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Determination of the Organoleptic Quality of Hard Dough Biscuits during the Shelf Life by Chemical Analysis

H.W.U.N. Senarathna, S.B. Navaratne

Department of Food Science & Technology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Abstract*—The variations of moisture, pH, free fatty acids (FFA), peroxide value (PV), total carbonyl content (TCC) and organoleptic properties of hard dough biscuits packed with double laminated wrapper, were studied for 56 days of storage under accelerated shelf life conditions at 40^0C and 90% relative humidity. Variations of FFA, PV and TCC were statistically analyzed using V-masks with time-weighted CUSUM control charts. Sensory test results revealed that the biscuits began to deviate from the organoleptic freshness from 22^{nd}day. Moisture content and FFA gradually increased and pH value slightly declined. The PV and TCC were remarkably elevated during the study. Only the variation of PV was statistically significant on 22^{nd} day which was compatible with the sensorily decay of freshness in biscuits from the 22^{nd}day onwards. However, PV declined after 42^{nd}day onwards while showing a compatible increment in TCC. Either PV ≥1.07meq/kg or TCC ≥0.25ppm were identified as the indicators of deviation from organoleptic freshness. Thus, the typical hard dough biscuits were considered to be organoleptically fresh when both PV and TCC remained <1.07meq/kg and<0.25ppm respectively. Accordingly, a color scale was developed to efficiently measure the TCC in typical hard dough biscuits.*

Keywords*— *Free fatty acids, Hard dough biscuits, Organoleptic properties, Peroxide value, Total carbonyl content.

I. INTRODUCTION

Biscuits are cereal based food products that are baked to a moisture content of less than 5%. The cereal component is variously enriched with two major ingredients, fat and sugar [1]. Hard dough biscuits have relatively high amounts of water and low amounts of fatand sugar by dough composition. Generally these biscuits areproduced through laminating, dusting, sheeting, and cutting processes.

Typical hard dough biscuits, which are unsweetened, fermented, aerated, thin, and crisp to eat, are highly susceptible for the changes in physical, chemical and organoleptic properties during the accelerated storage life due to their open-flaky texture [1]. In food technological aspects, freshness and organoleptic quality is measured with sensory evaluation by sensory panel which is practically time consuming, subjective, labor intensive and even difficult to perform routinely. At the time of this study, none of alternative or reliable chemical method was identified and practiced in industry to determine the sensible freshness of hard dough biscuits except labor-intensive sensory evaluation methods. Therefore, the study was focused to determine a quantitative chemical measure which would reliably represent the deviation of freshness in terms of organoleptic quality of typical hard dough biscuits. Another intention of this study was to modify the selected appropriate chemical measure as to perform quickly, saving the experimental time under the industrial processing conditions.

Biscuit deterioration is usually associated with different phenomena, where three incidents are predominant; loss of crispiness, fat bloom, and rancidity [2]. The latter is the most effective in generating off-flavors that would thereby most adversely contribute to the organoleptic quality of the hard dough biscuits leading to consumers'

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rejection. In dry bakery products like biscuits, the deterioration during storage is mainly caused by lipid oxidation and resultant rancidity [3]. Rancidity in fat is twofold, hydrolytic and oxidative rancidity, of which mechanisms are largely hypothesized, researched and documented in literature.

According to Dobarganes and Velasco (2002), the available physical and chemical methods to monitor lipid oxidation in foods can be classified into five groups based on what they measure, such as (i) absorption of oxygen, (ii) loss of initial substrates, (iii) formation of free radicals, (iv) formation of primary oxidation products, and (v) formation of secondary oxidation products [4]. Depending on the availability of resources, peroxide value (PV) test and total carbonyl content (TCC) tests were selected as indicators of primary and secondary oxidation respectively.

The carbonyl compounds are suggested to be the major contributors to off-flavors associated withthe rancidity of many food products [5]. TCC is measured by a colorimetric spectrophotometric procedure [6,7] developed by American Society for Testing and Materials (ASTM) for determination of trace quantities of carbonyl compounds with 2,4-dinitrophenylhydrazine (2,4-DNPH) based upon the work of Lappin & Clark (1951) [8]. With the special emphasis on this test method during this study, a color scale was developed for the quicker determination of TCC. This study contributed to identify the most correlated chemical test parameter(s) with organoleptic quality of hard dough biscuits, and to determine their critical level(s) which would distinguish the point of occurrence of sensory unacceptability.

II. MATERIALS AND METHODS

2.1 Samples

A typical variety of hard dough biscuits; namely plain crackers, freshly produced in commercial scale (containing 13.40% w/w% total fat (7.37% unsaturated fat) and 0.56% w/w% sugars as per the nutritional information claimed by the manufacturer) were taken and wrapped in double laminated and metalized (BOPP+MCPP) wrapper with 4 biscuits per each packet. These packets were stored at 40 ± 1^0C temperature and 90% relative humidity (RH) for 8 weeks (56 days) and samples were drawn weekly in order to analyze changes in freshness and sensory quality, moisture content, pH, FFA, PV and TCC. All quantitative outcomes of chemical analysis in the study were mean values of triplicates.

2.2 Triangular Sensory Test

Three biscuits were individually packed in flexible wrappers with two of which were identical and one was odd, and then coded in 3-digit random numbers. Trained panelists were supposed to identify odd biscuit through the forced-choice method. (Half of sensory panel were given with 1 fresh biscuit as odd sample and 2 old biscuits as identical samples, rest of panel was given with 1 old biscuit as odd sample and 2 fresh biscuits as identical samples). The number of correct replies per week was assessed as per the ISO 4120:1983 to determine whether there was a significant difference between the fresh and old biscuits at 0.01 significance level.

2.3 Determination of Moisture Content

Moisture content of finely ground biscuits were weekly determined with triplicates according to the AOAC method (1990).

2.4 Determination of pH value

The pH values in 10% (w/w) aqueous solution were weekly determined with triplicates, using a pH meter (HANNA instruments, USA).

2.5 Extraction of Fat for FFA

With a slight modification to the method carried out by Calligaris *et.al.* (2008), finely ground biscuit sample of 10g was extracted under room temperature, to obtain the fat, using solid-liquid direct extraction in two steps of 1 hour per each (first step with 1:3 (w/v) solid : liquid ratio and second step with 1:2.5 (w/v) solid : liquid ratio) with diethyl ether AR - petroleum ether AR mixture (1:1, v./v) [3]. Two fat-solvent fractions were pooled and filtered through a filter paper (Whatman, Qualitative), then evaporated at 45^0C$\pm5^0$C to separate fat(Gemmy, Taiwan).

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2.6 Determination of FFA

Three extracted fat portions from three samples per week were analyzed for titrimetric FFA level in 1.0 gram of sample with a slight modification to the AOCS method Ca 5a-40.

2.7 Extraction of Fat for PV

As per the method described by Mildner-Szkudlarz*et.al.*(2009), fat in 10g of finely ground sample was extracted twice by direct solid-liquid extraction with 30ml and 25ml of chloroform (Assay 99.2%) separately in two intervals of 30 minutes. Two fat-solvent fractions obtained in each interval were pooled and filtered through a filter paper (Whatman, Qualitative) [9]. The extraction was weekly carried out with triplications.

For PV, according to Patrignani*et.al.*(2015), direct solvent extraction was faster, simpler, and most suitable for low lipid biscuits extraction [10]. Thus, the directly extracted fat with chloroform was analyzed for PV.

2.8 Determination of PV

Three extracted fat-chloroform fractions were analyzed for iodometric PV in meq/kg of biscuits pooling with relevant proportionate volume of glacial acetic acid based on AOCS standard test method of Cd 8-53 and were averaged with respect to storage days. No evaporation of chloroform was followed in order to make the testing faster, which was one of the objectives of this study.

2.9 Standardization f TCC

The standard curve for total carbonyl compounds was spectrophotometrically determined with triplicates as per the test method described by the Lappin and Clark (1951) [8], referred by the standard test method; ASTM E411-05, using acetone (HPLC Grade, Assay 99.8%, 58.08 $gmol^{-1}$, Density 791 kgm^{-3}) as the reference carbonyl compound, through a carbonyl concentration series from 0.00 ppm (blank) to 0.60 ppm (with an error of 0.05 ppm).

As per the Beer's Law, a linear model was derived to depict the linear relationship between the two variables; TCC concentration and absorbance.

2.10 Development of a Color Scale for TCC Test

The standardized series of wine-red colored alkaline 2,4-DNPH solution filled into cuvettes (380-780nm; Vis, Nephstar) was captured with a digital camera (focal length 3.5mm, ISO 350) against the concentration of carbonyls (in ppm). The color series was used to develop a color scale with which the quantification of TCC would become quick and simple.

The corresponding colors captured against the concentration were electronically extracted for their RBG values for further validation.

2.11 Determination of Sample Extraction Method for TCC Test

The optimum solvent; either ethanol (Assay 99.99% v/v) or methanol (HPLC Grade, Assay 99.8%), optimum extraction time; either 10 minutes or 20 minutes, and optimum volume of aliquot from carbonyl extract from hard dough biscuit; either 3 ml or 5 ml were determined using 3-factor: 2-level factorial design. The results were statistically analyzed to identify the treatment combination which gave the maximum difference in spectrophotometric absorption between fresh biscuit and deteriorated (expired) biscuit. The chosen treatment combination was used for forth TCC test.

2.12 Determination of Carbonyls

Finely ground 15.0g sample was extracted by solid-liquid (3:5 w/v) direct extraction with 25ml of methanol (Assay 99.8%) for 10 minutes. The liquid layer was filtered out through a filter paper and 3ml aliquot of extract was reacted with reagents as per the ASTM E411-05 to determine the spectrophotometric absorption, and then concentration of carbonyls was quantified using the standard curve. TCC was weekly done with triplicates.

2.13 Statistical Analysis

All the data were statistically analyzed through MINITAB® Release 14.1 statistical software. The V-mask by time-weighted CUSUM control chart was used to determine where the each parameter had been significantly shifted by half of standard deviations.

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III. RESULTS & DISCUSSION

3.1 Organoleptic Freshness by Triangular Sensory Test

According to the Table 1, which represented the outcome of the sensory triangle test for overall acceptability, the test samples were significantly deviated from fresh samples **from 22nd day of storage onwards** with concern on the odor, taste and texture.

Table.1*: Triangular test results to determine freshness in hard dough biscuits in terms of organoleptic quality (overall acceptability)*

Age of biscuit (Storage in Days)	Total responses	No. of correct answers	Required No. of correct answers to be significant*	Decision on difference between fresh & old biscuits
1	10	4	8	Not significant
7	10	7	8	Not significant
14	10	7	8	Not significant
22	10	10	8	Significant
29	15	13	10	Significant
35	15	12	10	Significant
42	12	11	9	Significant
49	16	15	11	Significant
56	12	11	9	Significant

*Minimum number of correct replies to establish a difference at α=0.01 significant level for triangular test, at ISO 4120:1983 (Trained sensory panel)

3.2 Variations of Moisture Content

According to Fig.1, the moisture content of biscuits was gradually increased with time. Moisture is a critical quality parameter in biscuits as higher moisture contributes to the loss of crispiness and potentially causes for hydrolytic rancidity that probably leading to a lesser sensory quality. Since, biscuits are highly hygroscopic, they tend to absorb water vapor from the micro-environment (inside the pack), thereby developing a partial pressure gradient from macro-environment (outside the pack) to micro-environment through wrapper.

As a result of that, water vapor permeability occurs through the wrapper. Thus, a steady increase of moisture content in biscuits was observed throughout the storage.

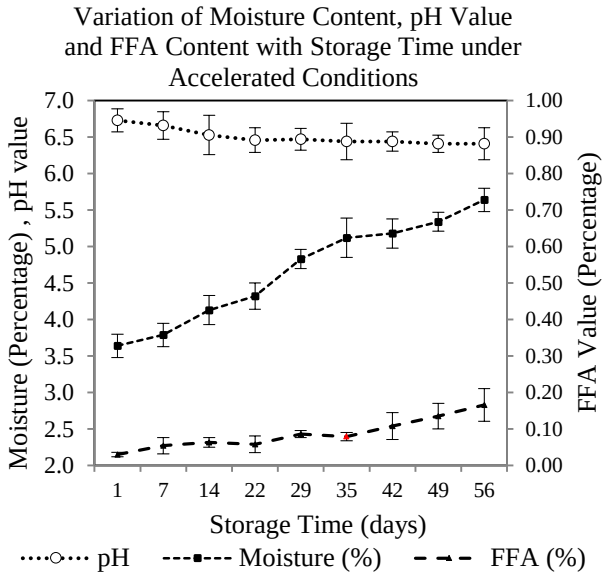

Fig. 1: Variation of moisture (wet basis), pH (in 10% aqueous solution) and FFA (in terms of oleic acid) during storage

3.3 Variations of pH Value

The pH value of the biscuits was gradually declined as illustrated in Fig.1, and this decline could be due to the increase of free fatty acids in the extracted fat from biscuits.

3.4 Variations of FFA

In this study, the fat extraction method was designed to avoid the extreme temperatures which the fat was exposed to; therefore, the observed increase of FFA was predominantly identified as the increase of hydrolytic rancidity of fat since the fat was continuously in contact with increasing moisture content within the biscuit matrix. Fatty acids were released to the biscuit matrix from the fat-hydrolyzation reaction.

As graphically presented in Fig.1, FFA value had a strong positive correlationship with moisture content (r=0.965; p=0.000), and a moderate negative relationship with pH (r=-0.764; p=0.017) at 0.05 significant level. More the moisture content, more hydrolyzation of fat and more free fatty acids, thereby lesser the pH value.

Fig.2 illustrates the V-mask by time-weighted CUSUM control chart for variations in FFA, which was used to

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monitor behavioral pattern of FFA during 56 days of shelf life. According to Fig. 2**,** for the first time, FFA value was significantly shifted on the 49th day under the accelerated storage conditions, when the biscuits had yielded only a mean FFA of 0.135% as demarcated in red color in Fig.1. Therefore, FFA was not an adequate measure to represent the freshness of biscuits as there was no significant deviation of FFA value occurred on 22nd day, at which the sensory panel was proficient to identify the turning point of deviation of freshness of biscuits.

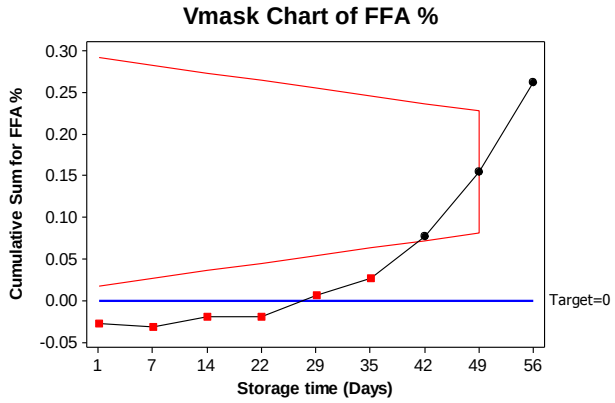

Fig.2: V-mask (on 49th Day) on CUSUM chart for variation of FFA% against storage (CUSUM plan with h=4.0 and k=0.5)

3.5 Variations of PV and TCC with Storage

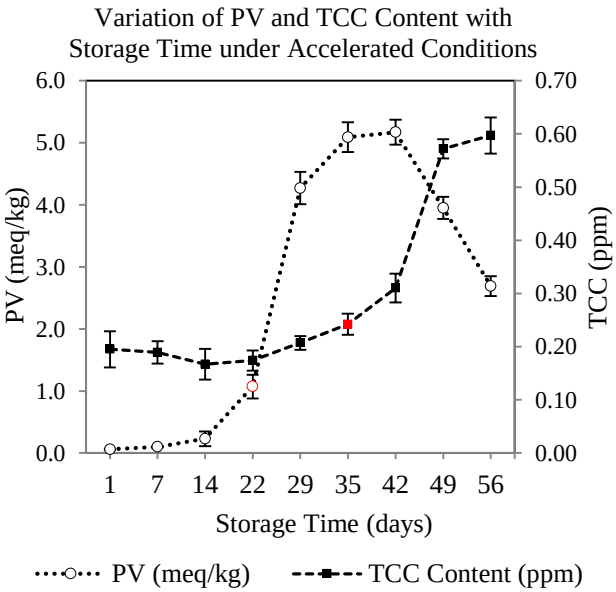

Fig.3: Variation of PV (meq/kg) and TCC (ppm) during storage

Variations of PV and TCC in hard dough biscuits during 56 days of storage are tabulated in Table 2 and further, graphically illustrated in Fig.3. The PV gradually increased up to a peak, followed by a gradual drop. TCC level started with a non-zero value, and then slightly declined, followed by a slight increase along with the acceleration of PV, finally drastically increased with the decline of PV.

3.5.1 Variation of PV

In this study, hydroperoxides in biscuits were quantified with PV, which is considered as an indicating test of the initial stages of oxidative change. The fat extraction method was designed to avoid the extreme temperatures to avoid acceleration of oxidation process at elevated temperatures, which is called as thermal oxidation [11].

Auto-oxidative increase in PV represents the continuation of spontaneous free radical mediated reactions which result in hydroperoxides, utilizing available triplet oxygen in the microenvironment inside the packaging [12].

However, these hydroperoxides are very unstable and susceptible to decomposition through secondary oxidation reactions. Therefore, a net decline of PV after 42nd day of storage in Fig.3 was observed as a result of a greater decomposition rate of hydroperoxides than the generation of them.

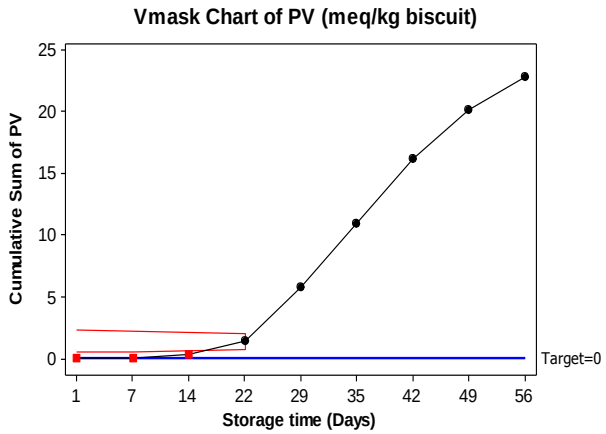

Fig.4: V-mask (on 22ndDay) on CUSUM chart for variation of PV against storage (CUSUM plan with h=4.0 and k=0.5)

Fig.4 shows the CUSUM control chart along with the V-mask for the development of PV during 56 days of storage of biscuits. According to the chart**,** the first time

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the PV significantly shifted was the 22nd day under the accelerated storage conditions. It had yielded a mean PV of 1.07meq/kg (on 22nd day) as illustrated in Fig.3. Therefore, PV was compatible with sensory test results.

Saxby (2012), O'Brien (2009) and Talbot (2011) have described that the peroxides (measured by the PV test), themselves, are generally tasteless and it is only when they are broken down further into aldehydes, ketones, etc., those impart off-flavors to the product [12, 13, 14]. Conversely, results of this study show that the PV can indicate the onset of organoleptic quality deviation or deterioration of hard dough biscuits. Similar results have also been reported by Calligaris *et. al.*(2008and 2007a) concluding and validating PV as a representative index for the quality of depletion of bakery products [3, 15].

However, the nature of PV observed was similar to that of being described in literature as a bell-shaped curve [16]. In observations, it initially moved up and thereafter moved down at 42nd day onwards. As widely described in literature, peroxides are unstable and further degrade into non-peroxide materials such as carbonyl compounds. Therefore, high PVs (>1.07meq/kg biscuits) sounds poor sensory quality, but a low PV (<1.07meq/kg biscuits) is not always an indication of good sensory quality.

3.5.2 Variations of TCC

3.5.2.1 Factorial Design for Sample Extraction Determinants for TCC

Carbonyl extraction method with alcoholic solvent which could obtain a maximum absorption difference in fresh and old biscuits, was investigated under three factor - two

Table.3:Analysis of 3-factor 2-level Factorial Design of 8 treatments for biscuit extraction determinants in TCC test (under 0.05 level of significance)

Treatment	Factor (X)			TCC of old biscuit Mean ± SD	TCC of new biscuit Mean ± SD	Absorbance Difference Mean ± SD
	Solvent	Volume of aliquot	Extraction Time			
1	Et	3	10	0.419 ± 0.081	0.351 ± 0.048	0.068 ± 0.033
2	Et	3	20	0.569 ± 0.096	0.414 ± 0.095	0.156 ± 0.054
3	Et	5	20	0.390 ± 0.068	0.303 ± 0.073	0.087 ± 0.010
4	Et	5	10	0.376 ± 0.112	0.500 ± 0.011	-0.124 ± 0.117
5	Me	3	10	1.404 ± 0.086	0.977 ± 0.052	0.427 ± 0.043
6	Me	3	20	1.247 ± 0.152	0.979 ± 0.134	0.269 ± 0.021
7	Me	5	10	1.339 ± 0.157	1.153 ± 0.152	0.186 ± 0.008
8	Me	5	20	1.346 ± 0.055	1.158 ± 0.049	0.188 ± 0.007
Main Effect[A]		P = 0.000	Solvent		P = 0.000	Me > Et
			Volume		P = 0.000	3 > 5
			Extraction Time		P = 0.101	-
Two-way interactions[B]		P = 0.000	Solvent * Volume		P = 0.475	-
			Solvent * Extraction Time		P = 0.000	Me: 10 > 20, Et:20> 10
			Volume * Extraction Time		P = 0.003	3:10>20, 5:20>10
Three-way interactions[B]		P = 0.661	Solvent * Volume * Extraction Time		P = 0.661	-

Me – Methanol, Et – Ethanol, 5 – Aliquot of 5 ml, 3 – Aliquot of 3 ml, 20 – 20 minutes, 10 – 10 minutes

All values are means of 3 determinations ± standard deviation (SD).

alternative Hypothesis on testing at 0.05 level of significance;

A - H_1: At least one pair of the different levels of X factor is significantly different with the absorbance difference.

B - H_1: At least one pair of the interactions of different levels of two or three factors is significantly different with the absorbance difference.

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level factorial design with 8 (as 2^3) treatments as indicated in the Table 3. The main effects of solvent (p=0.000) and aliquot volume (p=0.000), interaction effects between solvent and extraction time (p=0.000) and between volume and extraction time (p=0.003) were only statistically significant on the difference of absorbance (between fresh and old biscuit extracts) under 0.05 level of significance.

Main effects were identified to have a higher absorption difference between fresh and old hard dough biscuits for methanol solvent, and for 3 ml aliquot volume. In the "interaction effects plot" between solvent and extraction time, methanol solvent with 10 minutes extraction yielded the greatest absorbance difference. In the interaction effects plot between aliquot volume and extraction time, volume of 3 ml for 10 minutes extraction imparted the highest absorbance difference. Since there was no significant effect of extraction time as per the main effect analysis (p=0.101) according to the Table 3, 10 minutes of extraction time was more preferred than 20 minutes, as to save time.

According to the outcome, the treatment combination of **"extraction time of 10 minutes, with methanol solvent, by 3 ml aliquot"** was selected to extract carbonyls from hard dough biscuits prior to the TCC tests.

3.5.2.2 TCC Variation in Samples

Under the TCC test, the presence of carbonyl compounds such as aldehydes and ketones in biscuit matrix were quantified with 2,4-DNPH, which is recognized as a conventional method for measuring carbonyls, being most reliable and widely used too [17]. Extreme temperatures were avoided in fat extraction process to avoid thermal oxidation. The reagent; 2,4-DNPH, with a carbonyl compound, generates hydrozone compound, and then converts into a resonating quinoidal complex which is wine-red in color in the presence of excess alkali [8, 11].

The carbonyl compounds are incorporated into the biscuit matrix through several methods. Different volatile and non-volatile carbonyls are produced during the fermented hard dough biscuit manufacturing process**,** both at (i) fermentation by baker's yeast (*Saccharomyces cerevisiae)* and at (ii) sugar degradation while baking. The latter is two folds; as (ii.a) the caramelization of sugars and (ii.b) the series of Maillard browning reactions of sugars in combination with amino acids (specifically at the steps of Strecker reactions)while baking [1, 18].

According to Rothe and Thomas (1959), most of fermentation carbonyl products are volatilized during the latter stages of fermentation and baking [19]. Rooney *et.al.*(1967) has concluded that the Maillard-type browning is the major source of carbonyl compounds and brown color in starch paste model systems like breads [20]. Accordingly, the compounds with carbonyl groups are responsible for the yellow-brownish hue [1] on the biscuit and cracker-like aroma [21], which are prominently occurred during the production process of biscuits, especially at baking. Hence, the biscuit samples just after production had a non-zero TCC level; an initial TCC of 0.195 ppm, as indicated in Table 2 and Fig. 2.

Fig. 2 illustrates a lesser TCC of biscuits recorded in $7^{th}$and 14^{th}days of storage than the initial TCC level. This slight decline may be due to the loss of volatile carbonyls by volatilization from biscuit matrix when they are stored under accelerated conditions (at 40 ± 1^0C temperature).

With the acceleration phase of primary oxidation from 22^{nd} day to 42^{nd} day, a steady but slight increase in TCC level was observed. Within this phase, the formation rate of unstable hydroperoxides dominated over their degradation rate. Thus a little of carbonyls (secondary oxidation products) was being produced from degradation of hydro peroxides, which was represented with this slight increase in TCC level from 22^{nd} day to 42^{nd} day.

The TCC level was recorded to drastically increase on 49^{th} day onwards, while PV continued to drop severely. This drop of PV represented a lesser generation--rate with greater degradation rate of hydro peroxides, which lead to a net loss of hydro peroxides, and thereby, an escalation of the generation rate of carbonyls, as demonstrated in

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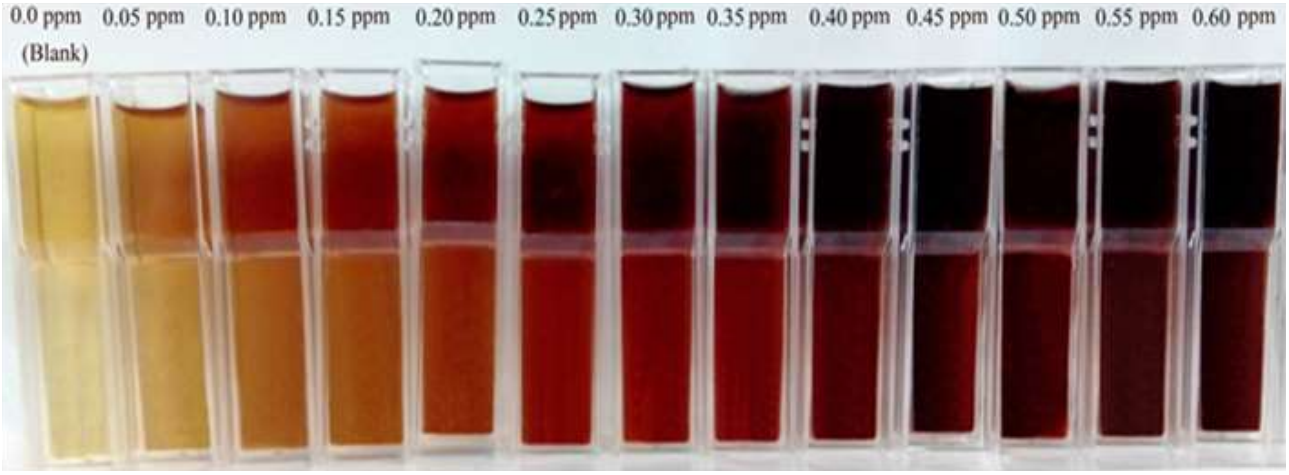

Fig. 6 :Series of known concentrations of carbonyl compounds (resulting alkaline 2,4-DNPH solutions are filled into cuvettes (380-780nm; Vis, Nephstar) and corresponding concentrations are noted above the cuvette)

Table.4: Development of color scale to measure TCC in hard dough biscuits

Concentration of carbonyls (ppm)	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	0.55	0.60
R	242	241	237	232	216	203	185	190	164	166	124	118	100
G	199	180	139	119	096	096	071	058	052	025	037	035	051
B	092	052	000	034	024	021	000	052	058	046	041	047	053
Color													

Fig. 2. Antolovich*et.al.* (2002), Saxby (2012) and Belitz (2009) have identified those carbonyl compounds as the contributors to off- flavors associated with the rancidity of many of food products [5, 12, 18].

Fig. 5 indicates the CUSUM control chart along with the V-mask for the development of TCC during 56 days of storage of hard dough biscuits. It indicates that TCC was significantly being shifted from the 35thday, where the carbonyls had been observed with a mean TCC value of 0.242 ppm as demarcated in red color in Fig. 3. Therefore, TCC was also not an adequate **single** measure to justify the sensory test results as there was no significant deviation occurring on TCC value on 22nd day.

Under this synopsis, when PV decreased, TCC increased as illustrated in Fig. 3. Therefore, a **combination of TCC**

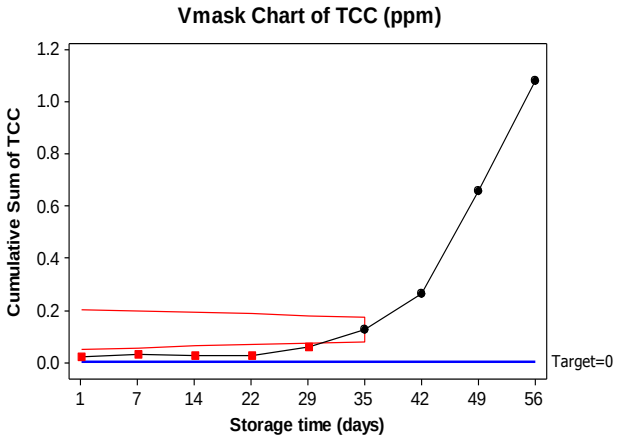

Fig. 5: V-mask (on 35thDay) on CUSUM chart for variation of TCC against storage (CUSUM plan with h=4.0 and k=0.5)

-and PV tests becomes a good measure of the decay of organoleptic freshness in hard dough biscuits.

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3.5.2.3 Color Scale as a Routine Measure for TCC Test

The wine-red color series illustrated in Fig. 6was the basis to develop the color scale for quantification of TCC. The corresponding colors captured against the concentration are also recorded in Table 4, with their RBG values. With the use of this color scale, the level of TCC in the extracted solution of hard dough biscuit sample would be easily quantified with an error of 0.05ppm. The concept requires performing both of "blank determination [Blank]" and "sample determination [Sample]" at a time to obtain the subtracted value [Sample - Blank] as the level of TCC in sample.

3.1 Chemical Analysis as a Measure for Organoleptic Quality in Quality Assurance

During this study, neither FFA nor TCC, but the PV was only compatible with sensory test results which could identify the onset of the deviation of organoleptic freshness in hard dough biscuits stored under accelerated conditions.

This result is compatible with the outcome of Calligaris *et. al.* (2007a) with evidence that the PV is well correlated to the organoleptic quality of bakery goods [15], and further this outcome has been validated by Calligaris *et. al.* (2008) for bread sticks [3]. However the bell-shaped behavior of PV was not been considered by Calligaris *et. al.* (2007a and 2008) since this decline was to be observed much later than the onset of organoleptic quality deterioration [15, 3].

With the quality assurance perspective throughout the shelf life in food industry, the declining nature of PV was also considered in this study. In case, PV requires verification from a second supportive measure, for which the TCC is proposed during this study. When PV is obtained lesser than the minimally acceptable point with <1.07 meq/kg of biscuits, the biscuits may either be too early to deteriorate, or else, too late from deterioration. This controversy can be solved by using TCC as a verification test.

In order to rapid up the entire testing time, TCC can be performed using the developed color scale within few minutes as the first test, and then PV as the verification test.

IV. CONCLUSION

Hard dough biscuits; the plain crackers stored for 56 days under accelerated storage conditions, were significantly deviated from freshness in sensory aspects, from 22ndday of storage onwards. Throughout the storage, moisture content and FFA were gradually increasing while pH value was slightly decreasing. PVrepresented a bell-shaped curve with the peak level on 42nd day. Rise of TCCwas remarkable from the 42nd day onwards, showing a negative correlationship with PV thereafter.

First significant shifts in CUSUM charts were observed on 49thday for FFA (mean 0.135%),on 22nd day for PV(mean 1.07meq/kg), and on 35th day for TCC (mean 0.242 ppm). Therefore, only the PV was compatible with the sensory test results. Since PV has a bell-shaped behavior through storage with a latter decreasing phase, it was recommended to be used**in combination** with TCC test**,**for determination of the organoleptic quality of hard dough biscuits.

For TCC test, 3ml aliquot from methanol extract from 10 minutes direct extraction of finely powdered hard dough biscuits was optimum, with which had resulted the highest color variance between fresh and deteriorated biscuits.

Hard dough biscuits are unacceptable organoleptically either if TCC [Sample - Blank] is 0.25ppm or more(with color scale) ***or*** if the PV is 1.07 meq/kg or more. Further, the organoleptic freshness of hard dough biscuits can be chemically identified if TCC [Sample - Blank] is less than 0.25 ppm (with color scale) ***and*** the PV is less than 1.07 meq/kg simultaneously.

As an extension, this study can be further validated to develop colorimetric testing equipment which can be used to determine the TCC in hard dough biscuits in a lesser experimental time.

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