PHYSICO-CHEMICAL CHARACTERISTICS OF BISCUIT INCORPORATED SODIUM CASEINATE HYDROLYSATE - ZINC COMPLEX

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ABSTRACT

Casein hydrolysates (CNH) - mineral complexes are a promising strategy for enhancement of mineral absorption & reduction of mineral deficiency. In the present investigation, an attempt was made to develop CNH-Zn complex incorporated biscuit. Among the various enzyme substrate used for the hydrolysis, the highest DH was obtained for 1:25 enzyme substrate ratio for 120 min and 700 mg added zinc showed highest zinc binding among all. Biscuit prepared by incorporating CNH-Zn complex showed moisture, protein, fat, carbohydrates, ash and peroxide value of 4.23, 7.58, 24.53, 0.97, 65.68 per cent and 2.10 meq O_2/kg fat, respectively.

Keywords: Casein hydrolysate, Zinc, Sodium caseinate hydrolysate - zinc complex, Biscuit.

I. INTRODUCTION

Zinc is needed for a variety of physiological functions that impact human growth and development. Subclinical zinc deficiency is common, particularly among the elderly and children (Maret and Sandstead, 2006). Growth retardation, compromised immune function, alopecia, dermatitis, increased morbidity, and reduced neurocognitive development are all possible consequences (Wang *et al.,* 2010). Zinc absorption is affected not only by the amount of zinc absorbed, but also by its bioavailability, which is influenced by the various components of dietary foods (Wang *et al.,* 2011). Zinc deficiency is normal in people who eat a plant-based diet. As a result, improving the quality of bioavailable dietary Zn is critical from a nutritional standpoint.

The bioavailability of dietary Zn in dairy food systems is affected by a number of factors. In intestinal basic conditions, some proteins or peptides may form complexes with minerals, making minerals soluble and increasing their absorption and bioavailability (Chaud *et al.,* 2002). Casein hydrolysis peptides have been shown to bind minerals like calcium and increase their absorption (Vegarud *et al.,* 2000). Many studies have centred on the role of casein phosphopeptides (CPP) derived from enzyme hydrolysis in increasing calcium or iron bioavailability (Narva *et al.,* 2003). (Chaud *et al.,* 2002). SpSpSpEE is a cluster sequence found in casein phosphopeptides (i.e., highly polar acidic sequence of 3 phosphoserines, followed by 2 glutamic acid residues).

Casein hydrolysates leads to the release of bioactive peptides that are involved in the regulation of blood pressure and the inhibition or activation of the immune response by serving as agonists or antagonists of opioid receptors, thus controlling the expression of genes that exert epigenetic control. Later, they bind to opioid receptor, increase the redox potential, and reduce oxidative stress. Casein hydrolysates are used in functional food ingredients, infant foods, and nutritional fortification, pharmaceutical and nutraceutical applications. CNH-Zn complexes could increase the stability, absorption and bioavailability of Zinc. Food based approaches may be an alternative way to improve zinc bioavailability (Wang *et al.,* 2011).

Direct addition of Zinc to a product leads to decrease in shelf life because of increase in oxidative stability, so in order to reduce the oxidative stability and to increase the bioavailability of Zinc in the bound form of complexes (Hotz *et al.,* 2005).

Bakery products, due to high nutrient value and affordability, are an item of huge consumption. Due to the rapid population rise, the rising foreign influence, the emergence of a female working population and the fluctuating eating habits of people, they have gained popularity among people, contributing significantly to the growth trajectory of the bakery industry. Bakery holds an important place in food processing industry and is a traditional activity. With regard to bakery products, consumers are demanding newer options, and the industry has been experiencing fortification of bakery products in order to satiate the burgeoning appetite of the health-conscious people (Bhole *et al.,* 2017).

Biscuits are prominent ready-to-eat baked snack among the people, globally. Biscuits have a long shelf life; are eaten straight from the pack; and are nutritious and available in many forms, both sweet and savoury. Biscuits are a rich source of fat and carbohydrate, hence are energy giving food and they are also a good source of protein and minerals. Biscuits occupy primary position, both for production and consumption as compared to other bakery products (Adeola *et al.,* 2018).

Therefore, this work was carried out to determine the proper enzymatic conditions to convert caseinate into a good source of Zn-binding peptides, and physico-chemical characteristics of biscuits incorporated CNH-Zn complex.

II. MATERIALS AND REAGENTS

The effect of incorporation of CNH-Zn complex on the sensory and physico-chemical from were evaluated in the present research.

Sodium caseinate was obtained from J.J. Chemicals, Peenya – Bengaluru-58, Trypsin enzyme was obtained from LOBA CHEMIE Pvt ltd, Colaba – Mumbai. Trypsin used in hydrolysis of sodium caseinate, Zinc salt used for fortification was zinc sulphate (99%) (food grade) was procured from Suchem Industries – Ahmedabad, Gujarat, Refined wheat flour (maida), icing sugar, vanaspathi, venila flavour, baking soda, milk powder and custard powder required for biscuit and biscuit preparation were purchased from local market – Bengaluru.

Preparation of Casein Hydrolysate

Casein hydrolysate was prepared according to the method of Mao *et al*. (2007), Casein was hydrolyzed with trypsin enzyme using a hydrolysis reactor equipped with a stirrer, pH electrode, and thermometer. The enzyme trypsin ratio 1:25 is used for the hydrolysis, commercial sodium caseinate (Na-CN) was subjected for hydrolysis using trypsin. Na-CN was dissolved in double distilled water at 7% level using trypsin at 1:25 enzyme-substrate (E: S) ratio and subjected for hydrolysis on a shaking water bath maintained at 37 ºC at 120 minutes (Aziza, 2019).

Formation of Casein Hydrolysate-Zinc Complexes

The CNH was treated with zinc sulphate (based on RDA value for adults) to produce the CNH- zinc (CNH-Zn) complex. To obtain CNH-Zn complex, food grade Zinc sulphate was added to each CNH solution at the levels of 0 mg (control) and 700 mg to get approximately the RDA for adults (recommended daily allowance) from 100 g of biscuits incubated at 37°C in shaking water bath for 40 min. The unreacted CNH was removed by centrifugation (3 times) at 3,500 – 4,000 RPM for 5 min. The supernatant containing CNH-Zn complex was separated and heated at 60˚C for concentration to 8 – 10 ml (Aziza, 2019).

Degree of Hydrolysis Determination

The degree of hydrolysis (DH) was determined by base consumption method followed by Mc Donagh and Fitz Gerald (1998) with necessary modifications in temperature and strength of alkali till optimum hydrolysis which was determined by sensory analysis of the casein hydrolysate (CNH).

Flowchart of biscuit preparation

Vanaspati (30g), icing sugar (25g) and soda (0.5g)

↓

Mixing till fluffy

↓

Addition of refined wheat flour (50 g) and other ingredients

↓

Kneading to smooth and soft dough

↓

Addition of CNH-Zn complex @ 10 %

↓

Mixing and aeration

↓

Sheeting/rolling (on biscuit frame)

↓

Cutting (using biscuit cutter)

↓

Baking (180 – 200°C / 20 min)

↓

Cooling

↓

Weighing

↓

Packaging in self-sealing poly propylene pouches

↓

Storage (room temperature)

Evaluation of the sensory and physicochemical attributes of the casein hydrolysate-zinc complex incorporated biscuits

Sensory attributes of biscuits

The sensory analysis was carried out by serving control sample along with experimental samples to a panel of 5 experienced judges on 9 point Hedonic Scale to adjudge the quality of biscuits with respect to colour and appearance, body and texture, flavour and overall acceptability.

Physical and textural parameters of the formulated biscuits were determined Physical parameters (Diameter, Thickness and Spread ratio)

The biscuits were analysed for the physical parameters like the diameter (D) and the thickness (T) in mm of biscuits using Vernier calipers and spread ratio (D/T) of the biscuit was calculated. Spread ratio is the ratio of diameter and thickness (D/T).

Texture profile analysis (Yield point, Fracturability and Hardness)

Yield point, Fracturability and Hardness of the biscuit was determined using MARK-10 texture profile analyser.

Chemical analysis for biscuits

The chemical components viz., moisture, protein, fat, ash, carbohydrates, zinc and peroxide value of the prepared biscuits were analysed by method as per ISI: SP 18 (Part XI) 1981 and The percentage of antioxidant activity (AA%) of each biscuit samples were assessed by DPPH free radical assay. This activity was performed according to method given by Wu *et al.* (2003).

Statistical analysis

The data obtained in the research work was analyzed using one way ANOVA using R software (R. version 3.5.3 Copyright (C) 2019. The R foundation for statistical computing to calculate mean and critical difference to prove significant or non-significant effect of the parameters were adopted in the present study.

III. RESULT AND DISCUSSION

Hydrolysis of sodium caseinate by using trypsin

The hydrolysis of sodium caseinate by using trypsin enzyme. The degree of hydrolysis was done by using the enzyme substrate ratio of 1:25 at 120 minutes of time interval. The DH obtained for 120 min for 1:25 E: S was 17.03 per cent respectively.

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Formation of Casein Hydrolysate-Zinc Complexes

The casein hydrolysate was added with level of 700 mg food grade zinc sulphate for the formation of CNH-Zn complex. The zinc content in the supernatant for 700 mg of added zinc obtained was 37.03 mg.

Optimization of biscuits incorporated with various levels of CNH-Zn complex based on sensory attributes

To optimize the biscuit with CNH-Zn complex, the levels of CNH-Zn complex solution were used at a rate of 10, 15 and 20 per cent. The results pertaining to effect of different levels of CNH-Zn complex in biscuits on sensory attributes is presented in Table 1.

Table 1: Effect of incorporation of CNH-Zn complex into biscuit on sensory attributes

Levels of CNH -Zn complex incorporation in biscuits	Sensory attributes			
	Colour and appearance	Body and Texture	Flavour	Overall Acceptability
	Scores on 9 – point hedonic scale			
Control (0%)	7.66^{a}	7.83^{a}	7.50^{a}	7.80^{a}
10%	8.50^{a}	8.33^{a}	8.54^{b}	8.30^{a}
15%	6.50^{bc}	7.50^{ab}	7.00^{ab}	7.50^{ab}
20%	6.10^{c}	6.50^{b}	6.66^{ab}	6.60^{b}
CD (*P=.05*)	**1.15**	**1.28**	**0.91**	**0.80**

Note:

CD: Critical Difference

All the values are average of three trials

Different superscripts indicate non-significant difference

The sensory scores with respect to colour and appearance of biscuit (Table 1) ranged from 8.50 to 6.10. The surface colour and appearance of the biscuits incorporated with CNH-Zn complex at 10 per cent level scored the highest (8.50) as compared to control and other experimental biscuits. This could be attributed to the increased brown colour. As the CNH-Zn provided higher amounts of proteins for non-enzymatic browning, and caramelization of sugars compared to control, with the increase in CNH-Zn complex solution the scores decreased due to the extensive non-enzymatic browning and also probably due to the greenish brown tinge of the CNH-Zn complex which appeared upon concertation of CNH-Zn complex solution at 60ºC. Lorenz *et al.,* (1979) reported similar results of increase in brown colour in cookies supplemented with faba bean protein concentrates. Gani *et al.,* (2015) similarly reported increased brown colour in cookies incorporated with whey and casein protein hydrolysates and attributed it to the extensive non-enzymatic browning and exposure of reactive sites on hydrolysis. Aziza (2019) who studied quality attributes of biscuits fortified with CNH- iron complex reported the scores ranged from 8.33 to 6.75 for colour and appearance by the incorporation of 0, 10 and 15 per cent CNH-Fe complex.

Biscuit prepared using 10 per cent CNH-Zn complex solution body and texture scored the highest *viz.,* 8.33 as against control. Biscuit containing 15 per cent and 20 per cent CNH-Zn complex solution secured 7.50 and 6.50 respectively. Body and texture of the biscuits was negatively altered by the incorporation of higher levels of CNH-Zn complex resulting in softer texture that caused lower scores. Aziza (2019) also reported quality attributes of biscuits fortified with CNH-Fe complex wherein the body and texture score of 8.20 for 10 per cent CNH-Fe complex incorporation level.

It is evident from (Table 1) that for biscuits with 10% CNH-Zn complex, the score of 8.54 obtained for flavour was the maximum. In biscuits containing 20 per cent, 15 per cent and 0 per cent CNH-Zn complex solution showed lower scores, i.e. 6.66, 7.00 and 7.50 respectively. Panellists described the biscuits containing more than 10 per cent CNH-Zn complex as having bitter after taste. In comparison with control, the flavour score was better for 10 percent CNH-Zn complex incorporated biscuits due to the release of increased levels of flavoured compounds like lactones, diketones, and esters by the Maillard browning. There was significant difference between the levels of CNH-Zn complex solution used for the incorporation in biscuits. The reason could be due to the masking of the flavour at higher levels of CNH-Zn complex incorporation. Aziza (2019) also reported quality attributes of biscuits fortified with CNH-Fe complex with a flavour score of 8.50 for 10 per cent CNH-Fe complex incorporation level.

The scores obtained for overall acceptability attribute of the biscuits for 10 per cent CNH-Zn complex incorporation was 8.30. Whereas the corresponding values were 6.60, 7.50 and 7.80 for biscuits containing 20, 15 and 0 per cent (control) respectively. The sample with 10 per cent CNH-Zn complex incorporation level secured highest for the overall acceptability attribute of the biscuits prepared. The reason may be attributed to colour and appearance, firm body and texture and flavour at 10 per cent of CNH-Zn complex solution. Similar values were observed by Divyashree *et al.* (2016) who studied buckwheat: chia seed fortified biscuits having an overall acceptability of 8.03 and Aziza (2019) also reported quality attributes of biscuits fortified with CNH-Fe complex with an overall acceptability score of 8.50 for 10 per cent CNH-Fe complex incorporation level.

Hence, the level of 10 per cent of CNH-Zn complex solution adjudged as the optimum level for biscuit preparation and this level was used in the further studies.

Physical and textural properties of biscuits incorporated with CNH-Zn complex

The diameter of control and optimized CNH-Zn complex incorporated biscuit observed was 43.60 and 45.00 mm respectively. It was observed that, an increase in diameter on the addition of CNH-Zn complex due to the water holding ability of CNH by exposure of hydrophilic groups. The values obtained were lesser than the values reported by Ahmed *et al.* (2019) who developed the biscuits made from the blends of WF (Wheat flour) and WPC (Whey protein concentrate) having a diameter of 65.30, 66.00, 65.60 and 65.60 mm for WF, WPC (5%), WPC (10%) and WPC (15%) respectively. It was observed that, an increase in diameter appears on addition of WPC to WF. Aziza, (2019) reported the diameter of the biscuits increased from 43.00 mm to 45.00 mm as the level of incorporation of CNH-Fe complex solution increased. This could be attributed to the higher spreadability by increased level of CNH-Fe complex due to the water holding ability of CNH.

Table 2: Effect of incorporation of CNH-Zn complex into biscuit on physical and textural properties

Sample	Properties analysed					
	Diameter (mm)	Thickness(mm)	Spread ratio (D/T)	Yield point (N)	Fracturability(N)	Hardness (N)
B_1	43.60^a	11.17^a	3.90^a	0.70^a	62.16^a	68.16^a
B_2	45.00^b	10.76^b	4.20^b	0.50^a	55.30^b	61.00^b
CD (*P=.05*)	**0.52**	**0.21**	**0.02**	**0.20**	**2.20**	**2.00**

Note

- CD: Critical Difference
- All the values are average of three trials
- Different superscripts indicate significant at the corresponding critical difference
- **B_1 -** Control biscuit without CNH-Zn complex
- **B_2 -** Optimized CNH-Zn complex incorporated biscuit

The thickness measured was 11.17 and 10.76 mm for the control biscuits and optimized CNH-Zn complex incorporated biscuit respectively. The thickness decreased by incorporation of CNH-Zn complex. As control biscuits had least moisture content, from control biscuits least moisture loss was found during baking as compared to experimental biscuits which had higher moisture content. The results of the present investigation are in accordance with Ahmed *et al.* (2019), who also reported that with increase in WPC levels from 0 to 15% in the biscuits the thickness of biscuits decreased from 9.40 mm to 7.60 mm respectively. Similarly Aziza, (2019) reported the thickness observed for the control biscuits and optimized CNH-Fe complex incorporated biscuit was 11.07 and 10.66 mm respectively. The highest thickness was obtained for the control biscuit as it had least moisture content.

The spread ratio calculated was 3.90 and 4.20 for the control biscuit and optimized CNH-Zn complex incorporated biscuit, respectively. The highest spread ratio was obtained for the optimized CNH-Zn complex incorporated biscuit. The increase in diameter and decrease in thickness affected the spread ratio of biscuits. Good quality biscuits should have a high spread ratio (Divyashree *et al.,* 2016). In the present study the spread ratio increased due to the substantial amount of moisture in the CNH-Zn complex. Also Ahmed *et al.* (2019), similarly reported the increase in spread ratio (D/T) in case of biscuits supplemented with WPC at 0 to 15 per cent with the values 6.94, 7.36 and 8.55 mm respectively.

The yield point, fracturability and hardness were higher for the control biscuit 0.70 N, 62.16 N, 68.16 N respectively when compared to the experimental biscuits 0.50 N, 55.30 N, 61.00 N respectively. The yield point, fracturability and hardness significantly decreased in the CNH-Zn complex incorporated biscuits probably due to the higher moisture content in the samples. The values obtained for yield point, fracturability and hardness were higher than the values reported by Baljeet *et al.,* (2010) who prepared biscuits by incorporating 20 per cent of buckwheat flour to the wheat flour which were 0.90 N, 59.00 N, and 63.00 N respectively. The values obtained for diameter, thickness, spread ratio, yield point, fracturability and hardness are in corroborative with the values reported by Aziza (2019) who studied CNH-Fe complex incorporated biscuit which were 43.60 mm, 11.07 mm, 3.93, 0.70 N, 68.16 N, 82.16 N respectively.

Chemical attributes of biscuit

Moisture

The moisture content of the optimized CNH-Zn complex incorporated biscuit obtained was 4.23 per cent. The moisture content of the control biscuit obtained was 3.35 per cent. The higher moisture content in the optimized CNH-Zn complex incorporated biscuits may be due to the water binding and holding property of the CNH by the exposure of hydrophilic groups. Low moisture content in biscuits (below 5 per cent) generally ensures the lesser probability from microbiological spoilage and has long shelf life. Aziza (2019) reported the moisture content of the optimized CNH-Fe complex incorporated biscuit and control biscuit was 4.60 and 3.11 per cent respectively. The higher moisture content in the optimized biscuit is due to the CNH-Fe complex solution.

Table 3: Major chemical parameters analysed in the biscuit

Biscuit sample	Moisture	Protein	Fat	Ash	Carbohydrates	Peroxide value (meq O_2/kg fat)	Antioxidant activity (% DPPH)
	Per cent (%)						
B_1	3.35^a	7.19^a	21.45^a	0.56^a	67.43^a	2.33^a	0.028^a
B_2	4.23^b	7.58^b	21.53^a	0.97^b	65.68^b	2.10^a	8.33^b
CD (*P=.05*)	**0.40**	**0.19**	**0.14**	**0.13**	**0.51**	**0.29**	**1.43**

Note:

- CD: Critical Difference
- All the values are average of three trials
- Different superscripts indicate significant difference
- **B_1 -** Control biscuit without CNH-Zn complex
- **B_2 -** Optimized CNH-Zn complex incorporated biscuit

Protein

The protein content of the control biscuit and optimized CNH-Zn complex incorporated biscuit was 7.19 and 7.58 per cent respectively. The optimized CNH-Zn complex incorporated biscuit was found to contain significantly higher level of protein, due to the incorporation of CNH-Zn complex. Similar values reported Aziza, (2019) the protein content was 7.10 and 7.48 per cent of control and CNH-Fe complex incorporated biscuits respectively.

Fat

The fat content of the control biscuit and optimized CNH-Zn complex incorporated biscuit was 21.45 and 21.53 respectively. There was no significant difference in the optimized CNH-Zn complex biscuit samples with respect to fat content. The values observed for the fat content was higher when compared to Aziza, (2019) reported the fat content of the optimized CNH-Fe complex incorporated biscuit and control biscuits was 19.57 and 19.45 per cent respectively.

Ash

The ash content of the control biscuit and optimized CNH-Zn complex incorporated biscuits obtained were 0.56 and 0.97 percent respectively. The significantly higher ash content in case of the CNH-Zn complex incorporated biscuits may be due to the supplementation of Zn as CNH-Zn complex. Similar increased levels of ash contents was reported by Aziza, (2019) who studied on the biscuits prepared incorporating CNH-Fe complex which was control and CNH-Fe complex biscuit was 0.09 and 0.56 per cent.

Carbohydrate

The carbohydrate content of the control biscuit and optimized CNH-Zn complex incorporated biscuit obtained was 67.43 and 65.48 percent respectively. The carbohydrate content of the optimized CNH-Zn complex incorporated biscuit was much lower than the control biscuits. The carbohydrate content of various biscuit ranges from 41.18 to 74.1 per cent (www.dietaryfitnesstoday.com). The results of the present investigation are in agreement with the values reported by Aziza, (2019) who studied on the biscuits prepared incorporating CNH-Fe complex which was control and CNH-Fe complex biscuit was 70.20 and 67.72 per cent.

Peroxide value

The peroxide value was slightly higher for the control biscuit when compared to optimized CNH-Zn complex incorporated biscuit. The peroxide value of the optimized CNH-Zn complex incorporated biscuit and control biscuit was obtained 1.10 and 1.33 meq of O_2/kg of fat respectively. The lower peroxide value in the experimental biscuits may be ascribed to the antioxidant activity of the CNH-Zn complex. The prooxidant activity of Zn is inhibited by antioxidant activity of CNH and as Zn is in complex form. Zn in free form may act as a prooxidant. The values observed for the peroxide value was lower when compared to Aziza, (2019) reported the peroxide value of the optimized CNH-Fe complex incorporated biscuit and control biscuits was 7.06 and 8.07 meq of O_2/kg of fat respectively.

Antioxidant activity

The optimized CNH-Zn complex solution incorporated biscuits had significantly higher antioxidant activity when compared to the control biscuits. The per cent DPPH radical scavenging activity was 8.33 and 0.028 for the optimized CNH-Zn complex incorporated biscuits and control biscuits respectively. The reason for this is the presence of casein which has antioxidant and free radical scavenging activity because of hydrogen donating capacity of hydroxyl group present in aromatic amino acids or phenolic anti-oxidants. Shahidi and Zhong (2008) reported higher antioxidant activity of protein hydrolysate prepared by alcalase as 16.80 per cent of DPPH. The values obtained for antioxidant activity of CNH-Zn complex biscuit were higher when compared to values reported by Aziza (2019) who reported antioxidant activity of CNH-Fe complex incorporated biscuits as 2.43 per cent of DPPH.

Chemical changes in optimized CNH-Zn complex incorporated biscuits during storage at ambient temperature (27±1˚C)

Effect of storage on moisture content of optimized CNH-Zn incorporated biscuits

The biscuits were stored in poly propylene self-sealing pouches at room temperature for a period of 60 days. Moisture greatly affects sensory, rheological and physico-chemical and microbiological characteristics of the biscuits. There was a slight increase in the moisture content of both the biscuit samples as the storage period proceeded and it increased from 3.36 to 4.30 per cent for the control biscuit, whereas for the optimized CNH-Zn complex incorporated biscuit it increased from 4.25 to 5.20 per cent during 60 days. The increase in moisture content might be due to the storage environment (water activity humidity and temperature), nature of packaging material used and also due to the hygroscopic nature of biscuits.

Similar report of increase in moisture content was observed by Balestra *et al.* (2016) who studied chemical and physical changes of biscuits stored in poly propylene pouches and it increased from 3.16 to 5.06 per cent during 60 days. The initial values of 3.36 and 4.25 per cent of moisture content are in agreement with the range for freshly baked biscuits that is typically between 1 and 5 per cent. Water uptake may be explained by the hygroscopic nature of dried products, environmental conditions related to temperature and relative humidity (RH) and characteristics of the packaging material.

Table 4: Effect of storage (27±1 ºC) on moisture content and peroxide value of biscuits

Biscuit sample	Moisture %					Peroxide value (meq O_2/kg fat)				
	Storage period (Days)									
	0	15	30	45	60	0	15	30	45	60
B_1	3.36^a	3.60^a	3.90^a	4.30^a	4.60^a	2.33^a	4.60^a	8.10^a	11.13^a	14.14^a
B_2	4.25^b	4.40^b	4.50^b	5.20^b	5.41^b	2.10^a	4.10^a	7.50^a	10.46^a	13.56^a
CD (*P=.05*)	**0.12**	**0.16**	**0.20**	**0.20**	**0.23**	**0.12**	**0.61**	**0.71**	**0.69**	**0.72**

Note:

- CD: Critical Difference
- All the values are average of three trials
- Different superscripts indicate significant at the corresponding critical difference
- **B_1** - Control biscuit without CNH-Zn complex
- **B_2** - Optimized CNH-Zn complex incorporated biscuit

Effect of storage on Peroxide value of optimized CNH-Zn incorporated biscuit

Peroxide value is the indicator of rate of auto oxidation. It increased significantly during storage from 2.33 to 14.14 and 2.10 to 13.56 meq O_2 per kg fat for control biscuit and optimized CNH-Zn complex biscuit respectively at the end of 60 days. The CNH-Zn complex might have played the antioxidant role, thereby reducing the peroxide value. The biscuit samples which were stored in poly propylene pouches, the rate of auto-oxidation is mainly controlled by the oxygen retained in the pack, which in turn is related to the headspace and oxygen permeability of the packaging material (Divyashree *et al.,* 2016). Similar report of increase in peroxide value was observed by Balestra *et al.* (2016) in study on chemical and physical changes during storage of biscuits packed in poly propylene pouches and it increased from 3.16 to 10.00 meq O_2 per kg fat at ambient temperature stored for 60 days.

IV. CONCLUSION

Thus, it can be concluded that, higher degree of hydrolysis can bind higher amount of zinc to the casein hydrolysate. If the higher amount of CNH-Zn complex solution is used in the biscuit preparation, it will lead to a biscuit with reduced sensorial properties. The use of CNH-Zn complex in the powder form rather than solution may be advantageous to achieve better physico-chemical and keeping quality of biscuits. Besides use of better packaging material instead of polypropylene pouches may enhance the shelf life of the biscuits.

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