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Research Article

Development of a rapid test kit for qualitative analysis of rancidity in fats and oils

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Abstract

Fats and oils are highly important for human health to regulate various bodily functions. Oxidative changes have a detrimental impact on the quality of oils and result in the production of off-flavors and a bad taste known as rancidity and consumption of such substandard fats and oils may lead to serious health complications e.g., cancer, cardiovascular diseases. The conventional laboratory methods to determine rancidity in fats and oils are laborious, expensive, and time-consuming, however, qualitative analysis is rapid, convenient, and time-saving. Main objective of the current study was to develop a Rapid Test Kit (RTK) for the detection of rancidity in fats and oils based on peroxide values (POV). During 1st phase, three different treatments (T1, T2, and T3) were prepared and best performing treatment (T1) was selected for further kit development. During storage study of the kit, shelf life of 3 months under uncontrolled atmospheric conditions (K_A), 4 months inside lab (K_L) (25 $^oC \pm 2$ ^oC), and 6 months in refrigerator (K_R) (2-4 $^oC \pm 2$ ^oC) was achieved. The optimized kit (K_R) was validated by comparing with the AOAC reference method on randomly selected oil samples. As a result, a semi-quantitative color chart was developed to assess the level of rancidity in oils and fats. The kit can be used at anywhere and anytime for quality control and monitoring of oil and ghee food safety standards and ultimately reduce health issues associated with the consumption of rancid fats and oils.

Keywords: Food safety; Peroxide value; Qualitative analysis; Rancidity; Test chart

Introduction

Dietary lipids being an important ingredient of our cuisine that not only provide energy but also essential fatty acids. They are also vital for transportation of fat soluble vitamins, and creation of complex organic molecules in human body such as prostaglandins and steroids **[1]**. Fats and oils when exposed to air, light, heat, moisture, or microbes undergo an oxidation termed as rancidity and provoke the generation of various toxic and oncogenic compounds such as aldehydes, ketones, peroxides, dimers and polymers etc. **[2, 3]**. As a result, quality of fats and oils is deteriorated leading to the production of unpleasant taste & odor **[4-6]**. Consumption of rancid oils pose serious health complications including

cardiovascular diseases, emergence of tumors, Alzheimer's, and Parkinson's diseases in humans as well as economic losses [7].

Detection of adulteration/contamination in foods especially rancidity in dietary fat & oils is vital to ensure food safety and quality [8]. Rancidity in cooking oils is determined by different quality characteristics such as POV and FFA [9]. POV indicate the level of oxidative compounds e.g. peroxide and hydro peroxides triggering incipient rancidity in fats and oils [10]. The conventional quantitative lab methods to determine rancidity in fats and oils are accurate and reliable, but time-consuming, costly and technically challenging to be performed by trained personnel in labs [11]. Therefore, a qualitative and cost effective tool having swift screening potential and convenience to perform at anytime and anywhere was needed [12].

In this background, the current study was aimed to develop a rapid test kit (RTK) that can qualitatively detect the level of rancidity based on the value of peroxides in fats and oils. The RTK was successfully developed, optimized and validated on different oil samples.

Materials and Methods

Analytical grade chemicals (HCl, NaOH, KOH, KI, and C_2H_5OH) were sought from Daejung Chemical and Metals Co. (South Korea) imported by Musaji Adam and Sons (Pakistan). Deionized water obtained from Thermo Scientific deionizer (DI-425) was used in all the experiments. Consumable items such as glass bottles, packing materials and oil samples were procured from the local market of Peshawar and stored in food nutrition lab for further storage studies.

Development of rapid test kit (RTK)

Initially three treatments of the rapid test kit for detection of rancidity in fats and oils were prepared. Clean and oven dried glass bottles were taken and labeled as per treatment plan (Table 1). Chemical reagents were filled in respective bottles by the auto-filling dispenser and bottles were stored at room temperature (25 ± 5 ^{0}C) for further analysis and screening of best treatment (Fig. 1).

Table 1: Treatment plan for the development of test kit

Treatments (T)	Reagents (R)	T * R
T1	R1	T1-R1 [Chemical-A (75 %) + Chemical-B (25%)]
	R2	T1-R2 [Chemical-C (100 %)]
	R3	T1-R3 [Chemical-D (0.5%) + Chemical-E (99.5%)]
T2	R1	T2-R1 [Chemical-A (50 %) + Chemical-B (50%)]
	R2	T2-R2 [Chemical-C (100 %)]
	R3	T2-R3 [Chemical-D (0.5%) + Chemical-E (99.5%)]
T3	R1	T3- R1 [Chemical-A (25 %) + Chemical-B (75%)]
	R2	T3-R2 [Chemical-C (100 %)]
	R3	T3-R3 [Chemical-D (0.5%) + Chemical-E (99.5%)]

T1 **T2** **T3**

Figure 1: Screening of the best treatment

Stability study of the kit

Shelf life and stability of the kit was determined by storing it at three different places i.e. inside the lab (25 ± 2 ^{o}C), outside the lab under uncontrolled atmospheric conditions, and in a refrigerator (Varioline Intercool, VCS-22) at 2-4 ± 2 ^{o}C for six months [13].

Comparison of the RTK with the AOAC reference method for validation

To validate the rapid test kit oil samples were simultaneously analyzed by qualitative and quantitative methods using the rapid test kit and AOAC reference method for determination of POV respectively. 20 replicates were analyzed under repeatability condition and outcomes were compared to achieve accurate and precise results. On the basis of these tangible findings a semi-quantitative test chart was developed to determine the level of rancidity based on POV (meq/Kg).

AOAC reference method

Oil samples were quantitatively analyzed for determining the POV using AOAC reference method No. 965.33 (2000) [14]. 2-3 g of oil sample was mixed with 25 ml of solvent mixture (acetic acid and chloroform (3:2) and swirled to dissolve the mixture. 0.5 ml saturated potassium iodide was added and shaken for 1 min, then 25 ml of distilled water was added to stop the reaction and titrated against standard 0.01N $Na_2S_2O_3$ using starch solution as an indicator. Titration of blank was also carried out and POV was calculated as per the given formulae:

$$POV\ (meq/Kg) = \frac{Titer\ (T) \times Normality\ (N) \times 1000}{Weight\ of\ Sample\ (w)}$$

Qualitative analysis by RTK

Qualitative analysis was performed by using the RTK [15]. 2-3 ml of homogenized oil sample was taken in a clean and dry test tube and 1 ml of R-1 was added into the test tube and gently shaken. Then 0.5 g of R-2 was added into the test tube and again gently shaken for 1 minute to uniformly mix the reagents and finally, 1 ml of R-3 was added into the test tube. Change in color of the solution in the test tubes was observed and compared with the developed test chart to determine the level of rancidity based on POV (Fig. 2).

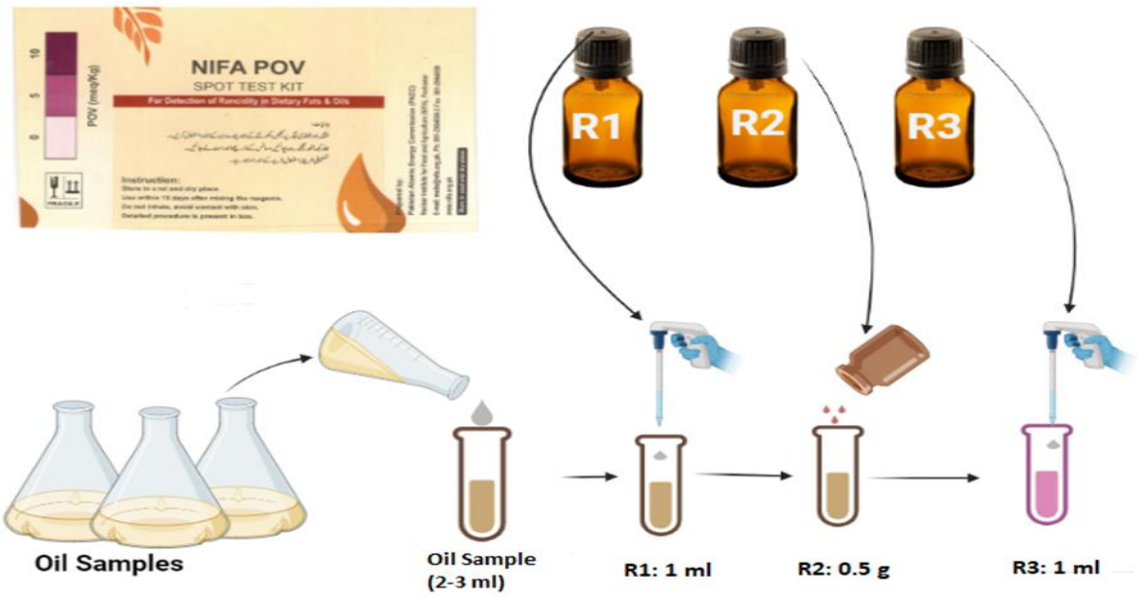

Figure 2: Schematic Diagram for qualitative analysis of oils samples by the rapid test kit

Statistical analysis

The data obtained was statistically analyzed to determine the level of significance through analysis of variance in completely randomized design (CRD) [16].

Results and Discussion

Development of rapid test kit (RTK)

Two different types of oil samples (fresh and used) were qualitatively tested against T1, T2 and T3. The analysis was performed at regular intervals of 7 days for 28 days storage period and repeated ten times on each day of analysis [8]. It was observed that no color was developed by any treatment in fresh oils however T1 developed light purple color in used oils on day-1. The intensity of color developed by T1 in both types of oils gradually increased over the passage of time as shown in (Fig. 3). While no color was observed for T2 and T3 during the entire analysis either in fresh or used oils. On the basis of these findings, T1 was selected for further kit development.

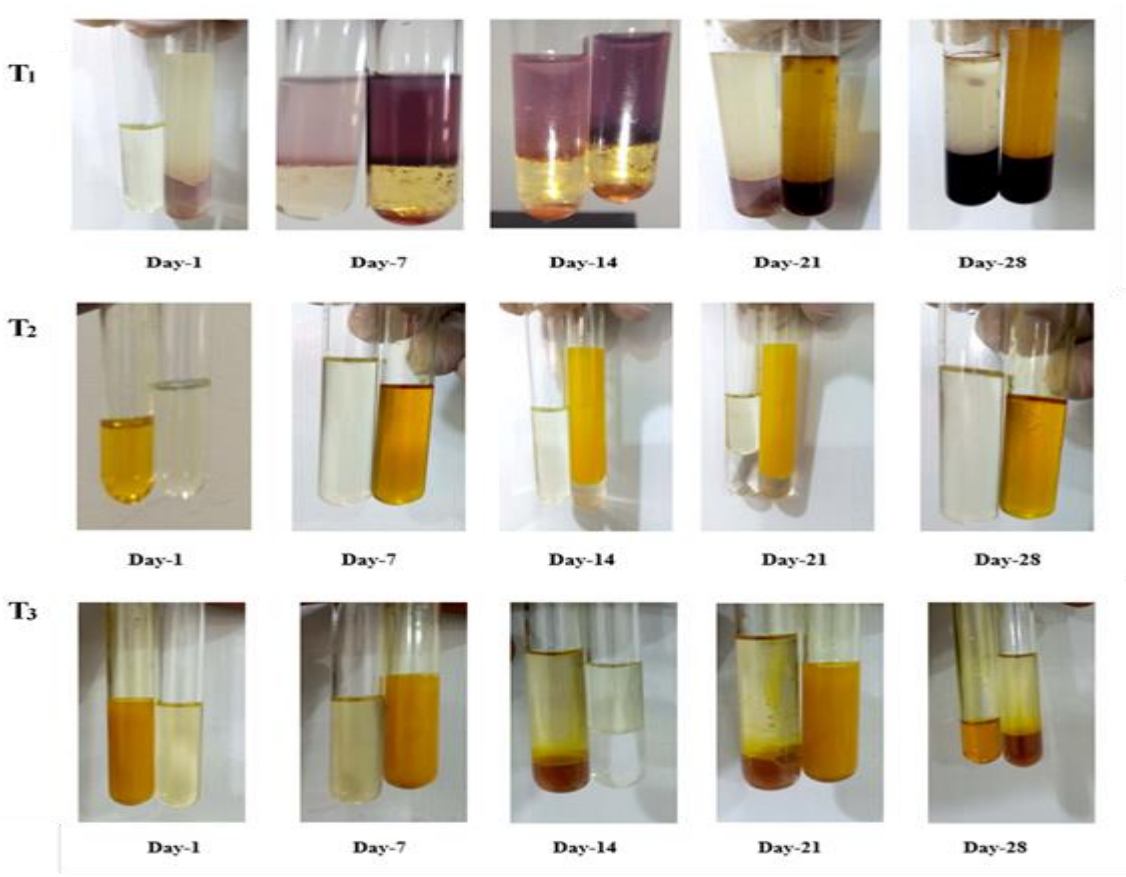

Figure 3: Initial screening of best treatment on the basis color development during storage

Stability study of the rapid test kit

Since T1 was selected for kit development and to determine stability and shelf life it was stored at 3 different storage conditions i.e. inside the lab at 25 ± 2 ^{0}C (K_L), outside the lab under uncontrolled atmospheric conditions (K_A), and in a refrigerator at 2-4 ± 2 ^{0}C (K_R) for six months [17]. All the stored kits were tested against randomly selected oil samples having quantitative values of per-oxides ≤5 meq/Kg, >5 but <10 meq/Kg and ≥10 meq/Kg as shown in (Table 2). It was observed that K_L, K_A, K_R produced different shades of color such as no color or light

purple at POV <5 (meq/Kg), purple or intense purple at POV between 5 to 10 (meq/Kg) and dark brown at POV ≥ 10 (meq/Kg) during 1st and 2nd month of storage. However, K_A developed color till 3rd month, K_L till 4th month and K_R throughout the storage period.

Table 2. Storage stability study of the developed rapid test kit

Treatments	Storage months	POV (meq/Kg)* / Color appearance in oil samples after addition of kit reagent		
		< 5	**≥ 5 but < 10**	**≥ 10**
QV	**Day-1**	0.15±0.01	5.21±0.09	10.29±0.13
K_L		No color	Purple	Purple turning to dark brown
QV	**1**	0.62±0.12	5.17±0.06	10.37±0.0.8
K_L		No color	Purple	Purple turning to dark brown
K_A		No color	Light purple	Purple turning to dark brown
K_R		Light purple	Purple	Purple turning to dark brown
QV	**2**	1.12±0.05	5.45±0.06	10.65±0.05
K_L		Light purple	Purple	Purple turning to dark brown
K_A		No color	Light Purple	Purple
K_R		Light purple	Purple	Purple turning to dark brown
QV	**3**	1.51±0.09	5.90±0.10	11.38±0.06
K_L		Light purple	Purple	Dark brown
K_A		No color	No color	Light Purple
K_R		Light purple	Purple	Dark brown
QV	**4**	2.71±0.06	7.36±0.08	13.28±0.09
K_L		No color	Purple	Dark brown
K_A		No color	No color	No color
K_R		Light purple	Purple	Dark brown
QV	**5**	2.23±0.04	6.51±0.12	11.56±0.13
K_L		No color	No color	Light purple
K_A		No color	No color	No color
K_R		Light purple	Purple turning to dark brown	Dark brown
QV	**6**	1.85±0.06	6.65±0.09	12.35±0.16
K_L		No color	No color	No color
K_A		No color	No color	No color
K_R		Light purple	Purple	Dark brown

*Values are Means ± Standard Deviation (S.D.); QV= Quantitative Value of POV(meq/Kg), K_L = kit stored inside lab at 25 ± 2 ^{0}C, K_A = kit stored outside the lab under uncontrolled atmospheric conditions and K_R = kit stored in refrigerator at 2-4 ± 2 ^{0}C

Comparison of the RTK with the AOAC Reference Method for Validation

Results of the comparative analysis of randomly selected oil samples are given in (Table 3). It was observed that no or light purple color was developed in oil samples when quantitative values of per-oxides were less than 5 meq/Kg, purple or purple turning

into dark brown for values more than 5 meq/Kg but less than 10 meq/Kg which is an indication of incipient rancidity. Similar results were reported by [18] while assessing the rancidity and other physicochemical properties of edible oils stored at room temperature. Oils having POV values >10 meq/Kg are acceptable for consumption as per the food regulatory guidelines [19, 20]. A dark brown color was noticed in oil samples having peroxide values ≥10 meq/Kg which may not be Generally Recognized as Safe (GRAS) for human consumption [21]. On the basis of these visual observations a semi-quantitative test chart was developed to estimate the level of rancidity in dietary fats and oils (Fig. 4).

Table 3. Qualitative and quantitative analysis between the test kit and reference method

Oil Samples	Rapid Test Kit	POV (meq/Kg) * by Standard Method
S1	No color	0.15±0.01
S2	Light purple	1.85±0.02
S3	Dark brown	6.51±0.07
S4	No color	0.62±0.01
S5	Purple	5.17±0.03
S6	Dark brown	10.29±0.06
S7	Purple turning into dark brown	7.36±0.04
S8	Purple	5.21±0.03
S9	Dark brown	13.28±0.07
S10	Light purple	2.71±0.02
S11	Purple turning into dark brown	8.62±0.03
S12	Purple	6.65±0.03
S13	No color	0.13±0.01
S14	Light purple	3.25±0.03
S15	Purple	6.02±0.02
S16	No color	0.17±0.01
S17	Purple turning into dark brown	8.45±0.04
S18	Dark brown	11.04±0.06
S19	Purple turning into dark brown	9.18±0.04
S20	Light purple	1.08±0.02

*Values are Means ± Standard Deviation (S.D.)

POV (meq/Kg)	< 5	≥ 5 but < 10	≥ 10
Color scheme			

Figure 4: Test chart to determine the level of rancidity

Analysis of oil samples using the rapid test kit and by the AOAC reference method yielded good correlation and agreement and it can be used to determine the level of rancidity in fats and oils samples based on peroxide values. According to PSQCA peroxide values for cooking oils shall be lower than the upper limit of 10 meq/kg [19]. Rapid test kit developed in the current study could be easily used to qualitatively judge the

standard limit of peroxide values i.e. appearance of dark brown color indicated that peroxide values ≥10 meq/Kg and oil samples were rancid and unfit for consumption. The results of the current study are compatible with the other finding **[22]** who reported that freshly refined oils had Peroxide values <1 meq/kg while >2.5 meq/kg showed excessive oxidation, potential lack of chip stability and >7.5 meq/kg indicated sufficient breakdown to aldehydes and production of rancid flavor in chips.

The rapid test kit is advantageous over the reference method in terms of simplicity, reliability and user-friendly as technicians with limited technical knowledge can analyse the samples and get valid results. This is an important aspect, as reference method require a high level of expertise and a well-established laboratory infrastructure (including high equipment and maintenance cost).

Conclusion

The developed rapid test kit (RTK) is a pragmatic approach for on-spot qualitative detection of rancidity during food processing. On-spot test by the kit can be performed at anytime and anywhere and did not require technical personnel & equipment making it a user friendly, efficient and reliable tool. It can be used for quality control purposes, monitoring and surveillance of food safety standards. RTK can be helpful for multi-sectorial stakeholders/end-users such as governmental ministries/departments working on food programs, provincial food regulatory authorities, and oil & ghee industries in Pakistan. RTKs would be helpful in mitigation of health issues associated with the consumption of rancid fats and oils.

Author's contributions

Conceived and designed the experiments: A Raza & Z Mehmood, Performed the experiments: A Raza & T Ahmad, Analyzed the data: MA Irshad & T Ahmad, Contributed materials/ analysis/ tools: M Khan, Wrote the paper: A Raza, T Ahmad & MA Irshad.

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