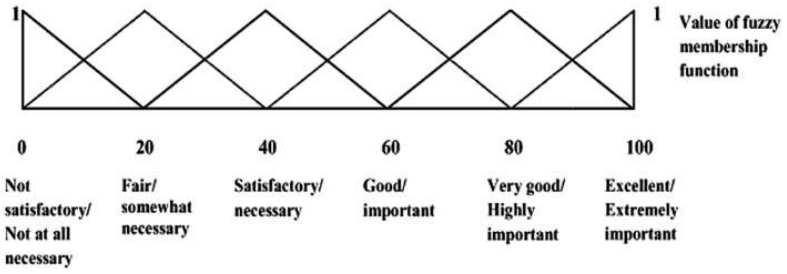

Fig 2. Standard Fuzzy scale.

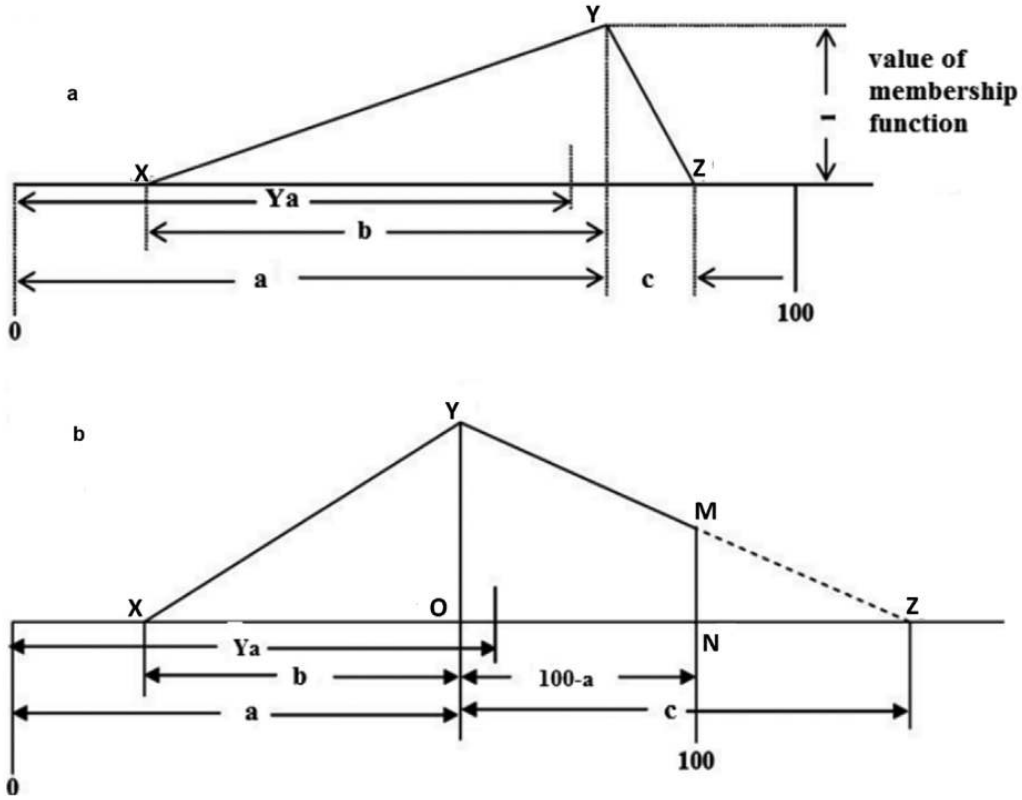

Fig. 3. 3 (a) Graphical view of overall sensory score as triangle ABC when the value of (a+c) is less than 100, the triangle XYZ lies within the sensory scale interval [0, 100], (b) Graphical view of overall sensory score as polygon XYMN when the value of (a+c) is greater than 100, a part of the triangle XYZ lies beyond the interval 0 to 100.

the value of membership function is 0 when abscissa is less than '(a–b)' or greater than '(a + c)'. The value of membership function, B_x at x = 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 obtained from overall sensory scores of the irradiated coconut oil samples were calculated from Eq. (10). After calculating the B_x values (as a set of ten values) for each sample, S_m were derived using Eq. (11). Determination of S_m comprises of the following steps: step1: determination of B_x values for each sample; step 2: multiplication of matrix F with transpose matrix of F (F x F^T); step 3: multiplication of matrix F with transpose matrix of B (F x B_x^T) and step 4: multiplication of matrix B with transpose matrix of B (B_x x B_x^T). Thus S_m values were calculated under six categories of sensory scales, employing these steps. The highest S_m was considered and its corresponding category described the quality of sample. This approach clearly demonstrated that determination of defuzzified scores using Eq. (8) is relatively simpler than determination of S_m values. Thus the advantage of using Eq. (8) [condition: (a + c) > 100] lies in its simplicity.

$$\begin{aligned} B_X &= \frac{X-(a-b)}{b} \ \text{for}(a-b) < x < a \\ B_x &= \frac{(a+c)-x}{c} \ \text{for} a < x < (a+c) \\ B_x &= 0 \text{for} x < (a-b) \ \text{and} \ x > (a+c) \end{aligned} \tag{10}$$

$$S_m(F,B) = \frac{F \times B^T}{\text{Max}\left(F \times F^T \text{and} B \times B^T\right)} \tag{11}$$

Validation of the newly developed equation

The conclusion derived from the results of newly developed equation (Eq. (8)) was validated from results of similarity value and also by odor profile analysis of the irradiated oil samples using e-nose (ENOVISION, C-DAC). The signal responses ($\Delta R/R$) of the irradiated coconut oil samples, obtained from

e-nose analysis have been reported by our research group [3]. Validation by S_m was carried out by standard fuzzy and sensory scales.

Ranking of quality attributes of irradiated coconut oil samples based on defuzzified scores

The triplets of sensory scores for four quality attributes (aroma, color, homogeneity and flowability) in general, were calculated using Eq. (1). Sensory scores, triplets associated with these scores of quality attributes of irradiated coconut oil samples and relative weightages of all quality attributes are presented in Table 2. Defuzzified scores for four quality attributes of each sample were calculated using Eq. (3) (Table 3). The Y_a values for aroma and homogeneity were obtained as 75.00 and 50.00, respectively. These results illustrated that aroma had received the highest importance in sensory acceptance followed by sample homogeneity. The defuzzified scores for other two quality attributes (color and flowability) were 8.33 and 25, respectively. Therefore, the following trend of preference of quality attributes of the irradiated coconut oil samples can be arrived at using linguistic representations of standard sensory scale.

Aroma (highly important) > homogeneity (important) > flowability (somewhat important) > color (not at all important)

Defuzzified scores of deodorized coconut irradiated oil samples and sample ranking

The sensory scores of five samples w.r.t four quality attributes (aroma, color, homogeneity and flowability) on each day of storage have been presented in Table 1.The triplets associated with sensory scores were determined using Eq. (1). Defuzzified scores for the stored irradiated-coconut oil samples were evaluated using Eq. (3) or Eq. (8), depending on the value of (a + c). For example, triplets of overall sensory score of IVCO 1 on day 0 ($SO1_0$) were found to be (24.57 32.08 45.56) from Eq. (2). Here, (a + c) = 70.13 (<100). Therefore, Eq. (3) was employed for finding defuzzified scores of IVCO 1 on day 0. For IVCO 3, Eq. (3) was applicable up to day 14. From day 21 onwards, (a + c) values were greater than 100. Triplets of overall sensory score ($SO3_{21}$) of IVCO 3 on day 21 were (45.28 47.92 56.25). The value of (a + c) was 101.53 (>100). Therefore, under this condition, Eq. (8) was used instead of Eq. (3) to determine the defuzzified scores (Ya) of IVCO 1.

In case of IVCO 1 and 2, defuzzified scores (Y_a) declined gradually up to day 40 (Table 4), meaning that sample acceptance with respect to all quality attributes deteriorated with time. Y_a values of IVCO 1 (27.22) and 2 (26.16) were found to be maximum on day 21 of storage period. It implied that the above two samples reached almost saturation level of deterioration on the said day. For IVCO 4 and 5, the defuzzified scores declined in a similar manner as those of IVCO 1 and 2 with a variation in slope (Fig. 4). From Table 4, it was found that the defuzzified scores of IVCO 4 and 5 were obtained under category 'satisfactory' up to day 14, which came down to 29.40 and 28.98, respectively on day 21 under category 'fair'. Therefore, 4.5 kGy and 5.0 kGy dose levels did not show effective deodorization effects in irradiated coconut oil samples with time, especially with respect to its rancid-acid aroma. Comparing the defuzzified scores of all the five samples on each day of storage study, it was observed that only IVCO 3 underwent continuous improvement with time, implying that 4.2 kGy dose level had effectively deodorized the oil sample with time. The defuzzified scores for IVCO 3 became high from day 28 onwards, i.e. 70.21 on day 28 and 74.70 on day at 40, respectively (both under 'very good'

Table 3
Defuzzified score of quality attributes of irradiated coconut oil samples.

Quality attributes	Q (for relative importance)			Defuzzified scores
	a	b	c	
Aroma	75	25	25	75.00
Colour	0	0	25	8.33
Homogeneity	50	25	25	50.00
Flowability	25	25	25	25.00

Table 4
Defuzzified scores of irradiated coconut oil sample.

Time	Sample no.	SO			Y_a/Y_a ·	
		a	b	c		Remarks
Day 0	IVCO 1	24.17	32.08	45.56	28.66	Fair
	IVCO 2	25.14	34.44	45.14	28.70	Fair
	IVCO 3	37.22	43.89	49.72	39.17	Satisfactory
	IVCO 4	36.11	42.92	49.03	38.15	Satisfactory
	IVCO 5	35.69	42.64	48.61	37.69	Satisfactory
Day 7	IVCO 1	24.31	32.50	45.00	28.47	Fair
	IVCO 2	24.31	32.78	44.72	28.29	Fair
	IVCO 3	39.74	45.26	52.01	41.99	Satisfactory
	IVCO 4	34.58	42.50	49.31	36.85	Satisfactory
	IVCO 5	33.06	41.94	48.89	35.57	Satisfactory
Day 14	IVCO 1	22.78	31.11	44.58	27.27	Fair
	IVCO 2	23.61	31.94	44.86	27.92	Fair
	IVCO 3	40.00	44.72	51.39	42.22	Satisfactory
	IVCO 4	31.94	41.81	48.61	34.21	Satisfactory
	IVCO 5	30.97	41.25	44.72	32.13	Satisfactory
Day 21	IVCO 1	24.03	31.53	41.11	27.22	Fair
	IVCO 2	22.78	31.25	41.39	26.16	Fair
	IVCO 3	45.28	47.92	56.25	**48.04**	Satisfactory
	IVCO 4	24.86	32.64	46.25	29.40	Fair
	IVCO 5	24.31	31.94	45.97	28.98	Fair
Day 28	IVCO 1	23.61	30.97	40.83	26.90	Fair
	IVCO 2	21.67	30.28	40.69	25.14	Fair
	IVCO 3	80.97	62.78	63.61	**70.21**	*Very good*
	IVCO 4	24.03	31.81	45.69	28.66	Fair
	IVCO 5	23.75	31.39	45.69	28.52	Fair
Day 40	IVCO 1	23.61	30.97	40.83	26.90	Fair
	IVCO 2	21.25	29.72	40.56	24.86	Fair
	IVCO 3	97.36	73.06	67.08	**74.71**	*Very good*
	IVCO 4	23.19	30.97	45.14	27.92	Fair
	IVCO 5	22.92	30.56	45.14	27.78	Fair

The bold values in Table 4 signify that defuzzified scores of IVCO 3 were found to exceptionally increase from day 21 compared to the rest of the samples (IVCO 1, 2, 4 and 5). In addition, the summation of first and third coordinates of overall sensory score of IVCO 3 was found to be greater than 100 from day 21 onwards until day 40.

category). The defuzzified scores for the rest of the samples were found to be below 50 throughout the study period and therefore they were under categories of 'fair' or 'satisfactory'. Thus, it was established that 4.2 kGy irradiated coconut oil sample alone had good sensory acceptance specifically with respect to deodorization (aroma) from day 28 onwards as had been inferred from our studies using e-nose (discussed later).

Validation of new developed equation by similarity principle

Values of overall membership function (B_x) and similarity values of five samples (IVCO −1, 2, 3, 4, 5) on each storage day were calculated using Eqs. (10) and (11), respectively. For example, the triplet of overall sensory score of IVCO 1 at day 0 was found as (24.57 32.08 45.56), i.e. a = 24.57, b = 32.08, c = 45.56. Using the value of this triplet in Eq. (10), the values of membership function for IVCO 1(at day 0) was obtained as $B1_0$ = (0.5582 0.8700 1 0.8720 0.6525 0.4330 0.2135 0.0000 0.0000 0.0000). Similar values for other samples on each day were evaluated using Eq. (10) and is presented in Table 5. Subsequently, S_m values of all five samples (IVCO-1, 2, 3, 4, 5) were calculated using Eq. (11) (Table 6).

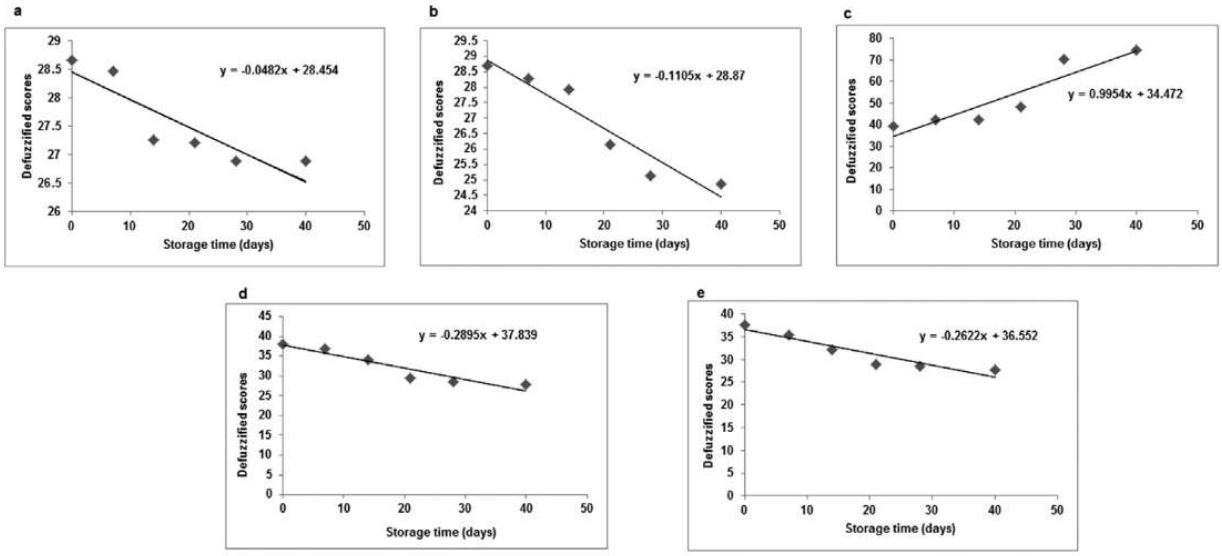

Fig 4. Plot of defuzzified scores of irradiated coconut oil samples with storage time (days) for (a) IVCO 1(0 kGy), (b) IVCO 2 (4.0 kGy), (c) IVCO 3 (4.2 kGy), (d) IVCO 4 (4.5 kGy), and (e) IVCO 5 (5.0 kGy).

Table 5
Overall membership function values of irradiated coconut oil samples.

Storage day	Values of overall membership functions (B_x)									
Day 0										
IVCO 1	0.5582	0.8700	1	0.8720	0.65254	0.4330	0.2135	0	0	0
IVCO 2	0.6001	0.9273	1	0.8238	0.5974	0.3710	0.1446	0	0	0
IVCO 3	0.3798	0.6076	0.8354	1	0.9440	0.7430	0.5418	0.3407	0.1395	0
IVCO 4	0.3916	0.6246	0.8576	1	0.9206	0.7170	0.5127	0.3087	0.1048	0
IVCO 5	0.3975	0.6320	0.8665	1	0.9113	0.7060	0.4998	0.2941	0.0884	0
Day 7										
IVCO 1	0.5597	0.8674	1.1751	0.8736	0.6513	0.4291	0.2069	0	0	0
IVCO 2	0.5635	0.8685	1.1736	0.8728	0.6492	0.4255	0.2019	0	0	0
IVCO 3	0.3578	0.5802	0.8025	1.1717	0.9783	0.7850	0.5916	0.3983	0.2049	0.0116
IVCO 4	0.3750	0.6058	0.8366	1.1412	0.9418	0.7423	0.5429	0.3434	0.1440	0
IVCO 5	0.3566	0.5859	0.8152	1.1594	0.9616	0.7638	0.5661	0.3683	0.1705	0
Day 14										
IVCO 1	0.5892	0.9106	1.2321	0.8380	0.6137	0.3894	0.1651	0	0	0
IVCO 2	0.5739	0.8870	1.2001	0.8576	0.6346	0.4117	0.1888	0	0	0
IVCO 3	0.3659	0.5930	0.8201	1.1575	0.9586	0.7597	0.5609	0.3620	0.1631	0
IVCO 4	0.3428	0.5692	0.7956	1.1772	0.9810	0.7848	0.5886	0.3924	0.1962	0
IVCO 5	0.3418	0.5696	0.7974	1.1744	0.9782	0.7820	0.5858	0.3896	0.1934	0
Day 21										
IVCO 1	0.5550	0.8721	1	0.8547	0.6115	0.3683	0.1250	0	0	0
IVCO 2	0.5910	0.9110	1	0.8255	0.5839	0.3424	0.1007	0	0	0
IVCO 3	0.2637	0.4724	0.6811	0.8898	1	0.9161	0.7383	0.5605	0.3828	0.2049
IVCO 4	0.5447	0.8511	1	0.8888	0.6726	0.4564	0	0	0	0
IVCO 5	0.5519	0.8650	1	0.8762	0.6586	0.4412	0	0	0	0
Day 28										
IVCO 1	0.5605	0.8834	1	0.8435	0.5986	0.3537	0.1087	0	0	0
IVCO 2	0.6146	0.9448	1	0.7953	0.5495	0.3038	0.0580	0	0	0
IVCO 3	0	0.0288	0.1881	0.3474	0.5067	0.6660	0.8253	0.9845	1.1438	0.8580
IVCO 4	0.5589	0.8733	1	0.8693	0.6505	0.4316	0.2127	0	0	0
IVCO 5	0.5620	0.8805	1	0.8632	0.6443	0.4255	0.2066	0	0	0

Table 5 (*Continued*)

Storage day	Values of overall membership functions (B_x)									
Day 40										
IVCO 1	0.5605	0.8834	1.2063	0.8435	0.5986	0.3537	0.1087	0	0	0
IVCO 2	0.6215	0.9579	1.2944	0.7843	0.5377	0.2912	0.0446	0	0	0
IVCO 3	0	0	0.0780	0.2149	0.3518	0.4886	0.6255	0.7624	0.8993	1.1097
IVCO 4	0.5741	0.8970	1.2199	0.8491	0.6276	0.4061	0.1845	0	0.0000	0
IVCO 5	0.5772	0.9045	1.2317	0.8432	0.6216	0.4001	0.1786	0	0.0000	0

Table 6
Similarity values of irradiated oil samples and their ranking.

Sample no.	Storage day	Six categories					
		Not significant	Fair	Satisfactory	Good	Very good	Excellent
IVCO 1	0	0.0000	0.3394	*1.0398*	0.9010	0.3914	0.0430
	7	0.0000	0.4011	*1.0428*	0.9040	0.3878	0.0420
	14	0.0000	0.4367	*1.0965*	0.8968	0.3598	0.0343
	21	0.0000	0.4303	*1.1190*	0.9340	0.3469	0.0269
	28	0.0000	0.4420	*1.1403*	0.9352	0.3361	0.0238
	40	0.0000	0.4420	*1.1403*	0.9352	0.3361	0.0238
IVCO 2	0	0.0000	0.4522	*1.1215*	0.8959	0.3464	0.0306
	7	0.0000	0.4032	*1.0457*	0.9038	0.3850	0.0451
	14	0.0000	0.4167	*1.0655*	0.9001	0.3762	0.0385
	21	0.0000	0.4610	*1.1543*	0.9171	0.3239	0.0220
	28	0.0000	0.4603	*1.2100*	0.9119	0.2909	0.0132
	40	0.0000	0.5088	*1.2303*	0.9103	0.2794	0.0102
IVCO 3	0	0.0000	0.2074	0.6471	*0.8290*	0.5846	0.1856
	7	0.0000	0.1879	0.5976	*0.8037*	0.5987	0.2012
	14	0.0000	0.1980	0.6268	*0.8221*	0.5924	0.1923
	21	0.0311	0.2539	0.5440	*0.6437*	0.4593	0.1563
	28	0.0000	0.0913	0.3100	0.5371	*0.6400*	0.2947
	40	0.0000	0.0000	0.0902	0.4151	*0.8151*	0.5244
IVCO 4	0	0.0000	0.2071	0.5883	*0.9461*	0.8608	0.3136
	7	0.0000	0.2058	0.6469	*0.8294*	0.5850	0.1864
	14	0.0000	0.1842	0.5972	*0.8131*	0.6043	0.2012
	21	0.0000	0.4080	*1.0790*	0.9622	0.3328	0.0000
	28	0.0000	0.4521	*1.1041*	0.9539	0.3231	0.0000
	40	0.0000	0.4220	*1.0759*	0.8993	0.3733	0.0380
IVCO 5	0	0.0000	0.2245	0.6912	*0.8484*	0.5686	0.1711
	7	0.0000	0.1942	0.6215	*0.8222*	0.5961	0.1945
	14	0.0000	0.1847	0.6003	*0.8153*	0.6044	0.2012
	21	0.0000	0.4178	*1.0951*	0.9580	0.3270	0.0000
	28	0.0000	0.4295	*1.1114*	0.9516	0.3206	0.0000
	40	0.0000	0.4270	*1.0846*	0.8983	0.3693	0.0373

The S_m values of both IVCO 1 and 2 were under “satisfactory” category throughout the period of the study. The similar trend was followed by IVCO 4 and 5 from day 21. IVCO 3 had the highest S_m under “good” category up to day 21. However, on day 28, S_m value was found to be maximum and therefore attested the VCO sample to “very good” category which remained unaltered until the last day of the study (day 40). These findings (obtained from defuzzified scores and S_m analyses) revealed that IVCO 3, i.e. VCO irradiated at 4.2 kGy had no objectionable odor and therefore was sensorically highly

accepted. This was in agreement with our previous findings [3] and we had reported that deodorization in this sample was owing to radiolysis of octanoic acid in the same.

Validation of new developed equation against our previous finding by e-nose technology

The new developed equation for deriving defuzzified scores under condition $(a + c) > 100$, unambiguously concluded that VCO sample irradiated at 4.2 kGy on day 28 of storage was the best quality sample, in complete agreement with the findings of sensory evaluation and e-nose technology [3].

From the above discussion, it can be concluded that the results of similarity value and the e-nose analyses were in agreement with that obtained by the new developed equation of fuzzy logic analysis. As per the new equation, the defuzzified scores were evaluated and it's corresponding descriptions implied that IVCO 3 was regarded as 'the best' with respect to deodorization (quality attribute: aroma) which was effective from day 28 of storage Similarity value analysis also designated IVCO 3 as 'very good' in terms of deodorization in accordance with six point standard fuzzy scale. This conclusion was further proved by the e-nose signal responses ($\Delta R/R$) of the irradiated coconut oil samples. This new developed equation can be widely adopted for ranking food samples unambiguously, rapidly and reliably, without any conflict with similarity value and e-nose approaches. It evades the complexity in evaluating defuzzified scores of samples, compared to use of similarity values.

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ORIGINAL ARTICLE

Acrylamide mitigation and 2,4-decadienal elimination in potato-crisps using L-proline accompanied by modified processing conditions

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Abstract Presence of toxic compounds such as acrylamide and 2,4-decadienal in fried products are dependent on frying temperature and time and the frying oil. Combination treatments such as aqueous pre-treatments of potato slices prior to frying; addition of L-proline to pre-treated samples; moisture reduction of samples pre-frying, replacement of refined soybean oil by deodorized-virgin-coconut oil (DVCO) as frying medium; and modification of frying time–temperature regime, were implemented to mitigate acrylamide and 2,4-decadienal in fried potato-crips, concomitantly enhancing their organoleptic quality. Based on similarity values of % acrylamide mitigation, experimental conditions were classified into four main clusters and the optimized conditions of the combination treatments obtained by central composite rotatable design were: blanching at 70 °C for 20 min; addition of 2% L-proline to pre-treated potato slices; and deep-frying in DVCO under modified frying conditions (140 °C, 5 min) successfully alleviated acrylamide ($\sim$ 99%) (confirmed by HR-MS and quantified by RP-HPLC) and 2,4-decadienal (quantified by RP-HPLC) in the fried potato-crisps, improving them sensorically. High Pearson's correlation co-efficient ($r = 0.9955$) was obtained between sensory scores and texture profile analyses data of the fried crisps. This mitigation strategy can be successfully extrapolated to industrial-scale frying for enhanced safety and sensory appeal of fried products.

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Keywords Potato-crisp · Acrylamide · 2,4-decadienal · L-proline · Deodorized virgin coconut oil · Modified SPE-QuEChERS

Introduction

Acrylamide (2-propene amide) is labeled as a human carcinogen-cum-a neurotoxin by the International Agency for Research on Cancer since 2002 (Claus et al. 2008). The Swedish National Food Administration and the University of Stockholm reports the formation of acrylamide in carbohydrate-rich foods at processing temperatures higher than 120 °C, as a product of Maillard reactions between reducing sugar(s) and free amino acid (L-asparagine). Fried potato-crisps are one of the most commonly consumed snacks globally. Among several strategies implemented for mitigation of acrylamide in fried potato products, aqueous pre-treatments such as rinsing, soaking and blanching are reportedly effective (Pedreschi et al. 2004). Many additives have proved to be useful in pre-treatment of potatoes prior to frying. For example, addition of 2% citric acid or 1% NaCl or $\sim$ 2% $CaCl_2$ to soaking solutions lowers subsequent acrylamide formation in fried potato chips by 60% (Rydberg et al. 2003), 75% (Mestdagh et al. 2007) and 85% (Gökmen et al. 2007), respectively. Another strategy for mitigation of acrylamide in food products (fried and baked) is use of the enzyme asparaginase which effectively reduces acrylamide in fried and baked food products by 99% (Zyzak et al. 2009). However, all the above-mentioned methodologies adversely affect sensory quality of the fried product and therefore could not be adopted successfully.

Other approaches of mitigation of acrylamide formation in fried potato products include reducing the formation of acrylamide and modification of thermal processing

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conditions (Skog and Alexander 2005). Addition of amino acids in glucose-asparagine model system could successfully mitigate acrylamide formation (Koutsidis et al. 2009). Nine amino acids- histidine, alanine, methionine, glutamic acid, aspartic acid, proline, phenylalanine, valine and arginine could individually reduce acrylamide by 22–78% in a glucose (0.4%)-L-asparagine (0.18%) model system when heated at 160 °C (Elder et al. 2007). Similar experiment on mitigation of acrylamide formation was conducted with 5.0% aqueous suspension of waxy maize starch accompanied by reactants such as glucose (50 mmol/kg) and amino acids (25 or 75 mmol/kg) to elucidate the effect of each amino acid on acrylamide formation (Koutsidis et al. 2009). The findings of Koustsidis et al. (2009) complemented the findings of Elder et al. (2007) that acrylamide formation is strongly correlated with the presence of amino acid. Kim et al. (2005) reported that 0.5% glycine and 3.0% lysine in combination when used for pre-treatment of potatoes could mitigate acrylamide formation in potato chips by more than 70% and 80%, respectively. Acrylamide in French fries can be reduced by 40% using 2% glutamine (Bråthen and Knutsen 2005). A maximum concentration of 3.0% (w/d.w of potato flakes) of L-cysteine, glycine, glutamine and lysine have been used for thermally processed foods such as potato chips and bread. However, none of these reported methods could reduce acrylamide by more than 90% and also compromised the fried chips organoleptically. To the best of our knowledge, there is no published literature on alleviation of acrylamide in fried potato-crisps by addition of L-proline accompanied by multiple stage treatments.

The commonly used oil for deep frying potato-crisps is refined soybean oil due to its high smoke point (216–252 °C) and reasonable price (Serna-SaldivarSergio and Chuck-Hernandez 2019). Being a linoleic acid-rich oil (C18:2; 51%) (Woodfield and Harwood 2017), it produces a potentially toxic aldehyde, namely 2,4-decadienal through peroxidation of C18:2 fatty acid which eventually concentrates in the fried products (Boskou et al. 2006). In the current investigation, deep frying was carried out using deodorized-virgin-coconut oil (DVCO) instead of soybean oil. DVCO was processed in our laboratory by deodorizing VCO using 4.2 kGy dose of gamma-irradiation and subsequent storage of the irradiated oil at 23 ± 2 °C for 28 days, in accordance with the method elaborated by Ghosh et al. (2014). It is reportedly known that the predominant presence of medium chain fatty acid (MCF), principally lauric acid (C12:0; 47.8%) (Narayanankutty et al. 2018); Ghosh et al. (2014) in VCO does not support the formation of any toxic aldehyde. DVCO was preferred over VCO since the latter renders a typical rancid odor which limits acceptability of VCO-fried products by consumers at large.

The current investigation reports for the first time on the application of L-proline amino acid with unique treatment combinations for mitigation of acrylamide with concomitant absence of 2,4-decadienal in DVCO-fried potato-crisps. The study examined the feasibility of formulating fried potato-crisps using exclusive treatment combinations for delivering safe fried products while preserving their sensory appeal.

Material and methods

Materials

Potato tubers (Kufri jyoti variety) were purchased from a local super market of Jadavpur, Kolkata, India. Refined soybean oil of King brand (manufactured by Adani Wilmar) and DVCO, processed in our Green Technology Laboratory at Jadavpur University [following the procedure developed by Ghosh et al. (2014)], were used for the current study. A potato cutter was procured from M/s Jas Enterprises, Ahmedabad, India. Frying was carried out in a countertop electric deep fat fryer procured from M/s Shiva Kitchen Appliances, Kolkata, India. All chemicals and solvents used were of AR grade. Specialty chemicals such as acrylamide (∼99% pure), 2,4-decadienal (> 90% pure) and L-proline were procured from M/s Sigma-Aldrich, Bengaluru, India, M/s Aldrich, Steinheim, Germany and M/s Merck, Mumbai, India, respectively. HPLC grade solvents namely *n*-hexane, acetonitrile, methanol, phosphoric acid, 2-propanol and deionized water were procured from M/s Merck, Mumbai, India. Extraction and clean-up (for acrylamide) were achieved with an Agilent Bond Elut QuEChERS extraction kit (p/n 5982–5950) and dispersive SPE kit (p/n 5982–5022). All were purchased from M/s Agilent Technologies, California, USA.

Preparation of conventional set of fried potato-crisps

Fresh potato tubers were washed under running tap water for 5 min, peeled and sliced to average dimensions of 70 × 5 × 9 mm using a potato cutter. The sliced tubers were washed in distilled water and then dried using tissue papers. Thereafter the dried slices of potatoes were subjected to deep frying in DVCO at 170 °C for 3 min [deep frying conditions; surface: volume (S/V) = 1.0 cm^{-1}] in an electric deep fat fryer (2270–2500 W, 50 Hz, 220–230 V).

Preparation of experimental (treated) and control sets of fried potato-crisps

Aqueous pre-treatments of potato slices

It is well known that incorporation of aqueous pre-treatment steps viz. rinsing, soaking and blanching of potato slices contribute to reduction in acrylamide formation. Rinsing and soaking of potato slices up to 90 min can reduce sugar content by 20% via leaching of precursors from the surfaces of cut potatoes (Pedreschi et al. 2004). Blanching is reportedly known to be more effective compared to the former processes in reducing the contents of acrylamide precursors (L-asparagine and reducing sugar) (Jackson and Al-Taher 2005). In this study, steps of aqueous pre-treatment, viz. immersion and blanching; and time and temperature of treatment of potato slices were optimized through several preliminary trials. The rinsed potato slices were immersed in distilled water for 40 min and subsequently blanched at 70 °C for 20 min. Post blanching, the potato slices were subjected to further treatments.

Treatment by L-proline followed by frying under modified conditions

The aqueous pre-treated sets of potato slices (under optimized aqueous pre-treatment conditions), were then soaked in L-proline of different concentrations (Table 1). The temperature of the soaking solution was maintained at 80 °C and the time of immersion was 30 min for aqueous pre-treated potato slices (time–temperature of immersion was fixed based on texture of potato slices). Water was drained off from the aqueous pre-treated and L-proline treated potato slices; then air-dried for 15 min at ambient condtions (23 ± 2 °C, 70 ± 2% RH) and finally tray-dried at 60 °C for 130 min under optimized drying conditions (process of optimization not revealed) to a moisture content of ∼ 40% [moisture analysis of the potato slices and crisps of three different sets including conventional (vide infra), control and treated sets (prior to frying and post frying) were conducted in accordance with AOAC methods (AOAC 1998)].The dried potato slices were then fried in DVCO under varying frying conditions (s/v = 1.0 cm^{-1}).

Preparation of control (experimental) sets of fried potato crisps

Non-treated set of potato slices fried under modified and optimized frying conditions (s/v = 1.0 cm^{-1}) has been designated as the control set. The importance of the control set was to assess the impact of modified frying conditions on percent acrylamide mitigation and physicochemical characteristics of the fried product, vis-à-vis the conventional frying conditions (170 °C for 3 min).

Quantification of asparagine and reducing sugar contents in aqueous-pretreated and control/conventional set of potato slices

Acrylamide precursors were quantified to study the effects of aqueous pre-treatment on potato slices. Analyses of L-asparagine and reducing sugar contents in potato tuber, control/conventional set of potato slices (washed in water) and in aqueous pre-treated potato slices were performed according to the methods described by Boos et al. (1996) and McFeeters' (1993), respectively. The experimental results are provided in supplementary data (Table S1).

Acrylamide extraction from conventional, control and treated fried potato-crisps using "solid phase extraction-quick, easy, cheap, effective, rugged, and safe"(SPE-QuEChERS) method

Quantity of sample and ratio of water-acetonitrile needed for extraction of acrylamide were optimized to perform SPE-QuEChERS extraction method. A sample (grounded) of ∼ 15 g was transferred into a 50-mL Falcon tube. Then 5 mL *n*-hexane was added and vortexed for 5 min followed by addition of 20 mL binary mixture of water and acetonitrile (ACN) in 4:1 ratio. Thereafter Bond Elut QuChERS salt packet was added and the mixture was vortexed for 1 min. The tubes were centrifuged for 1 min at 5000 × g. The upper layer of hexane was discarded and 1 mL of aqueous-ACN layer was pipetted out a into a 2 mL miocrocentrifugal vial pre-packed with 50 mg PSA and 150 mg MgSO4. The sample was vortexed for 1 min followed by centrifugation at 3000 × g for 5 min. The filtrate was collected through 25 µ filter-paper. It was transferred into an amber-colored screw capped glass vial in an inert atmosphere of nitrogen and stored at 4 °C in the dark. It was subjected to high resolution mass spectrometry (HR-MS) and reverse phase-high performance liquid chromatography (RP-HPLC) for qualitatitive and quantitative analyses of acrylamide, respectively.

Qualitative analysis of acrylamide by HR-MS

HR-MS is invaluable to prove the identity of acrylamide. In this current study, standard acrylamide (concentration: 200 µg/mL) in acetronitrile was pumped to HR-MS (Model: QTOF Micro YA263) at a flow rate of 5 µL/min. Orbitrap MS equipped with a heated electrospray interface was operated in the positive mode and scanned the ions in the m/z range of 50–200. The resolving power was set to 50,000 full width (Troise et al. 2014). The interface

Table 1 Experimental results of % acrylamide mitigation of treated set of fried potato crisps

Treatment No	Concentration of L-proline (% W/W)	Frying time (Min)	Frying temperature (°C)	Acrylamide mitigation (%)
T-1	1.0	4.0	140	84.32 ± 0.04
T-2	1.0	4.0	150	76.88 ± 0.04
T-3	1.0	4.0	160	74.57 ± 0.07
T-4	1.0	5.0	140	83.72 ± 0.06
T-5	1.0	5.0	150	75.49 ± 0.07
T-6	1.0	5.0	160	73.77 ± 0.03
T-7	1.0	6.0	140	83.12 ± 1.09
T-8	1.0	6.0	150	74.95 ± 0.08
T-9	1.0	6.0	160	73.04 ± 0.05
T-10	1.5	4.0	140	90.04 ± 0.05
T-11	1.5	4.0	150	82.99 ± 0.04
T-12	1.5	4.0	160	80.62 ± 1.0
T-13	1.5	5.0	140	88.60 ± 0.09
T-14	1.5	5.0	150	81.75 ± 0.08
T-15	1.5	5.0	160	78.74 ± 0.07
T-16	1.5	6.0	140	87.48 ± 0.04
T-17	1.5	6.0	150	80.89 ± 0.05
T-18	1.5	6.0	160	78.01 ± 0.06
T-19	2.0	4.0	140	99.03 ± 0.07
T-20	2.0	4.0	150	92.07 ± 0.08
T-21	2.0	4.0	160	89.96 ± 0.09
T-22	**2.0**	**5.0**	**140**	**98.82 ± 0.03**
T-23	2.0	5.0	150	91.75 ± 0.06
T-24	2.0	5.0	160	88.76 ± 0.05
T-25	2.0	6.0	140	97.79 ± 0.04
T-26	2.0	6.0	150	90.90 ± 0.07
T-27	2.0	6.0	160	87.92 ± 0.05

Bold signifies the optimum experimental conditions of L-proline concentration (%w/w) and frying operations

All the results are expressed in mean ± SD

parameters were- spray voltage: 4.0 kV; capillary voltage: 41.0 V; skimmer voltage: 13 V; capillary temperature: 300 °C; heater temperature: 250 °C; sheath gas flow: 35 (arbitrary units); and auxiliary gas flow: 5 (arbitrary units). These conditions were similar when the SPE-QuEChERS extracted sample solution (conventional) was analyzed for identification of acrylamide (Fig. 1).

Quantification of acrylamide in fried potato-crisps by RP-HPLC

Quantification of acrylamide in the purified extract of defatted [Soxhlet method (Method 3540)] conventional, control and different treated sets of fried potato-crisps was performed by RP-HPLC analysis. The RP-HPLC method for detection of acrylamide reported by Khoshnam et al. (2010) was adopted with few modifications. The HPLC system (100 V-240 V 50/60 Hz 28 W; MD 2015 plus, M/s JASCO, Easton, US) consisted of a reversed-phase C18 column (25 cm × 0.46 cm I.D.) with a HPLC pump (PU 2080 plus, M/s JASCO Easton, US) and an injector with a 20 µL sample loop. Retention time of acrylamide was determined using HPLC–PDA detector (M/s JASCO MD 2015 plus, Easton, US) at 200 nm. The mobile phase (HPLC grade water) was purged at a constant flow rate of 1 mL/min. A standard curve of pure acrylamide (10–200 µg/L) was prepared by dissolving a pure standard of acrylamide (99% purity) in HPLC grade deionized water. Dilutions were made immediately before injection into the LC column for quantification. The limit of detection (LOD) was determined to be 10 µg/L.

Hierarchical cluster analysis (HCA) analysis of acrylamide contents obtained different treatment conditions

In this study, HCA was employed as an 'unsupervised classification' method to assess the association of %

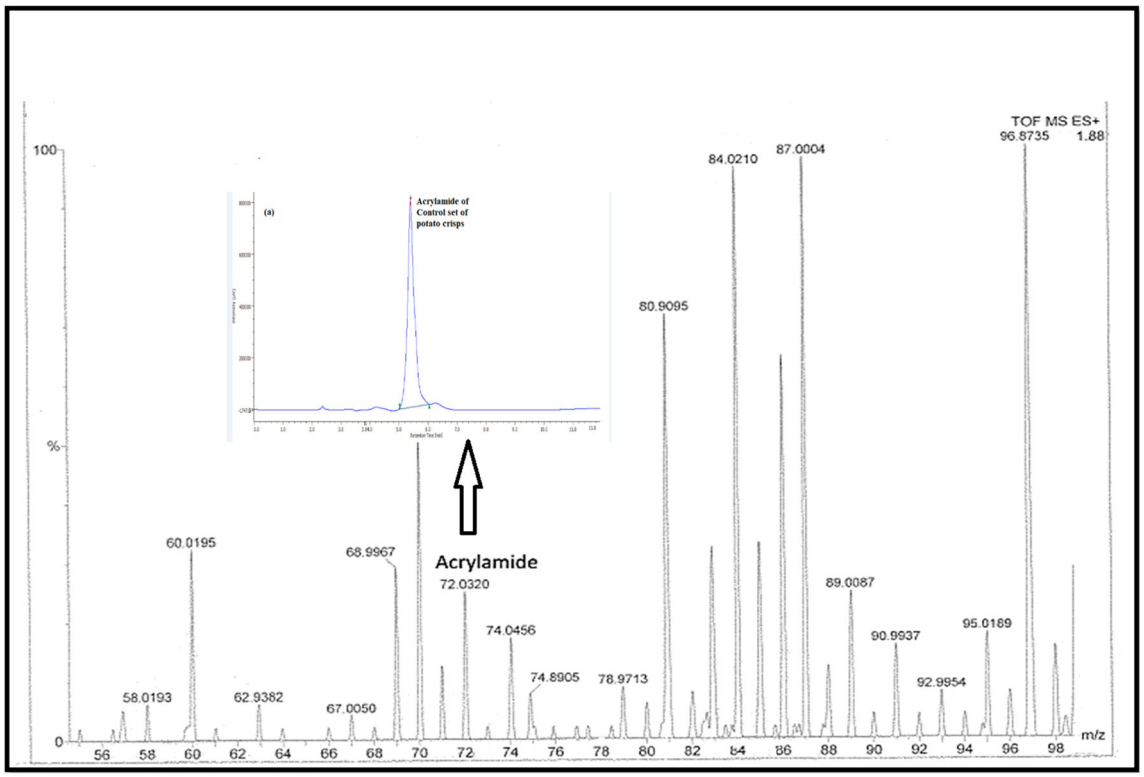

Fig. 1 Qualitatitive analysis of acrylamide of conventional set of fried potato crisps by HR-MS

similarity in acrylamide mitigation among 27 different treatment conditions (Table 1). The results of HCA are presented in a dendogram (Fig. 2).

Optimization of the treatment conditions (concentration of L-proline, frying time and temperature) for acrylamide mitigation in fried potato-crisp

The optimization process for % acrylamide mitigation in fried potato-crisps was verified and validated using central composite rotational design (CCRD) with three parameters and response surface methodology (RSM) (Fig. 3a–c). Three factors namely % L-proline (g/100 g of blanched potato slices), frying time (min) and temperature (°C) were considered as independent variables and % acrylamide mitigation in fried potato-crisps as the response variable (Tables 1 and 2). Preliminary trials suggested that significant percentage of acrylamide mitigation ($p < 0.05$) was obtained when the potato slices were treated with L-proline at a concentration ranging from 1 to 2% (w/w) and subsequently fried at 140–160 °C for 4–6 min. Significant changes in % acrylamide mitigation ($p < 0.05$) was found when % L-proline, frying temperature and time were varied at 0.5% (w/w) (1, 1.50 and 2%), 10 °C (140, 150 and 160 °C) and 1.0 min (4.0, 5.0 and 6.0 min) intervals, respectively.

Extraction and quantification of 2,4-decadienal in treated (T-22) set of fried potato-crisps

Total 5 batches of treated (under optimized treatment conditions) sets of fried potato-crisps were successively deep fried in the same oil (implying that the same oil was subjected to repeated frying 5 times as is practiced industrially) in DVCO and refined soybean oil, individually to assess formation of 2,4-decadienal in the frying oils. Frying operation was performed in duplicate for each batch of sample in each of these two oils. The cooking oil was allowed to cool prior to each new frying operation. The excess oil was drained off from the fried samples using cross-linked S.S. wire mesh. Samples of each batch was packaged in LDPE-bags under nitrogen flushing, labelled accordingly and stored at − 24 °C for analysis of 2,4-decadienal therein.

The methanol based extraction of 2,4-decadienal from treated (designated as T-22) set of fried potato-crisps and its quantification was performed by RP-HPLC using HPLC–PDA detector at 280 nm (Table 3) in accordance with the method described by Boskou et al. (2006).

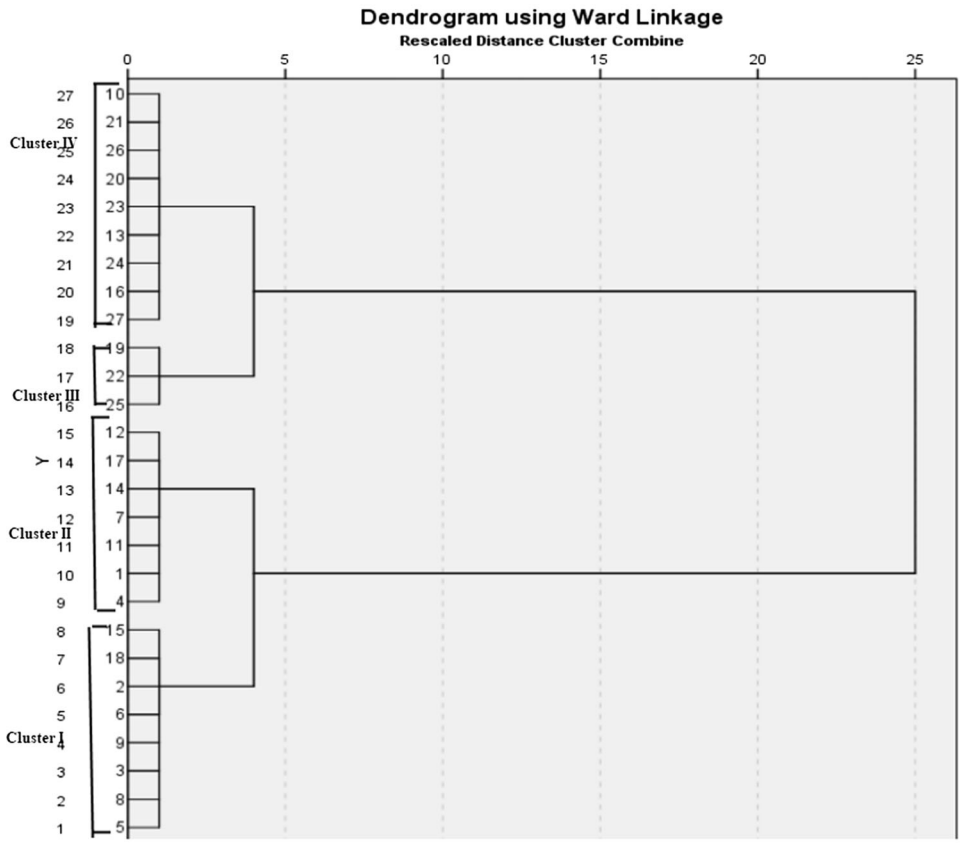

Fig. 2 Hierarchical cluster analysis of different treatment conditions based on % acrylamide mitigation (Dendogram)

Characterization of physicochemical properties of conventional, control and T-22 set of fried potato-crisps

Assessmet of color

The CIE color values (L*, a* and b* values) of the conventional, control and T-22 sets of fried potato-crisps were determined with a CM-5 spectrophotometer (M/s Konica Minolta Inc., Osaka, Japan) at a 10° inclination from the light source. The color co-ordinates of these fried potato-crisps were calibrated against a standard white plate (100% calibration). Chroma (C*) values and hue (h) angles were calculated (provided as supplementary data in Table S2) using standard equations (Chatterjee and Bhattacharjee 2014).

Analysis of texture

The texture profile analysis (TPA) of the conventional, control and T-22 set of fried potato-crisps were conducted using texture analyzer (TA.XT Express; TA XTplus Texture Analyzer Stable Micro System) with a 5 g load cell (P/5) and 5 mm diameter of cylindrical probe. The conditions for performing TPA of fried potato-crisps were- pre-test: 1.0 mm/s; test: 0.5 mm/s; post-test: 10.0 m/s; distance: 2.0 mm; trigger force: auto; data acquisition: 400 ppb. The texture of the potato-crisps was quantified in terms of hardness, fracturability (as function of crispiness), adhesiveness, cohesiveness and chewiness (Table S3). Texture analysis data were subjected to one-way ANOVA to ascertain differences between the control and T-22 sets of fried potato crisps in textural attributes (five). Correlations between the TPA parameters and sensory scores (related to texture judgment) of treated (T-22) set of fried potato-crisps were also evaluated using Pearson's correlation matrix (Serna-SaldivarSergio and Chuck-Hernandez 2019).

Sensory evaluation of fried potato-crisps

Sensory evaluations by 30 semi-trained panelists (comprising of University staff, faculty and students) were carried out for conventional, control and T-22 set of fried potato-crisps in an air-ventilated room under white light. Unsalted crackers and water were supplied to panelists for refreshing their palates before tasting subsequent samples (Ranganna 1987). Frying ime and temperature contributes to sensory perception of the fried product, including it's

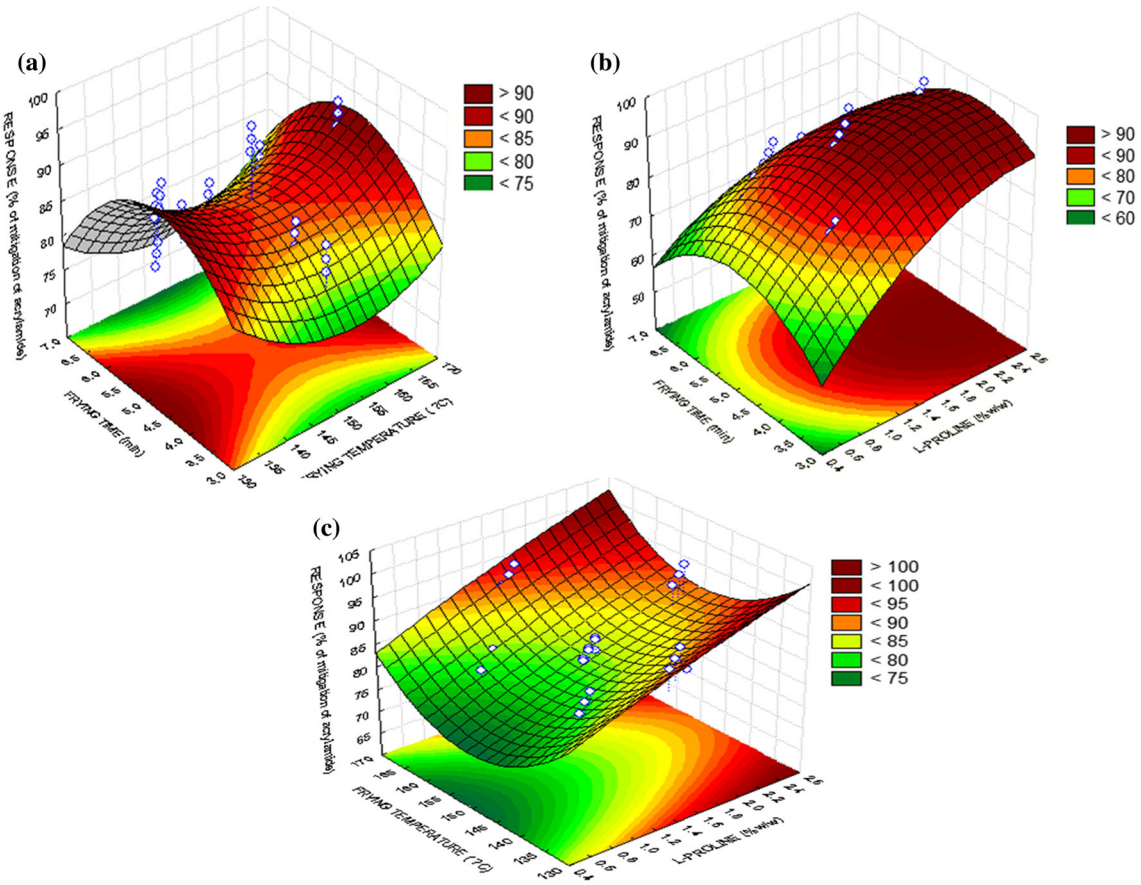

Fig. 3 Plot of response surface indicating percentage of acrylamide mitigation in treated set of fried potato-crisps (**a**) as a function of frying time and frying temperature at 1.50% (w/w) L-proline concentration, **b** as a function of frying time and L-proline concentration at 150 °C frying temperature, **c** as a function of frying temperature and L-proline concentration at 5 min frying time

Table 2 Anova study of surface methodology

Effect	Degree of freedom	Yield of acrylamide on extraction (G/Kg Of dry potato crisp) SS	Yield of acrylamide on extraction (G/Kg Of dry potato crisp) MS	Yield of acrylamide on extraction (G/Kg Of dry potato crisp) F	Yield of acrylamide on extraction (G/Kg Of dry potato crisp) P
X_1	1	586.665	586.665	102.5962	0.0000
X_1^2	1	16.688	16.688	2.9185	0.095730
X_2	1	26.687	26.687	4.6670	0.037119
X_2^2	1	2.886	2.886	0.5048	0.481744
X_3	1	1416.119	1416.119	247.6489	0.00000
X_3^2	1	1.516	1.516	0.2651	0.609597
X_1 X_2	1	33.957	33.957	5.9384	0.019608
X_1 X_3	1	88.117	88.117	15.4098	0.000352
X_2 X_3	1	16.715	16.715	2.9231	0.095477
Error	38	217.294	5.718		
Total	47	3190.753			

texture. In the current investigation, a total of 27 runs were performed under nine time–temperature combinations (Table 1) which differed from conventional deep frying conditions. Sensory evaluation of the fried potato crisps was conducted for each set of sample and corresponding average sensory scores were calculated (average sensory

Table 3 Quantity of 2,4-decadienal of treated (T-22) set of fried potato crisps

No. of frying	Refined Soybean oil Deep frying (μg/g)	Deodorized-virgin-coconut oil (DVCO)
1	4 ± 0.01^{a}	0
2	8 ± 0.02^{b}	0
3	11 ± 0.05^{c}	0
4	14 ± 0.08^{d}	0
5	15 ± 0.07^{d}	0

All the results are expressed in mean ± SD on basis of three standard deviations of the mean value

'0 μg/g' implies the quantity is less than LOD (0.3 μg/g)

a, b, c, d Different letters in a column indicate significant differences at $p < 0.05$

scores of conventional, control and treated sets having more than 98% acrylamide mitigation are displayed in Table S3). Total six quality attributes such as color, odor, texture, taste, after-taste and overall acceptability of the aforementioned sample sets were adjudged on the basis of the standard 9-point hedonic scale (Stone and Sidel, 2004).

Statistical analysis

In this investigation, HCA was performed using SPSS 20.0 software (M/s IBM). Statistical analysis for ascertaining optimum treatment conditions (based on % acrylamide mitigation in fried potato-crisps) was conducted by one-way analysis of variance (ANOVA), response surface methodology (RSM) and regression modeling using STATISTICA 8.0 software (M/s Statsoft, OK, USA). The ridge analysis for saddle point analysis was performed using R 3.4.3 software. A p value $\leq$ 0 0.05 was used to verify the significance of the tests. Pearson correlation analysis between the results of five sensory attributes of texture acquired from sensory evaluation and texture analyzer (instrumental tests), respectively, was conducted using SPSS 20.0 software (M/s IBM).

Results and discussion

Determination of acrylamide precursors (L-asparagine and reducing sugar) in potato tuber, control/conventional set of potato slices and blanching water

It was found that quantities of L-asparagine and reducing sugar varied in both treated and control/conventional set of potato-crisps (from data of Table S1) at significant levels ($p < 0.05$). The aqueous pre-treatments resulted in loss of precursors of acrylamide: 43.25% for L-asparagine and 42.77% for reducing sugar. These results affirmed that aqueous pre-treatments are potential methods to reduce the contents of precursors significantly ($p < 0.05$) which in turn resulted in effective mitigation of acrylamide in the said food matrix.

Qualitative analysis of acrylamide by HR-MS

The lens voltage was optimized by setting the mass to 72.0 (theoritical values of m/z ~ 72.03217–72.03261) by manual tuning. In case of standard acrylamide solution, peak having m/z value 72.0320 was identified (Fig. 1) and the same was also detected in SPE-QuEChERS extracted sample (control set of fried potato crisps).

Classification of treatment conditions causing significant acrylamide mitigation by hierarchical cluster analysis

Four individual clusters (I, II, III and IV) were obtained from cluster analysis based on the correlation coefficient distances among them (Fig. 2). Cluster I presents a similarity level having the least acceptable level (73.04%–78.74%) of % acrylamide mitigation and is associated with treatments 2, 3, 5, 6, 8, 9, 15 and 18. This association is probably influenced by the effects of combinations of lowest % L-proline concentration (1%) coupled with moderate frying temperature (150 °C); and lowest % L-proline concentration (1%) with highest frying temperature (160 °C). Cluster II represents a similarity level higher than 80% acrylamide mitigation by grouping together the treatments 1, 4, 7, 11, 12, 14 and 17 and corresponded to the third highest acceptable level of % acrylamide mitigation (80.62%–84.32%) among all clusters. The high similarity level is probably related to the consequence of combination of moderate concentration of % L-proline (1.5%) and lowest frying temperature (140 °C) and opposite combination of these factors (% L-proline: 1% and frying temperature: 150 °C). Cluster III is associated with treatments 19, 22 and 25, representing a high similarity level (> 95% acrylamide mitigation). It

corresponded to the highest acceptable level of % acrylamide mitigation (97.79–99.03%). This association is influenced by the effect of combination of highest % L-proline concentration (2%) and lowest frying temperature (140 °C). Cluster IV includes treatments 10, 13, 16, 20, 21, 23, 24, 26 and 27, signifying greater than 85% acrylamide mitigation implying second highest acceptable level (87.48%–92.07%) of the fried crisps. The high similarity level is possibly related to the consequence of combination of moderate concentration of % L-proline (1.5%) and highest frying temperature (160 °C) and opposite setting of these two factors (% L-proline: 2% and frying temperature: 150 °C). These findings suggested that the combination of highest % L-proline with lowest frying temperature yielded the most desirable result of highest (> 95%) acrylamide mitigation in the fried potato-crisps. These findings were further validated through CCRD-RSM optimization of treatment conditions to acheive maximum possible mitigation of acrylamide under given experimental conditions.

In cluster III, among T-19, T-22 and T-25 sets, T-19 and T-22 were chosen since they exhibited > 98% acrylamide mitigation. Sensory analysis revealed superiority of T-22 over T-19 (Table S3). On the basis of acrylamide mitigation and sensory acceptance, T-22 was adjudged to be the best among all treatments.

Optimum conditions of treatment for mitigation of acrylamide in treated set of fried potato-crisps

Table 1 provdies the % acrylamide mitigation in fried potato-crisps subjected to 27 different treatment combinations obtained by varying of % L-proline, frying temperature and frying time. ANOVA study (Table 2) revealed that % acrylamide mitigation in treated set of fried potato-crisps increased as % L-proline amount (X_1) ($p = 0.000$) increased and frying time (X_2) ($p = 0.037$) and temperature (X_3) ($p = 0.000$) decreased. The maximum % acrylamide mitigation (99.03%) was obtained in 2% L-proline pre-treated samples when fried for 4 min at 140 °C in DVCO. However the texture (crispiness) of the same was not adequate and received no sensory acceptability. The second highest % acrylamide mitigation (98.82%) was obtained under following conditions: pre-treatment with 2% L-proline, 5 min frying time and 140 °C frying temperature. The fried potato-crisps under these conditions received appreciable sensory acceptability. Considering good sensory acceptability and > 95% acrylamide mitigation, the combination of 2% L-proline, 5 min frying time and 140 °C frying temperature was regarded as the best for mitigation of acrylamide under the given experimental conditions.

The above finding indicates that % acrylamide mitigation in low frying (deep) temperature increased with increasing concentration of % L-proline used in pre-treatment and with decreasing frying time. Therefore, high % L-proline as well as reduced frying time and temperature played important roles in reducing acrylamide formation in potato crisps.

Generation of response curves

The response surfaces of % acrylamide mitigation in relation to the % L-proline concentrations (w/w), frying temperature and time are shown in stereoscopic figures (Fig. 3a–c). The % L-proline concentrations (w/w), frying temperature and time were fixed at their middle values of 1.50%, 150 °C and 5 min in respective stereoscopic figures Fig. 3a–c and showed significant ($p < 0.05$) effects on % acrylamide mitigation, evidenced by the test statistics for the regression models (vide supra).

Regression modeling of mitigation of acrylamide in treated set of fried potato-crisps

The second-order polynomial equation that fitted the coded variables is stated as follows:

$$Y = B_0 + \sum B_i X_i + \sum B_{ii} X_i^2 + \sum B_{ij} X_i X_j \quad (1)$$

where Y represents the response variables (% acrylamide mitigation in treated set of potato-crisps), B_0, B_i, B_{ii}, and B_{ij} are constants and regression coefficients of the model, and X_i and X_j are independent variables in coded forms. The expanded model includes linear, quadratic and cross-product terms as shown below (with intercept):

$$Y = 189.26 + 83.09\ X_1 - 7.35\ X_2 - 1.43\ X_3 + 4.06\ X_1^2 - 0.46\ X_2^2 + 0.003\ X_3^2 - 3.53\ X_1X_2 - 0.45\ X_1X_3 + 0.11\ X_2X_3 \quad (2)$$

The significance of the investigated factors and their interactions were examined. It was seen that there was a significant ($p < 0.05$) dependence of % acrylamide mitigation (response variable) in fried potato-crisps on % L-proline concentration (X_1) ($p = 0.000$), frying time (X_2) ($p = 0.037$) and temperature (X_3) (p = 0.000). However, all the second-order terms (X_{12}, X_{22}, X_{32}) of the three processing parameters did not have significant effects on the same. The two-level interactions showed that % L-proline concentration and frying temperature (the cross-product term X_1X_3) ($p = 0.000$) were found to be the most significant followed by interdependence of % L-proline concentration and frying time (the cross-product term X_1X_2) ($p = 0.019$). However, the interdependence of frying time and temperature (X_2X_3) did not have significant ($p = 0.095$) effect on the response variable. ANOVA study showed a good F value for the cross-product term X_1X_3

(F = 15.40), signifying that this interaction was very important (Table 2). The cross-product term X_1X_2 showed a moderate F value (5.938) representing interactions among the variables to be significant. However, a poor F value (2.923) was obtained for the remaining interaction (X_2X_3) indicating that its effect on the response variable was negligible. A plot of observed vs. predicted values of responses (Y) showed a close fit of the observed values with the predicted ones. Thus, a statistically significant multiple regression relationship (r = 0.91) between the independent variables and the responding variable could be established. The complete quadratic model showed a very good fit.

Analysis of response surfaces

From the test statistics for the regression models (F-test and ANOVA), it can be reasonably concluded that % L-proline and frying temperature in combination had the most significant effect (p = 0.000) on % acrylamide mitigation.

Optimization of mitigation of acrylamide in treated set of fried potato-crisps

The stationary point obtained following the method of Montgomery (2001)] was X_{1S} = 1.28%, X_{2S} = 6.34 min and X_{3S} = 142.52 °C. Percentage acrylamide mitigation at this stationary point was found to be 79.66.

Characterizing the response surface

In this study, the eigen values determined in accordance with the method described by Montgomery (2001) were 4.6842, − 0.0080 and − 1.0704. Different signs of eigen values indicated X_S to be a saddle point.

Optimal conditions of mitigation of acrylamide

Since the conditions of the saddle point (X_{1S} = 1.28%, X_{2S} = 6.34 min and X_{3S} = 142.52 °C) cannot be considered as optimum conditions for maximum mitigation of acrylamide in treated set of fried potato-crisps, a ridge analysis was performed to identify the point of maxima. Ridge analysis indicated that % L-proline (X_1) if increased along with decreasing frying time (X_2) and temperature (X_3), % acrylamide mitigation would be maximum. It revealed the optimum conditions for obtaining the point of maxima to be: X_1 = 2.02%, X_2 = 4.63 min, X_3 = 139.77 °C. The predicted % acrylamide mitigation at this point was found to be as 98.28 and the experimental value was also found to be 98.82% under conditions of 2% L-proline pre-treatment, 5 min frying time and 140 °C frying temperature. Thus the findings based on the concept of canonical ridge analysis are strongly in accord with the experimental findings.

RP-HPLC analysis of acrylamide of conventional, control and treated set of fried potato-crisps using SPE-QuEChERS extraction method

Identification and quantification of acrylamide in SPE-QuEChERS extracts of conventional, control and 27 different treated sets of fried potato-crisps were carried out by RP-HPLC. The second highest % reduction of acrylamide was observed for the following treatment combination: 2% L-proline pre-treatment, 140 °C frying temperature and 5 min frying time [provided as supplementary information in Fig. S1 (a) and (b)]. Acrylamide was quantified using standard curve having high correlation coefficient (r = 0.9984). The concentration of acrylamide in the conventional set of fried potato-crisps was 1739.12 µg/kg (8695.6 µg/L) which post application of modified frying conditions alone (for control set) and along with the combination treatment, reduced to 1182.60 (5922.6 µg/L) and 20.52 µg/kg (102.94 µg/L), respectively. Compared to the conventional set, 38.1% and 98.82% (∼ 99%) mitigation of acrylamide occurred in the control and treated sets of fried potato-crisps under optimized treatment conditions of- blanching temperature: 70 °C, blanching time: 20 min, concentration of L-proline amino acid: 2% (w/w), drying conditions: 60 °C for 130 min, frying temperature: 140 °C and frying time: 5 min. Thus it is evident that modified frying conditions played a crucial role in controlling acrylamide level in the fried product. This combination treatment strategy proved to be effective in reducing acrylamide content by ∼ 99% without compromising sensory acceptability of the fried potato-crisps.

2,4-decadienal contents in T-22 set of fried potato-crisps

2,4-decadienal contents are expressed as mean values of µg decadienal per g French-fries. Table 3 reveals that 2,4-decadienaol in each batch of T-22 set of fried potato-crisps, prepared successively in the same batch of DVCO could not be quantified by RP-HPLC since the concentration of this compound was lower than its LOD (considered as 0 µg/g). However, this toxic aldehyde was detected in each batch of fried crisps, fried successively in the same batch of frying oil (refined soybean oil). Significantly higher quantity (11–15 µg/g) of this toxic aldehyde detected in fried potato-crisps during 3–5 (times) repeat frying was influenced by successive thermal peroxidation of C18:2 present (Woodfield and Harwood 2017) in refined soybean oil. On the contrary, this phenomenon was not supported by DVCO in repeat frying process since DVCO contains

insignificant amount of C18:2 ($\sim$ 1.5%) (Ghosh et al. 2014; Narayanakutty et al. 2018).

Physical characteristics of fried potato-crisps

Color profiles of conventional, control and T-22 set of fried potato-crisps

The color profiles of conventional, control and optimized treated sets of fried potato-crisps are represented by L*, a*, and b* values, chroma and hue angles (Table S2). It was found that T-22 set of fried potato-crisps was brighter and more yellowish with higher L* [L*: 68.68 $\pm$ 4.90] and b* values (b*: 13.40 $\pm$ 0.68), compared to those in the conventional and control sets. The T-22 set of fried potato-crisps had significantly higher ($p = 0.0001$) chroma value, implying higher color intensity. The hue angle of T-22 set of fried potato-crisps was in the range of 88.53 $\pm$ 6.02° indicating golden yellow color of the samples. These results corroborated well with the sensory scores.

Texture analyses of conventional, control and T-22 set of fried potato-crisps

Chauvin et al. (2010) established that crispness, hardness and fracturability were significantly correlated ($r = 0.80$–0.90) with each other. Crispiness is a prime factor among all textural attributes of fried potato crisps. However, in a double compression test, crispiness cannot be quantified directly from the TPA graph. Fracturability is defined as the tendency of a material to shatter, crack, crumble or fail upon the application of a relatively small magnitude of force, encompasses crispiness and can be measured directly from TPA graph. It is usually displayed by a product of high degree of hardness and low degree of cohesiveness (Walter et al. 2002). Thus crispy foods require less force to fracture. Hardness and fracturability are diretly correlated with each other whereas crispiness is inversely proportional to fracturability and hardness (Chauvin et al. 2010). Comparative and quantitative assessments of TPA of conventional, control and T-22 sets of fried potato crisps revealed that there were no significant differences ($p > 0.05$) between conventional and T-22 sets w.r.t. all five textural attributes. The conventional and T-22 sets had significantly lower ($p < 0.05$) hardness and fracturability (implying higher crispiness) vis-à-vis the control set of fried potato crisps (Table S3). This was due to difference in the moisture content of treated potato slices before and after frying which were influenced by drying (prior to frying) and frying conditions, respectively. It is evident from the results of moisture analysis (Table S4) that conventional and control sets of fried potato crisps were found to have higher and comparable ($p > 0.05$) moisture contents prior to frying, which differed significantly ($p < 0.05$) from that of the T-22 set. On the basis of final moisture content after frying, it is seen that conventional frying conditions (170 °C @ 3 min) led to a significant moisture loss of $\sim$ 46.8%, higher than that of the control and T-22 sets ($\sim$ 19.5%). These observations showed that higher frying temperature (170 °C) could more effectively remove moisture than a lower frying temperature of 140 °C (optimized frying condition). This was attributed to similar moisture contents (31.9–32.2%) of conventional and T-22 sets of fried potato crisps and thus accounted for similar texture profiles of the same [excessive lower or higher moisture content of fried product ($< / > 32\%$) hampered the texture, color and overall acceptability of the product]. The control set of fried potato crisps however, possessed higher moisture content i.e., $\sim$ 49.1% (post frying) since the same did not undergo drying prior to frying and was fried at a comparitively lower temperature (140 °C). Consequently, adhesiveness and chewiness of conventional and T-22 set of fried potato crisps were lower than that of the control set. Cohesiveness being indirectly proportional to adhesiveness, followed an opposing trend in each case and the values were statistically different ($p < 0.05$) between control and conventional (or considering T-22 set) sets. Therefore, it is evident that moisture content and frying conditions strongly influenced the physicochemical properties of the fried potato products effectively. These findings unambiguosly suggest that potato crisps should be fried at 140 °C for 5 min immediately after drying (to a moisture content of $\sim$ 32.2%), for good texture appeal (in terms of crispiness) and safety (98.82% acrylamide mitigation).

Sensory analysis of conventional, control and T-22 set of fried potato crisps

Sensory scores strongly supported the findings of TPA. T-22 set of fried potato crisps received higher sensory scores (with respect to texture) compared to the control set, but was very similar to that of the conventional set. T-22 set of fried potato crisps achieved the highest sensory scores for color, odor, taste and after-taste among all three sample sets. This was attributed to development of attractive golden yellow color as well as unique odor-cum-flavor over and above the characteristic ones possibly owing to addition of L-proline during pre-treatment. When overall acceptability of the product was adjudged on the basis of five sensory attributes, it revealed that T-22 set of fried potato crisps had the highest sensory acceptance followed by the conventional and control set consecutively (Fig. S2).

Correlation between sensory and texture analyses data

The statistical correlations between sensory and instrumental analysis (two variables) for each attribute of texture (hardness, fracturability, adhesiveness and cohesiveness) were strongly positive ($r = 0.9955$) for each attribute These results successfully validated the sensory scores for textural attributes of the control and T-22 set of fried potato-crisps.

Combined effect of three treatments on acrylamide formation in treated set of potato-crisps and effect of usage of DVCO as cooking-medium on 2,4-decadienal content in fried crisps

The combined effects of four treatments viz. aqueous pre-treatments- treatment with L-proline amino acid, drying and modified frying conditions effectively mitigated acrylamide in fried potato crisps. This could be attributed to three different reasons. Firstly, the aqueous pre-treatments profoundly reduced the acrylamide content in the said food matrix because these treatments reduced free L-asparagine and glucose (main precursors of acrylamide formation) significantly. Secondly, immersion of potato slices in the solution of 2% L-proline limited the acrylamide formation in treated set of fried potato-crisps. Acrylamide has two reactive sites viz. the conjugated double bond and the amide group. Therefore, L-proline formed an adduct via Michael addition reaction with acrylamide via alkylation of it's secondary amine group (Koutsidis et al. 2009), promoted by increased nucleophilicity of the same (Fig. S3). Consequently L-proline amino acid played a crucial role in mitigating acrylamide in treated set of fried potato-crisps. Thirdly, the influence of moisture content of potato slices (prior to frying) in relation to acrylamide formation and overall acceptability (judged on the basis of five sensory attributes, mentioned earlier) (Fig. S4) was investigated (Table S4). The figure shows $\sim$ 40% to be the optimum moisture content of potato slices (prior to frying) considering both acrylamide formation and sensory acceptability. Moisture is known to impact the chemical route, namely, hydrolysis of imine (intermediates of Maillard reaction) as well as molecular mobility of chemical constituents associated with the formation of acrylamide (Ciesarová et al. 2006). T-22 set of potato slices having $\sim$ 40% moisture content (prior to frying) when fried had mimimum acrylamide formation and was sensorically most acceptable (Fig. S4). Thus modified frying conditions of 140 °C for 5 min instead of conventional deep frying condition (170 °C for 3 min) contributed to lower acrylamide formation. However, the lower frying conditions compared to optimal one (< 140 °C & 5 min) rendered the fried crisps organoleptically unacceptable especially in terms of texture. On increase of frying time beyond 5 min at 140 °C, the textural characteristics improved (sensorically judged) with concomitant increase of acrylamide content in the final products by $\sim$ 5% of the same obtained at 140 °C, 5 min.

Holistically considering all parameters, this study established that the following conditions: aqueous pre-treatments [rinsing, soaking and blanching (at 70 °C for 20 min)]; addition of 2% L-proline; drying at 65 °C for 130 min; 140 °C frying temperature and 5 min frying time effectively reduced acrylamide formation by 98.82% ($\sim$ 99%). These conditions also improved the sensory attributes (in terms of color profile, aroma and taste) of the fried product significantly ($p < 0.05$). In addition, low frying time and temperature (140 °C at 5 min) and usage of DVCO as frying oil did not allow formation of the potential toxic aldehyde i.e., 2,4-decadienal in fried potato-crisps. Outcome of this work if adopted by food processing industries for manufacture of fried crisps would certainly aid in mitigating the health-debilitating effects associated with these toxicants.

Conclusion

The new approach for acrylamide mitigation in fried potato-crisps comprised of incorporation of aqueous pre-treatments steps, usage of L-proline, drying and employment of modified deep frying conditions. This combination treatment contributed to $\sim$ 99% acrylamide mitigation with concomitant enhancement of organoleptic properties of characteristic color and unique flavor, which undoubtedly holds promise of utilization in snack food industries. The potato-crisps with minimum acrylamide content, absence of 2,4-decadienal and excellent sensory appeal could be a novel fried product for global consumers.

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Author's contribution AB designed and executed the entire work and PB provided guidance.

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Declarations

Conflict of interest There is no conflict of interest to disclose.

Ethical approval Our University does not need ethical approval for sensory evlauation.

Consent for publication Authors provide consnet for publication to JFST.

Data availability Data related to this current research are provided herewith in tables and figures.

Consent to participate The consent was obtained from all 30 semi-trained panelists, who participated in sensory evaluation.

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RESEARCH ARTICLE

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Cookies Formulated with Gamma-Irradiated Virgin Coconut Oil are Less Rancid: Analysis By Metal Oxide-Based Electronic Nose and Support Vector Machines

*Kona Mondal, Anupama Bose, Dipshikha Tamili, Soumi Chakraborty, Dipan Chatterjee, Kaninika Paul, Nabarun Bhattacharyya, and Paramita Bhattacharjee**

In this study, hydrogenated vegetable fat in cookie is replaced by gamma-irradiation-induced (4.2 kGy) deodorized (rancid-acid odor removed) virgin coconut oil (VCO) with the aim to formulate antioxidant-rich cookies with good sensory acceptability up to 150 days, *vis-à-vis* the hydrogenated fat- and VCO-formulated cookies. Cookies formulated with hydrogenated fat, non-irradiated VCO and 4.2 kGy irradiated VCO serve as control, positive control, and sample sets, respectively. Rancidity of three sets of cookies is assessed for a period of 180 days at 25±2°C, ≈75–80% RH using metal oxide gas sensor-based electronic nose (e-nose). The best combinations of sensors are selected employing support vector machines analysis to accurately predict rancidity development in the three sets of cookies during storage. Analyses of principal component analysis (PCA) plots of the e-nose sensor responses reveal that the progression of rancidity in the control and positive control sets of cookies is detected on day 90 and 120, respectively; whereas, the onset of rancidity is detected for sample set of cookies on day 150. Findings of sensory evaluation and e-nose analyses affirm that the newly formulated cookies can be safely consumed up to 150 days when stored at 25±2 °C, 75–80% RH. *Practical applications*: Gamma-irradiated deodorized VCO successfully enhances storage-life of drop cookies by more than 60 days *vis-à-vis* its hydrogenated fat counterpart. These newly formulated cookies which can be stored and consumed for a longer period would certainly be more commercially viable. Gamma-irradiated VCO can also be used as an alternative to hydrogenated fats in preparing similar bakery-cum-confectionary products. The methodology developed for assessment of rancidity using electronic nose technology would be extremely useful considering high industrial throughput and can be safely extrapolated to other bakery and confectionary products for accurate assessment of rancidity, forgoing cumbersome biochemical assays.

1. Introduction

Cookies (a popular bakery product) formulated with saturated fats are health debilitating.[1] Virgin coconut oil (VCO) could be an alternative to hydrogenated fats for cookie preparation owing to its high content of rheologically (in terms of plasticity)-compatible[2] saturated fat content (about 90% of the total fatty acids is saturated).[3] Moreover, VCO-formulated cookies could be potentially healthier since VCO is reportedly known to be rich in several phytochemicals.[4] However, VCO could impart an unacceptable rancid-acid odor (octanoic acid) note to the cookies during processing and storage[5,6] and would thus render the same commercially unacceptable.

Earlier investigations conducted in our research laboratory have successfully deodorized VCO employing gamma (γ)-irradiation at a dose of 4.2 kGy which could eliminate the typical rancid-acid odor in VCO, without compromising its characteristic lactonic aroma, as well as its physiochemical and phytochemical properties (chiefly antioxidant potency).[4,5] The deodorized VCO had been successively utilized in designing a long shelf-life green tea-based beverage.[7]

However, 4.2 kGy-deodorized VCO still had significant amounts of $C_{18:1}$ and $C_{18:2}$ (which did not undergo complete radiolysis during γ-irradiation to yield all characteristic long chain hydrocarbons) unsaturated fatty acids[5] which could render the VCO-formulated cookies rancid. Oxidative rancidity which follows a free radical chain mechanism could thus occur in this confectionary product and thereby extension of shelf-life of the same would primarily depend on delay of onset of rancidity therein. This warrants accurate assessment of rancidity in this high-fat product forgoing the limitations associated with biochemical analyses (primarily fat extraction from cookies for assays), chiefly determination of peroxide values (PVs) and acid values (AVs) by conventional titrimetric methods.[8] To circumvent the above

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limitation, researchers world-wide have assessed rancidity of high-fat food products such as nuts, crisps, cookies, and oils using electronic nose (e-nose) technology.[9–13]

In the current investigation, drop cookies were formulated using deodorized γ-irradiated (4.2 kGy) VCO, as a replacement of hydrogenated fat with the aim to provide healthy cookies fortified with VCO-antioxidants. There is also an interesting work reported by Sung and Lin[14] who formulated cookies with beeswax-VCO organogels; however, assessed rancidity using conventional biochemical assays.

Rancidity in the hydrogenated fat- and VCO-formulated cookies has been analyzed for a period of 180 days at 25±2 °C, employing an e-nose set up known as ENOVISION, developed by the Centre for Development of Advanced Computing (C-DAC), Kolkata, India. The working principle of this technology and its related configurations has been elaborated in detail by Chatterjee et al.[8] and Bhattacharyya et al.[15] ENOVISION comprised of a sensor array of 8 metal oxide sensors (MOS) reportedly suitable for detection of volatile organic compounds (VCOs) emanating from food samples. The array of sensors (TGS 823, TGS 2620, TGS 816, TGS 2602, TGS 2611, TGS 2610, TGS 832, and TGS 2600) provides responses to volatile organic compounds (VOCs) via appropriate odor channelization-cum-recognition systems. This causes changes in conductivities across the sensors' surfaces and the variations in conductivities are recorded as changes in resistances (R) with respect to their original values, i.e., $|\Delta R|/R$ (a dimensionless quantity, known as sensor response). The requisite airflow is maintained using mini air compressor for three purposes: (a) during headspace generation time- the blowing of regulated flow of air on the cookie samples ensures an adequate concentration of VOCs in the sample holder; (b) during sampling time- the uniform airflow distribution in sensor array renders the sensors saturated with VOCs of the cookie samples; (c) during purging time- sensor heads are cleared with the blow of fresh air to enable sensors to return to their base line values. The $|\Delta R|/R$ values are converted into voltage and subsequent transmission to computational mode is effected by pulse code modulation technique.

Screening of the e-nose sensors was first conducted based on sensor responses towards the VOCs generated from the cookies. The screening method was further validated using a supervised machine learning classifier (SMLC). The conventional (standard) biochemical assays for rancidity assessment in terms of PV, AV, and malonaldehyde (MDA) content were also carried out. A principal component analysis (PCA) of the e-nose sensor data was performed to assess alterations in VOCs generated from the cookies during 180 days of storage. Multiple regression equations corresponding to the aforesaid biochemical parameters were developed as a function of the e-nose sensor responses.

The study targets to predict the above biochemical parameters of rancidity for the cookies directly from the e-nose sensor responses, forgoing the cumbersome routine biochemical assays. This will enable food processing industries to accurately and reliably assess rancidity in cookies for large sample throughputs and thus provide consumers with accurate storage life on product packages.

The final outcome of the work would be to obtain cookies formulated with γ-irradiated VCO (as a replacer of hydrogenated fat) and correlation equations of biochemical parameters (rancidity assay values) with e-nose sensor responses (to VOCs generated from cookies consequent to rancidity).

Table 1. Ingredients of cookies prepared with γ-irradiated VCO.

Ingredients	% Composition (w/w of dough weight), wet basis
Wheat flour	50
Table salt	0.6
Ammonium bicarbonate	0.8
Sodium bicarbonate	0.4
Milk powder	2
Powder sugar	20
Coconut oil (as replacement of hydrogenated fat)	20
Butter	2
Water	8

2. Experimental Section

2.1. Materials

Expeller-pressed VCO and *vanaspati* (Dalda, India) were purchased from a supermarket close to Jadavpur University, Kolkata, India. The manufacturer of VCO, viz. M/s KPL Oil Mills Pvt. Ltd., Kerala, India, confirmed that the VCO used for this study had been extracted from coconut copra of the "West coast tall" variety. Amber bottles, aluminum foil, and low density polyethylene (LDPE) self-sealable bags (20 μm thickness) were also purchased from the supermarket. All analytical grade chemicals were used in this work. Food grade nitrogen was procured from M/s Linde India Ltd., Kolkata, India.

2.2. Formulation of Drop Cookies Using γ-Irradiated VCO

To remove rancid odor from VCO, 500 mL sample was filled in amber-colored screw-capped glass bottles (10 bottles), purged with nitrogen, sealed with teflon and γ-irradiated at the pre-optimized dose of 4.2 kGy. Gamma irradiation was conducted using ^{60}Co as the radioactive source inside an irradiation chamber (GC-5000) in accordance with the methodology reported by Ghosh et al.[5]

Drop cookies were prepared as per procedure detailed by Chatterjee et al.,[8] with few modifications. The ingredients used for cookie preparation have been listed in **Table 1** and the process flow sheet for formulation of VCO-cookies has been presented in **Figure 1**. Three sets of cookies were prepared: two control sets and one sample set. Conventional cookies formulated with hydrogenated fat (*vanaspati*) was the positive control set (control sets of cookies formulated with hydrogenated fat (HC)); those formulated with non-irradiated VCO comprised of the experimental control set (control sets of cookies formulated with non-irradiated VCO (CC)); and those formulated using γ-irradiated VCO served as the sample set (sample sets of cookies formulated with deodorized γ-irradiated VCO (SC)). The training sets (described in Section 2.4.2.) of HC, CC, and SC have been designated as training sets of cookies formulated with hydrogenated

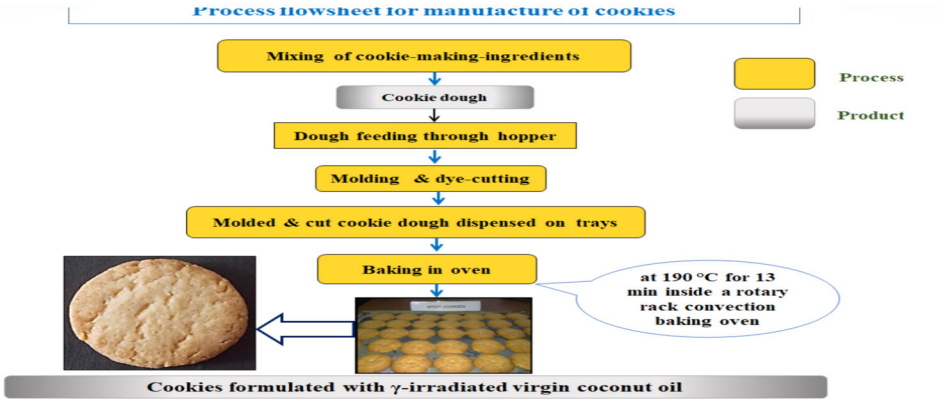

Figure 1. Process flow sheet for formulation of VCO-cookies.

fat (THC), training sets of cookies formulated with non-irradiated VCO (TCC), and training sets of cookies formulated with deodorized γ-irradiated VCO (TSC), respectively. Details of different batches of cookies are presented in **Table 2**. Post formulation, the cookies were placed on a greased pan and baked in a rotary rack convection type baking oven (Model CMHS-108, M/s Chanmag Bakery Machine Co. Ltd., New Taipei City, Taiwan) at 190 °C for 13 min (baking temperature and time were optimized after several preliminary trials) and then cooled to room temperature (25±2 °C, ≈80% RH) before packaging. The mean diameter and thickness of the cookies were 50±0.20 and 10±0.10 mm, respectively. The mean weight of the sample and control sets of cookies was 20±0.80 g. The cookies were first wrapped in aluminum foils and then packaged in self-sealable Ziploc pouches (M/s Johnson & Johnson Ltd., Mumbai, India) and finally flushed with food grade nitrogen. For assessment of rancidity, the control and sample sets of cookies were stored for a period of 180 days in laboratory environment conditions (25±2 °C, 75–80% RH).

HC, CC and SC sets of cookies were subjected to sensory evaluation, e-nose analyses and routine biochemical analyses for assessment of their rancidity. Sampling of cookies for the aforesaid analyses were conducted as shown in **Figure 2**. Post sampling, cookie sample sets were subjected to the above-mentioned analyses: the two control sets (HC and CC) and the sample set (SC) comprised of 100 cookies each, of which 90 cookies in each set were subjected to sensory evaluation; and biochemical and e-nose analyses were conducted in tandem with the remaining 10 cookies, post grinding (to powder of diameter particle (d_p) ≈ 0.500 mm) in an electric grinder (HL 1618, M/s Philips, India).

Prior to e-nose analyses, it was necessary to train the e-nose with the VOCs emanating from rancid cookies. Thus, a training set of cookies was generated which comprised of deliberately-made rancid cookies.[9,16] These were prepared by storing freshly prepared HC, CC and SC sets of cookies for 1, 3, 10, 20, and 30 days inside a chamber at 40±2 °C under UV light of wavelength 254 nm to accelerate rancidity (lipid oxidation) development in them. At the end of the rancidity (ageing) treatment, the samples were withdrawn at intervals of 1, 3, 10, 20, and 30 days from the chamber and transferred into a conservation chamber maintained at 25±2 °C, 75–80% RH, to arrest the rancidity stage(s) of the samples on the said days. These deliberately-made rancid cookie sets withdrawn from the conservation chamber were coded as THC, TCC, and TSC (labelled apposite to their control and sample sets), which comprised of 10 cookies in each set. Five cookies from each set were subjected to biochemical analyses and the VOCs generated from the remaining five cookies were analyzed by e-nose technology (Figure 2).

2.3. Sensory Evaluation of the HC, CC, and SC Cookie Sets

Sensory evaluation of the sample and control sets of cookies was conducted in accordance with the method reported by Ghosh et al. (2016)[17] and Nwakalor and Chizoba (2014).[18] In the present study, HC, CC. and SC cookie sets were assessed by a semi-trained panel comprised of our university faculty members and research scholars (15 men and 15 women), aged 20–45 years, who evaluated the three sets of cookies, using effective method-based acceptance test (9-point hedonic scale rating).[19]

The sensory attributes judged by the panelists were overall appearance, color, odor, texture, taste and after taste. The sensory evaluation was conducted between 10 a.m. and 12 noon in a ventilated room under white light. The selection of panelists was conducted on the basis of two parameters: (a) interest of the panelists and (b) their performance in screening tests conducted with training sets (discussed in Section 2.4.2.) of HC, CC, and SC sets. The panelists were familiarized with the quality parameters of cookie samples prior to analysis and provided scores for different attributes (mentioned above) of both fresh and rancid cookies (characterized by a typical rancid-acid odor note, metallic taste and an oily mouthfeel). For sensory evaluation, three cookies (selected randomly from a set of 100 cookies as shown in Figure 2) were placed in small white plates labelled with three-digit codes and were served randomly to the panelists in triplicate. Panelists were provided a rest period of 5 min in between consecutive samples; and with water and unsalted crackers to cleanse their palate.[17]

2.4. Analysis of Cookie Samples by ENOVISION

2.4.1. Selection of Appropriate Sensors of ENOVISION

Although all eight sensors (TGS 823, TGS 2620, TGS 816, TGS 2602, TGS 2611, TGS 2610, TGS 832 and TGS 2600) of

Table 2. Different batches of cookies prepared.

Sample Codes	Sets of cookies	Sample descriptions
Control sets of cookies (prepared with hydrogenated fat)		
THC1	Training Set	Deliberately made rancid in accelerated rancidity chamber for 1 day
THC3	Training Set	Deliberately made rancid in accelerated rancidity chamber for 3 days
THC10	Training Set	Deliberately made rancid in accelerated rancidity chamber for 10 days
TCC20	Training Set	Deliberately made rancid in accelerated rancidity chamber for 20 days
THC30	Training Set	Deliberately made rancid in accelerated rancidity chamber for 30 day
HC0	Experimental Set	Hydrogenated fat added and stored for 0 day
HC10	Experimental Set	Hydrogenated fat added and stored for 10 days
HC20	Experimental Set	Hydrogenated fat added and stored for 20 days
HC30	Experimental Set	Hydrogenated fat added and stored for 30 days
HC45	Experimental Set	Hydrogenated fat added and stored for 45 days
HC60	Experimental Set	Hydrogenated fat added and stored for 60 days
HC75	Experimental Set	Hydrogenated fat added and stored for 75 days
HC90	Experimental Set	Hydrogenated fat added and stored for 90 days
HC120	Experimental Set	Hydrogenated fat added and stored for 120 days
HC150	Experimental Set	Hydrogenated fat added and stored for 150 days
HC180	Experimental Set	Hydrogenated fat added and stored for 180 days
Coconut Control sets of cookies (prepared with coconut oil)		
TCC1	Training Set	Deliberately made rancid in accelerated rancidity chamber for 1 day
TCC3	Training Set	Deliberately made rancid in accelerated rancidity chamber for 3 days
TCC10	Training Set	Deliberately made rancid in accelerated rancidity chamber for 10 days
TCC20	Training Set	Deliberately made rancid in accelerated rancidity chamber for 20 days
TCC30	Training Set	Deliberately made rancid in accelerated rancidity chamber for 30 days
CC0	Experimental Set	Non-irradiated virgin coconut oil added and stored for 0 day
CC10	Experimental Set	Non-irradiated virgin coconut oil added and stored for 10 days
CC20	Experimental Set	Non-irradiated virgin coconut oil added and stored for 20 days
CC30	Experimental Set	Non-irradiated virgin coconut oil added and stored for 30 days
CC45	Experimental Set	Non-irradiated virgin coconut oil added and stored for 45 days
CC60	Experimental Set	Non-irradiated virgin coconut oil added and stored for 60 days
CC75	Experimental Set	Non-irradiated virgin coconut oil added and stored for 75 days
CC90	Experimental Set	Non-irradiated virgin coconut oil added and stored for 90 days
CC120	Experimental Set	Non-irradiated virgin coconut oil added and stored for 120 days
CC150	Experimental Set	Non-irradiated virgin coconut oil added and stored for 150 days
CC180	Experimental Set	Non-irradiated virgin coconut oil added and stored for 180 days
Sample sets of cookies (prepared with irradiated virgin coconut oil)		
TSC1	Training Set	Deliberately made rancid in accelerated rancidity chamber for 1 day
TSC3	Training Set	Deliberately made rancid in accelerated rancidity chamber for 3 days
TSC10	Training Set	Deliberately made rancid in accelerated rancidity chamber for 10 days
TSC20	Training Set	Deliberately made rancid in accelerated rancidity chamber for 20 days
TSC30	Training Set	Deliberately made rancid in accelerated rancidity chamber for 30 days
SC0	Experimental Set	Irradiated virgin coconut oil added and stored for 0 day
SC10	Experimental Set	Irradiated virgin coconut oil added and stored for 10 days
SC20	Experimental Set	Irradiated virgin coconut oil added and stored for 20 days
SC30	Experimental Set	Irradiated virgin coconut oil added and stored for 30 days
SC45	Experimental Set	Irradiated virgin coconut oil added and stored for 45 days
SC60	Experimental Set	Irradiated virgin coconut oil added and stored for 60 days
S75	Experimental Set	Irradiated virgin coconut oil added and stored for 75 days
SC90	Experimental Set	Irradiated virgin coconut oil added and stored for 90 days
SC120	Experimental Set	Irradiated virgin coconut oil added and stored for 120 days
SC150	Experimental Set	Irradiated virgin coconut oil added and stored for 150 days
SC180	Experimental Set	Irradiated virgin coconut oil added and stored for 180 days

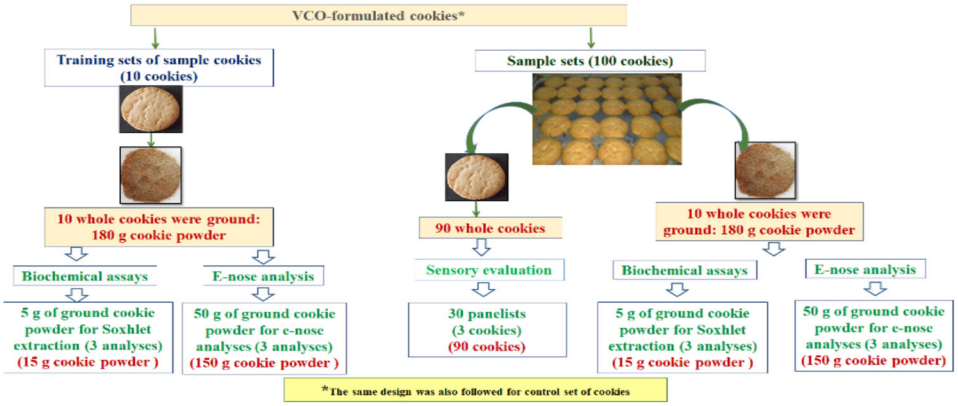

Figure 2. Sets of cookies subjected to 180 day storage study.

Table 3. Classification results for each classifier.

Classifier	Classifier type	Accuracy [%]	Prediction speed [Objects/s]	Training time [s]
Decision tree	Fine Tree	81.20	3800	6.32
	Medium Tree	81.20	4400	3.15
	Coarse Tree	78.80	3700	2.16
SVM	Linear SVM	92.30	5100	3.19
	Quadratic SVM	87.50	1600	2.09
	Cubic SVM	87.50	1600	2.96
	Fine Gaussian SVM	78.80	1400	4.18
	Medium Gaussian SVM	86.20	1600	3.23
	Coarse Gaussian SVM	86.20	1600	5.43
Ensemble classifiers	Boosted Trees	32.50	2300	2.63
	Bagged Trees	88.80	350	4.66
	Subspace discriminate	82.50	350	3.48
	Subspace KNN	63.70	230	3.89
Naïve Bayes	Kernel	90.00	1700	3.21
Neural network	Narrow	81.20	2900	4.83
	Medium	85.00	3800	4.48
	Wide	87.50	3700	5.15
KNN	Fine	91.20	1700	2.29
	Medium	82.50	2500	2.53
	Coarse	32.50	2500	3.36
	Cosine	86.20	1800	2.54
	Cubic	82.50	2200	1.95
	Weighed	91.20	2200	1.79

The best classification method was judged based on the highest prediction speed and highest accuracy Prediction speed: It involves checking the value of one predictor (variable) in unit time. It is the combination of computational speed and accuracy.

ENOVISION are reportedly known to respond to VOCs of cookies;[20] in this work, sensor screening was performed using SMLC to identify those sensors that specifically respond to VCO-formulated cookies. Different classifiers or models of SMLC were run using five-fold cross-validation method for evaluating performance of each model on the basis of root mean square error (RMSE) values. The optimum model was selected based on % accuracy and prediction speed of each classifier. Data sets of $|\Delta R|/R$ obtained from each sensor for the VOCs generated from cookie sets on 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, and 180 days of storage were trained by different models of SMLC such as decision tree, support vector machines (SVM), KNN (k-nearest neighbors), neural network, ensemble classifiers and naïve bayse. Recognition of the best combination of sensors was realized using the best fit model (**Table 3**). Validation of the sensor screening method was performed by SMLC using Matlab R2020a (Mathworks, Inc. Natick, MA, USA).

Table 4. E-nose screened sensor responses ($|\Delta R|/R$) of control and sample sets of cookies that had been made rancid deliberately.

Storage period [Days]	Sensor responses ($|\Delta R|/R$) of cookies prepared with hydrogenated fat				Sensor responses ($|\Delta R|/R$) of cookies prepared with coconut oil				Sensor responses ($|\Delta R|/R$) of cookies prepared with irradiated VCO			
	TGS 816	TGS 823	TGS 832	TGS 2600	TGS 816	TGS 823	TGS 832	TGS 2600	TGS 816	TGS 823	TGS 832	TGS 2600
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
1	0.6325	0.1746	0.1105	0.1567	0.5201	0.1824	0.1208	0.2500	0.4908	0.2098	0.1942	0.2534
3	0.6536	0.2430	0.1714	0.3111	0.5475	0.2401	0.1804	0.2980	0.5158	0.2491	0.2022	0.2687
10	0.7047	0.4595	0.2381	0.4991	0.5700	0.5673	0.2250	0.3001	0.5802	0.2986	0.2177	0.3188
20	0.7769	0.7114	0.5124	0.6296	0.6100	0.5278	0.4920	0.5909	0.6757	0.3339	0.2322	0.3910
30	0.8491	0.9870	0.8571	0.9667	0.6204	0.7111	0.6300	0.7025	0.7523	0.4782	0.2961	0.4456

SD was not mentioned in "±" form here as it is insignificant (<0.0000)

Training sets of samples (vide infra in Section 2.4.2.) were subsequently analyzed using the selected sensors on days 1, 3, 10, 20, and 30 of storage (**Table 4**). The different storage conditions of training sets and experimental sets of HC, CC, and SC influenced the total duration of storage period of the cookie sets and the time-intervals of sample analyses.

2.4.2. Detection of Rancidity in Cookies using ENOVISION

E-nose was calibrated by several preliminary trials using different batches of powdered cookies and optimization of the parameters (such as sample size, mesh size, or granularity of ground cookies, time needed for generation of VOCs, time for saturation of headspace of sample vial, and sampling and purging time) was performed on the basis of the maximum response ($|\Delta R|/R$) received from each sensor. A 50 g sample of ground cookie powder [particle diameter (d_p) = 0.50±0.02 mm] was placed inside a 100 mL vial and heated for 450 s at 50±2 °C using a 50 W miniature halogen lamp. The acquisition rate (number of data acquired per sample) was 600. The adequate amounts of VOCs were generated from powdered cookie samples for 30 s (headspace generation time). The VOCs were allowed to reach the sensors via pipelines and saturate the sensors' surfaces for 50 s (sampling time). Purging operation was conducted for 500 s at a flow rate of 5 mL s^{-1} of fresh air to restore the base line values of the sensors.

As mentioned earlier, prior to rancid-acid odor analyses of HC, CC, and SC cookie sets by e-nose, it was necessary to train the e-nose with deliberately-made rancid cookies viz. by THC, TCC, and TSC sets of cookies first so as to enable reliable discrimination between rancid and non-rancid cookies by e-nose. Post screening and training, e-nose analyses were performed for the HC, CC, and SC cookies sets. Labelling of samples withdrawn from the said sets were performed in accordance with their respective day of analysis, viz. sample labels corresponded to their storage time in days. For example, HC cookie set analyzed on day 1 was labelled as HC1. The samples withdrawn from the sets THC, TCC, and TSC cookie sets were similarly labelled.

2.5. Determination of Moisture Contents of the Cookies

Since most of the e-nose sensors were moisture sensitive, it was necessary to ensure that response signal of the sensors was only due to rancid-acid odor and not due to moisture present in the cookies. Hence the moisture contents of all batches of cookies were determined during storage using the classical Dean and Stark method (Association of Official Analytical Collaboration (AOAC) official method 986.21).[21]

2.6. Assessment of Rancidity in Cookies by Routine Biochemical Assays

Standard biochemical assays (AVs, PVs, and MDA contents) were also performed to determine rancidity in the cookies and to establish apposite correlations with the e-nose data. The above assays were performed with HC, CC, and SC sets of cookies (on days 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, and 180) and their deliberately-rancid counterparts (on days 1, 3, 10, 20, and 30). To analyze the above rancidity parameters of the cookies, fat was extracted from 5 g of powdered cookies using a Soxhlet extraction assembly in accordance with AOAC 963.15 (2000)[22] and the extracted fat was subjected to biochemical assays. PVs and AVs were determined in accordance with standard methods[8,9,23] and expressed in terms of meq. O_2/kg of sample and mg of KOH/g of sample, respectively. To determine the secondary oxidation products in the cookies, the MDA contents (mmol g^{-1} cookie) of the cookies were quantified (from its standard graph prepared using a pure standard of the said chemical) by thiobarbituric acid-reactive substances (TBARS) assay, in accordance with the method reported by Dutta et al. (2017)[9] and Che-Man et al. (1999).[24]

2.7. Statistical Analysis

The biochemical assays for determination of PVs, AVs, and MDA contents and the e-nose analyses of cookies were performed in triplicate for three independent samples of cookies withdrawn from HC, CC, and SC sets. Data are expressed as means ± SD of the data obtained from the said three samples. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Duncan's multiple-range test was conducted using STATISTICA 8.0 software (Statsoft, Oklahoma, USA) to determine significant differences among the means of different biochemical parameters of rancidity (for the cookies) with storage time. Three individual multiple regression equations were generated to predict the PVs, AVs, and MDA contents of cookies as a function of e-nose

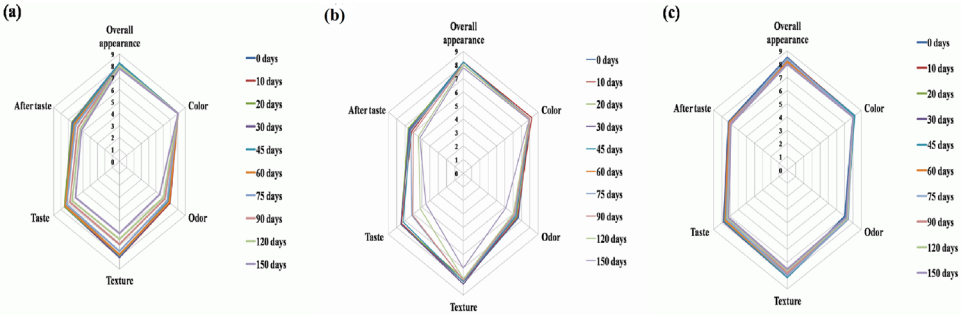

Figure 3. Effect of storage on sensory characteristics of a) HC, b) CC, and c) SC cookies.

responses for the entire storage period. The test of significance was verified using p value of ≤ 0.05.

In the present study, normality test was performed with the e-nose data ($\Delta R/R$ obtained from each sensor on different storage days) using XLSTAT 2014.5.03 (Microsoft Corporation, USA). Since the data followed normal distribution (data not shown), PCA has been conducted using the same software to enable us to discriminate between rancid and nonrancid cookie samples during 180 days of storage.

3. Results and Discussion

3.1. Sensory Analysis of Cookies

The mean sensory scores for the control (HC and CC) sets and sample set (SC) of cookies have been presented in radar plots (**Figure 3**). A detectable rancid-acid odor note developed in HC cookie sets on day 90 and increased with storage time. The CC set of cookies had a distinct odor note of VCOs which persisted up to 90 days but was unacceptable to the panelists. The typical odor note of VCO was suppressed by a strong rancid-acid odor note in the CC set of cookies from day 120 onwards with concomitant development of bitter taste. However, SC set of cookies had acceptable sensory properties vis-à-vis the CC set which was attributed to the absence of typical odor note of VCO. This set was acceptable by the panelists owing to less oily mouthfeel and pleasant lactonic aroma which remained unaltered up to day 150 and also owing to undetectable rancid-acid odor note in the same. However, a strong rancid-acid odor note and a metallic taste with oily mouthfeel were perceived by the panelists in the SC set of cookies only on the last of storage, viz. day 180.

3.2. Moisture Content of the Cookies During Storage

Since the cookies were stored in Al-coated pouches filled with nitrogen gas, the moisture contents of the three sets of cookies remained unaltered throughout the storage period. The initial moisture content of the cookies on day 0 was around 2.0–2.5% which underwent insignificant ($p > 0.05$) variation till the last day of storage (day 180). This also suggested that signal responses of e-nose sensors were not affected by the moisture content of the cookies.

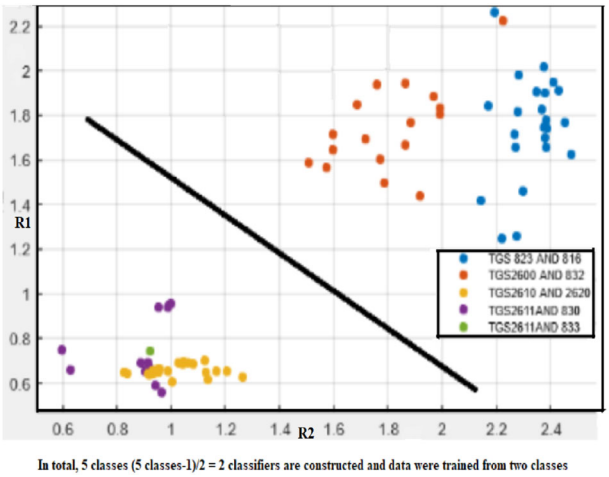

Figure 4. SVM classification discriminates the good sensors providing better sensor responses towards the rancidity odor of cookies during shelf-life using maximum margin separating hyperplane within a two-class separable dataset (R_1 and R_2) [R_1: comprises sensor responses 0.70-0.40; R_2: comprises sensor responses 0.40-0.00].

3.3. Electronic Nose Analyses of the Cookies with Storage

3.3.1. Screening of e-Nose Sensors for Detection of Rancidity in the Cookies

The sensor responses ($|\Delta R|/R$) for the control and sample sets of cookies on different storage days have been presented in **Figure 4**. The trend lines and slope equations were obtained by plotting the storage time on X-axis and $|\Delta R|/R$ on Y-axis. Among the eight sensors- TGS 816, TGS 832, and TGS 2600 sensors exhibited relatively higher $|\Delta R|/R$ values with minimum variations. Sensor TGS 816 showed highest sensor response throughout the storage period of 180 days, followed by the sensors TGS 2600 and TGS 832. The responses of each sensor towards VOCs of the cookies withdrawn on different storage days were then trained by 26 SMLC models (Table 3). The different models were run and cross validated using "fitcsvm" syntax programming (provided in File S1 in the Supporting Information) (Table 4).

SVM performs classification by finding the hyperplane that maximizes the margin between the two classes; herein classes containing lower and higher$|\Delta R|/R$ values of sensors (Figure 4). In this regard, the data points or vectors that affect the position

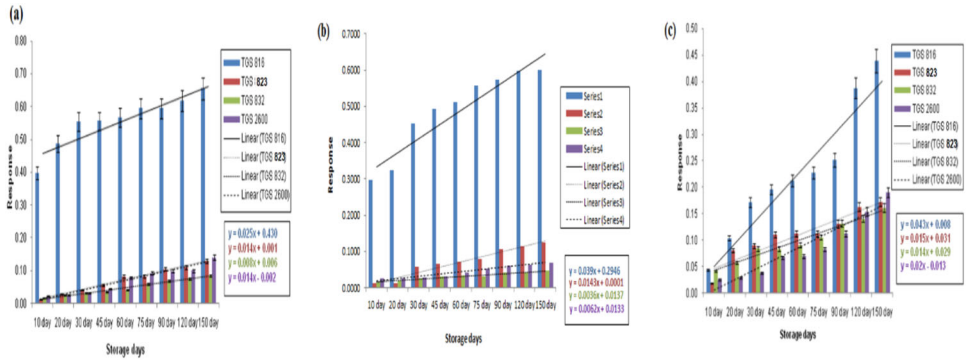

Figure 5. Plot of variation in signal responses of a) control set of cookies, b) cookies prepared with non-irradiated VCO, and c) sample set of cookies formulated with γ-irradiated VCO and analyzed by sensors TGS 816, TGS 823, TGS 832, TGS 2600.

of the hyperplane are termed as support vectors. The best fit model was found to be SVM(C) (linear) [support vector machines] with an accuracy of 92.3%, post facto, which considers "one-versus-one" approach for multiclass classification.[25] This technique has its roots in statistical learning theory developed by Vapnik (1992).[26] A scatter plot obtained using "plt" syntax programming (provided in File S2 in the Supporting Information) for $|\Delta R|/R$ values of all eight MOS was segregated by a linear hyper-plane with maximal margin (maximum marginal hyperplane, MMH = $D^+ + D^-$) using the following formula

$$w^T \cdot (x) + b = 0 \quad (1)$$

where, b = intercept and bias term of the hyper-plane equation; w = classification vector/weightage of attributes.

It was evident from Figure 4 that TGS 816 and 823; and TGS 832 and 2600 sensors were classified with the highest accuracy because their $|\Delta R|/R$ values were appreciably high (greater than 1.5000) and each vector (point) was discrete in nature. The set comprising of the remaining sensors was regarded as the "opponent group" w.r.t the above-mentioned groups since the sensors in the former set displayed lower $|\Delta R|/R$ values of overlapping nature, implying that the responses of the said sensors to the VOCs of cookies were indistinguishable from each other. Thus, the SVM analysis successfully allowed recognition of the best combination of pairs of sensors, viz. TGS 816 and 823; and TGS 832 and 2600 for assessment of rancidity of CC, HC and SC sets during storage.

Similar study on the use of four MOS, i.e., MOS2, MOS6, MOS7, and MOS8 have been reported by Lirong et al. (2016)[27] for detection of rancidity of different edible oils, including coconut oil. The current study too has considered responses of four selected sensors (TGS 816, TGS 823, TGS 832, and TGS 2600) of an 8-sensor array suitable for detection of rancidity in cookies (**Figure 5**). The data obtained from the above e-nose sensors were then subjected to PCA to discriminate rancid cookies from their non-rancid counterparts during 180 days of storage.

3.3.2. Discrimination of Rancid Cookies from Non-rancid Ones by PCA Analysis

The data obtained from the selected sensors were analysed by PCA-based odor maps and the PCA plots have been presented in **Figure 6**. Analysis of the PCA plots showed that the discrimination indices were 99.27%, 97.88%, and 99.15% for the HC (Figure 6a), CC (Figure 6b), and SC (Figure 6c) cookie sets, respectively, indicating clear discrimination between nonrancid and rancid cookie samples based on $|\Delta R|/R$ values of the four selected sensors. The data points have been found to be clustered in different quadrants: quadrant I comprised of a cluster of THC3, THC10 cookie sets; quadrant II constituted of two distinct clusters of HC20, HC30, HC45, HC60 cookie sets; and another of HC90, HC120, HC150, HC180, THC1 cookie sets; quadrant III comprised of a cluster of HC0, HC10 cookie sets; and quadrant IV constituted a cluster of THC20 and THC30 cookie sets. The above analyses confirmed that HC90 had rancid-acid odor note similar to that of THC1 since they occupied the same cluster (II). Thus, it is evident that storage-life (in terms of rancidity) of the HC set of cookies is less than 90 days under our experimental storage conditions.

In Figure 6b similarly, four well-defined clusters in different quadrants were evident in CC sets. Quadrant I comprised of a cluster of TCC20 and TCC30 cookie sets; quadrant II consisted of a cluster of CC0, CC10, CC20 cookie sets; quadrant III comprised of a cluster of CC30, CC45, CC60, CC75, and CC90 cookie sets; and quadrant IV comprised of a cluster of CC120, CC150, CC180, TCC1, TCC3, and TCC10 cookie sets. From the PCA plots, it can be deciphered that progression of rancidity in the CC set occurred from day 120 onwards since it belonged to the same cluster occupied by TCC1, TCC3, and TCC10. These findings indicated that progression of rancidity in the CC set occurred from day 120 onwards at 25±2 °C (≈75–80% RH). Therefore, it could be concluded that storage-life of HC sets was less than 120 days under the above-mentioned storage conditions.

In a similar manner, PCA plots for the SC sets have shown three distinct clusters in different quadrants (Figure 6c). Quadrant I consists of a cluster comprising of TSC10, TSC20, TSC30 sets of cookies; quadrant II consists of a cluster comprising of SC20, SC30, SC45, SC60, SC75, SC90 sets of cookies, wherein SC0 and SC10 were distinctly separated from this cluster in the same quadrant; quadrant III consists of SC120; and quadrant IV constituted a cluster of TSC1, TSC3 and SC180. SC150 was distinctly separated from this cluster in the same quadrant.

It is evident from the above findings that onset of rancidity in the SC cookie set occurred from day 150 onwards; however, the

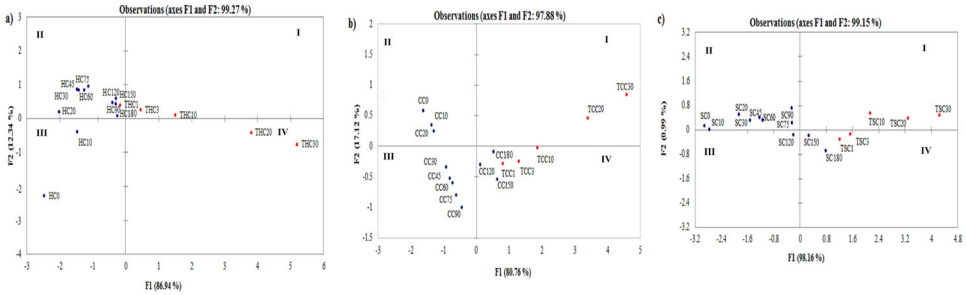

Figure 6. PCA plots of a) HC, b) CC, and c) SC cookies.

Table 5. AVs, PVs, and MDA contents of cookies prepared with hydrogenated fat (HC), coconut oil (CC), and irradiated VCO (SC) and their deliberately rancid counterparts, during storage period of 150 days.

Storage period [days]	PVs [ppm × 10^{-4}] of cookies			AVs [% oleic acid] of cookies			MDA contents [mmole g^{-1} dried sample) of cookies]		
	HC	CC	SC	HC	CC	SC	HC	CC	SC
0	2.01±0.10^{a}	2.20±0.10^{a}	2.00±0.10^{a}	0.34±0.01^{a}	0.81±0.02^{f}	0.33±0.01^{a}	1.09±0.04^{a}	1.05±0.04^{a}	0.85±0.03^{a}
10	5.31±0.25^{a}	5.00±0.25^{a}	3.33±0.15^{a}	0.45±0.02^{b}	0.31±0.01a	0.34±0.01^{a}	1.48±0.06^{b}	1.18±0.03^{b}	0.96±0.04^{a,b}
20	11.04±0.55^{b}	13.21±0.55^{b}	19.21±0.95^{b}	0.45±0.01^{b}	0.39±0.02^{b}	0.34±0.02^{a}	1.64±0.07^{c}	1.34±0.07^{c}	1.03±0.04^{a,b}
30	18.02±0.90^{b}	21.00±0.90^{b}	20.00±1.00^{b}	0.55±0.02^{c}	0.41±0.01^{b}	0.44±0.02^{b}	1.69±0.08^{c}	1.54±0.08^{c}	1.12±0.05^{b}
45	43.02±2.15^{c}	40.21±2.15^{c}	39.11±1.95^{c}	0.56±0.02^{c}	0.45±0.02^{c}	0.45±0.02^{b}	1.67±0.07^{c}	1.57±0.07^{c}	1.41±0.06^{c}
60	52.11±2.60^{d}	49.21±2.60^{d}	53.22±2.65^{d}	0.63±0.03^{d}	0.47±0.02^{c}	0.45±0.02^{b}	1.70±0.08^{c}	1.69±0.05^{c}	1.43±0.06^{c}
75	56.41±2.80^{d}	55.13±2.80^{d}	54.21±2.70^{d}	0.66±0.01^{d}	0.51±0.03^{d}	0.50±0.02^{c}	1.76±0.08^{c}	1.73±0.08^{c}	1.59±0.07^{d}
90	66.12±3.30^{e}	65.22±3.30^{e}	59.10±3.35^{e}	0.67±0.02^{d}	0.52±0.01^{d}	0.56±0.02^{d}	1.84±0.09^{d}	1.82±0.06^{d}	1.62±0.08^{d}
120	77.21±3.95^{f}	73.12±3.95^{f}	63.33±3.85^{f}	0.76±0.03^{e}	0.58±0.02^{e}	0.56±0.02^{d}	1.94±0.10^{e}	1.90±0.10^{e}	1.66±0.08^{e}
150	79.21±3.75^{f}	74.01±3.05^{f}	66.11±3.55^{f}	0.79±0.03^{e}	0.61±0.03^{e}	0.57±0.02^{d}	2.14±0.^{11ef}	2.01±0.09^{f}	1.82±0.09^{e}
180	80.12±2.83^{ef}	75.55±3.06^{ef}	68.01±1.10^{ef}	0.81±0.02^{f}	0.69±0.03^{f}	0.59±0.03^{e}	2.26±0.13^{ef}	2.14±0.05^{ef}	1.99±0.04^{f}
	Deliberately made rancid cookies (training set)				0.72±0.02g				

Storage period [days]	PVs [ppm × 10^{-4}] of cookies			AVs [% oleic acid] of cookies			MDA contents [mmole g^{-1} dried sample] of cookies		
	HC	CC	SC	HC	CC	SC	HC	CC	SC
1	85.11±4.25^{g}	81.11±4.25^{g}	82.17±4.10^{g}	0.88±0.04^{f}	0.77±0.04^{f}	0.67±0.03^{e}	2.29±0.10^{ef}	2.23±0.11^{g}	2.19±0.09^{g}
3	95.02±4.75^{h}	88.10±4.75^{h}	85.12±4.25^{g}	1.01±0.05^{g}	0.85±0.05^{g}	0.75±0.03^{f}	2.68±0.11^{f}	2.25±0.10^{g}	2.21±0.10^{g}
10	97.21±4.85^{h}	91.02±4.85^{h}	86.22±4.30^{g}	1.13±0.06^{h}	0.98±0.06^{h}	0.79±0.04^{f}	3.39±0.15^{g}	3.33±0.13^{h}	3.11±0.14^{h}
20	101.22±5.05^{i}	97.07±5.05^{i}	91.09±4.55^{h}	1.20±0.04^{f}	1.09±0.04^{f}	0.90±0.04^{g}	5.18±0.23^{h}	4.89±0.^{21gh}	4.62±0.23^{i}
30	106.12±5.30^{i}	99.11±5.30^{i}	94.02±4.70^{h}	1.26±0.06^{i}	1.18±0.06^{i}	1.01±0.05^{h}	5.52±0.26^{i}	5.31±0.19^{i}	5.18±0.24^{j}

Mean ± S.D of three samples of one experimental set; $^{a-i)}$ Different letters in a row indicate significant difference ($p \leq 0.05$).

panellists could not detect the rancid-acid odor note by sensory evaluation in SC cookie set on the same day. With progression of rancidity in the same, sensory detection of rancid-acid odor note was possible only on the last day of storage, viz. on day 180. These findings attest to the superiority of the e-nose technology over sensory evaluation in fast and reliable detection of rancidity in cookies. The study showed a promising application of e-nose technology in combination with PCA analysis for accurate detection of both onset as well as progression of rancidity in cookies, which could not be detected and ascertained reliably by sensory analysis and biochemical assays. Moreover, this study also confirmed the role of deodorized VCO in delaying onset of rancidity in cookies and hence provided a new shelf-stable nutraceutical confectionary for consumers. The SC set of cookies can be consumed safely up to 150 days.

3.4. Changes in Biochemical Parameters of the Cookies during Storage

The PVs, AVs, and MDA contents of the cookie sets (HC, CC, and SC) analysed on 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, and 180 days of storage have been presented in **Table 5**. It was evident from these analyses (vide infra) that there were positive correlations of these biochemical parameters with time, owing to progression of oxidative rancidity. The findings were in consonance with those of Sibian and Riar, 2020[28] who studied changes in biochemical

parameters of cookies (formulated with kidney bean and chickpea) with storage time.

3.4.1. Changes in PVs of Cookies During Storage

The PVs of all sets of cookies also increased slowly and steadily till the last day of storage (day 180). On this day, PVs of the positive control and control sets of cookies were 0.0080 meq. O_2/kg (HC) and 0.0076 meq. O_2/kg (CC), respectively, which were lower than their respective deliberately-rancid counterparts (Table 5). The PV (mean value) of the SC cookie set was 0.0068 meq. O_2/kg on day 150 of storage. In this study, low PVs (i.e., below 1.0 ppm) in the sample set of cookies on day 150 of storage certainly attested to the sensory acceptability of the same up to 180 days. These findings corroborated well with the findings of Divyashree (2014)[29] who studied rancidity in buckwheat-chia seed-fortified cookies for 35 days.

3.4.2. Change in AVs of Cookies During Storage

On day 0, the AV values of HC, CC, and SC sets of cookies were 0.34, 0.31, and 0.33 mg of KOH/g of sample, respectively. However, the AVs increased significantly with storage and reached 0.81, 0.72, and 0.59 mg of KOH/g of sample, respectively, on day 180 (Table 5). The AVs of all sets of cookies on day 180 was significantly ($p < 0.05$) lower than those of day-1 deliberately-rancid cookie sets. Nagi et al.[30] reported similar trends of AVs in cereal bran-fortified cookies at the end of 90 days of storage.

3.4.3. Changes in MDA Contents of Cookies During Storage

The MDA contents of HC (1.09–2.26 mmol/g of cookie), CC (1.05–2.14 mmol/g of cookie), and SC (0.85–1.99 mmol/g of cookie) sets of cookies increased significantly ($p < 0.05$) up to day 180 (Table 5) implying the formation of secondary oxidation products (i.e., MDA) therein during storage. The MDA contents of HC, CC, and SC sets of cookies on day 150 were 2.14, 2.01, and 1.82 mmol g^{-1} of cookie, respectively. This increased trend of MDA values with progression of storage time was in consonance with the findings of Izreen and Noriham (2011)[31] who investigated oxidative rancidity in cakes for a storage period of 15 days. However, in both control sets of cookies, the MDA contents of 150-d stored cookies were lower than those of 1-d stored deliberately rancid cookies (Table 5). Sample sets of cookies on the other hand have shown minimum development of MDA (0.85–1.82 mmol g^{-1} of cookie) during the entire storage period, advocating the efficacy of γ-irradiated VCO in thwarting progression of rancidity. SC sets of cookies on the other hand have shown minimum development of MDA (0.85–1.99 mmol g^{-1} of cookie) during the entire storage period, reaffirming the efficacy of γ-irradiated VCO in delaying progression of rancidity.

3.5. Merits of γ-Irradiated VCO-Formulated Cookies

The saturated and unsaturated fatty acids of VCO when subjected to γ-irradiation were converted into long chain hydrocarbons by radiolysis.[5] The SC set of cookies formulated with γ-irradiated VCO had relatively lower amount of $C_{18:1}$ *vis-à-vis* its non-irradiated counterpart. Additionally, the unsaturated long chain hydrocarbons generated consequent to radiolysis would offer protection to the residual unsaturated fatly acids in the γ-irradiated VCO, possibly delaying oxidative rancidity of the SC set of cookies.

HC and SC cookie sets were successively analysed for their antioxidant potencies (in terms of 2,2-diphenyl-1-picrylhydrazy i.e., DPPH radical scavenging activity) in accordance with the procedure reported by Dutta et al. (2017)[9] and were found to be 91.20 mg mL^{-1} (day 90) and 35.45 mg mL^{-1} (day 150), respectively. From the findings it is evident that the HC cookie set had much lower antioxidant potency vis-à-vis the SC set of cookies. Therefore, it can be concluded that incorporation of γ-irradiated VCO in the dough of SC cookie set rendered the same less prone to development of rancidity vis-à-vis the HC and CC cookie sets with 150 days storage stability. This could be attributed to cumulative effects of radiolysis of unsaturated fatty acids and antioxidant potency of VCO-formulated cookies.

3.6. Correlations Among the e-Nose Sensor Responses and Biochemical Assay Values

From the experimental data obtained by e-nose analysis and biochemical analyses of the sample set of cookies (SC) at different storage intervals, linear correlations were established among e-nose sensor responses and values of biochemical parameters (PV, AV, MDA) to allow prediction of the rancidity status of the cookies directly from the e-nose responses. The regressions involved the use of the entire data set (from 0 to 180 days of storage period) related to $|\Delta R|/R$ values of selected sensors and biochemical parameters (provided in Figure 5 and Table 5, respectively). Multiple regression equations (shown below) were developed to predict the PVs, AVs, and MDA contents of the SC and HC sets as a function of selected (four) sensor responses (on the basis of high $|\Delta R|/R$ values) during 180 days of storage. Equations 2–7 represent the correlations of the screened e-nose sensor responses (to the VOCs generated during storage of the cookies) with the biochemical assay values of the cookie sets.

$$Y_{PV} = -0.0004 - 0.0165 \times X_1 + 0.0472 \times X_2 - 0.0014 \times X_3 + 0.0386 \times X_4 \quad (2)$$

$$Y_{AV} = 0.2953 - 0.0858 \times X_1 - 0.3538 \times X_2 + 1.7882 \times X_3 + 0.2041 \times X_4 \quad (3)$$

$$Y_{MDA} = 0.8062 - 1.4512 \times X_1 + 3.2785 \times X_2 - 2.3149 X_3 + 3.9042 \times X_4 \quad (4)$$

$$Y'_{PV} = -0.0005 - 0.0177 \times X_1' + 0.0489 \times X_2' - 0.0016 \times X_3' + 0.0356 \times X_4' \quad (5)$$

$$Y'_{AV} = 0.2773 - 0.0785 \times X_1' - 0.3336 \times X_2' + 1.7920 \times X_3' + 0.2066 \times X_4' \quad (6)$$

$$Y'_{MDA} = 0.8102 - 1.4600 \times X_1' + 3.2793 \times X_2' - 2.332 \times X_3' + 3.9502 \times X_4' \quad (7)$$

where, Y_{PV} and Y'_{PV} are the PV (ppm) data for SC and HC sets, respectively; Y_{AV} and Y'_{AV} are the AV (% oleic acid) data for SC and HC sets, respectively; and YMDA and Y¢MDA are the MDA (mmol malondialdehyde/g dried chips) contents of SC and HC sets, respectively. X_1, X_2, X_3, and X_4 are the e-nose responses for the sample cookies obtained from sensors TGS 816, TGS 823, TGS 832, and TGS 2600, respectively.

The fitted models were validated by *p*- and *F*-values. The *p*-values showing less than 0.05 and reasonably high *F*-values of 13.69, 15.37, and 15.47 corresponding to Equations (2)–(4), respectively, indicated that regression models fit the data significantly with good correlations (R^2 values of Equations (2)–(4) were 0.95, 0.97, 0.96, respectively) between the independent and the dependent variables. Similarly, Equations (5)(7) with high *F* values of 14.22, 16.01, and 15.66, respectively, and *p* values less than 0.05, implied that regression models fit the data significantly with good correlations (R^2 values of Equations (5)–(7) were 0.96, 0.98, and 0.97, respectively) of PVs, AVs and MDA values of HC with the responses of four selected sensors of e-nose ($|\Delta R|/R$ values). The regression equations generated in this study could accurately predict the formation of primary (PV, AV) and secondary oxidation products (MDA contents) of the cookie sets (SC and HC) during storage.

Recent works of other research groups have also reported on appreciable potentiality of e-noses to detect different contaminations, and defects in food products and quality such as in beer, meat, fruit, bakery and animal products using MOS and metal oxide-semiconductor field-effect transistor (MOSFET) sensors.[32] This state-of-the-art technology can successfully overcome the problems (human errors) associated with normal biochemical assays for determination of rancidity and can thus forgo biochemical assays. This study is a systematic effort for unambiguous assessment of rancidity (at low detectable levels) in VCO-formulated drop cookies using e-nose technology, validated by classical sensory evaluation and biochemical assays of rancidity parameters. E-nose data clearly differentiated rancid cookies from their non-rancid counterparts and established that γ-irradiated VCO could certainly enhance shelf-life of cookies (by delaying onset of rancidity) vis-à-vis the HC and CC samples.

The cookies formulated with γ-irradiated VCO could be consumed safely for 150 days under ambient storage conditions (25±2 °C, 75–80% RH). This cookie set retained good antioxidant potency (35.45 mg mL^{-1}) attesting to its nutraceutical value. The regression equations generated using the biochemical parameters (PVs, AVs, and MDA content) and the e-nose data can successfully predict these parameters eliminating the need to perform their respective biochemical assays.

3.7. Validation of Equations Developed for Sample Set of Cookies using Market Samples

The equations developed in this study were validated using several branded packaged coconut cookies purchased from local markets and departmental stores of Kolkata, India (data not shown). To illustrate this, let us consider the package of cookies purchased after three months from its manufacturing date bearing the label of best before seven months This set of cookies were subjected (post-grinding) to e-nose analysis on different storage days, i.e., 60, 120, 180, and 240 and their corresponding PVs, AVs, and MDA contents were determined by biochemical assays and also by using the equations developed in our study (Equations (2)–(4)). The equations could successfully predict the biochemical parameters of rancidity in the cookies. The data also revealed that there was no onset of rancidity in the cookies up to 180 days of storage. Rancidity was first detected in the 240-d stored samples (25±2 °C, 75–80% RH) (extended shelf-life of these commercial cookies is attributed to the commercial 4-layer laminate packaging in biaxially oriented polypropylene/polyester/aluminium foil, vis-à-vis our laboratory-packaged cookies). Therefore, it could be conjectured that the equations developed in this study could be used directly for prediction of rancidity parameters of market cookie samples, by performing e-nose analysis alone, forgoing sensory and biochemical assays of rancidity assessment. VCO-formulated cookies are not commercially manufactured and thus comparative evaluation with our newly formulated cookies could not be performed.

4. Conclusions

In the current investigation, γ-irradiated (4.2 kGy) deodorized VCO has been successfully used to replace hydrogenated fat in cookies and thus it may find application as a shortening replacer in several bakery and confectionery products. E-nose technology has also been successfully used to analyse rancidity in the cookies. Among the eight different MOS in ENOVISION employed in this study, four sensors viz. TGS 816, TGS 823, TGS 832, and TGS 2600 were considered based on SVM classification of each sensor's response ($\Delta R/R$ value) towards VOCs generated from cookie samples at different storage time. E-nose analysis established that onset of rancidity in the VCO-formulated cookies occurred on day 150 when stored in laboratory conditions (25±2 °C, 75–80% RH). Thus these cookies were safe for consumption for 150 days, vis-à-vis the control and positive control sets of cookies (complemented by sensory analysis of the above-mentioned three sets of cookies). The findings from e-nose data analysis and sensory evaluation correlated well with the assay values of biochemical parameters of rancidity. The zero-chemical-use methodology developed in this study could be safely employed for several high-fat bakery and confectionery products to overcome time and expense of routine biochemical assays of large sample sets on an industrial scale.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

K.M.: Data curation; Formal analysis; Investigation; Methodology; Validation; Writing – original draft. A.B.: Data curation; Formal analysis; Investigation; Methodology; Validation; Writing – original draft. D.C.: Data curation; Formal analysis; Investigation; Methodology; Writing – review and editing. S.C.: Data curation; Formal analysis; Methodology. K.P.: Investigation; Methodology; Writing – original draft. P.B.: Conceptualization; Project administration; Resources; Supervision; Visualization; Writing – review and editing

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