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EFFECT OF BAKING ON ANTIOXIDANT AND SENSORY ATTRIBUTES OF CURCUMIN ENCAPSULATES ENRICHED DESIGNER BREAD FORMULATIONS

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ABSTRACT

Dietary intrusions emphasize on dynamic facets of phytonutrients due to strong antioxidant capacity. For the purpose, turmeric polyphenol *i.e.* curcumin was firstly extracted through conventional and supercritical extraction techniques following the preparation of microcapsule using maltodextrin and gelatin as enrobing material by freeze drying method. Afterwards, four types of designer bread weremade; turmeric (T_1), microencapsulated nutraceutical$_{CSE}$ (T_2) and nutraceutical$_{SFE}$ enriched breads (T_3) along with control (T_0). Physicochemical profile of resultant prototypes showed that addition of microencapsulated nutraceutical$_{SFE}$ enhanced volume and phenolics from 572.60±7.56 to 686.20±8.25 cm^3 and50.22±1.70 to 81.12±3.48 mg GAE/100g whilst hardness decreased from 2.97±0.11 to 3.36±0.10 kg/cm^2 compared to control. Furthermore, hedonic response also improved with maximum scores for bread volume, crust color, symmetry and evenness in bake;8.01±0.39, 7.04±0.21, 3.83±0.16 and 1.94±0.05, respectively. Regarding internal parameters, highest scores for grain, crumb color, aroma, taste and texture were 12.32±0.38, 8.14±0.26,8.05±0.26, 16.41±0.61 and 13.02±0.52, correspondingly. Conclusively, microencapsulated nutraceutical$_{SFE}$ enhances both antioxidant and sensorial profile of designer bread.

Keywords: *turmeric, nutraceutical, microencapsulation, antioxidant, CSE and SFE*

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INTRODUCTION

As customary dietary recommendations, healthy living pattern and modern nutritional science are always connected to good health. Nowadays, people are more inclined towards the use of natural ingredients as a prophylactic approach against various ailments (Manjula and Suneetha 2011). In this context, nutraceutics and nutraceutic enriched designer foods have gained importance over the globe due to their health enhancing potential beyond the provision of basic nutrition. Scientific investigations have provided an insight regarding therapeutic potential of bioactive moieties nevertheless, development of designer foods ensuring target delivery is required to manage disease burden (Al-Obaidi *et al.,* 2021; Prescott 2009).Designer foods are enriched with naturally occurring disease preventing constituents and similar in appearance to conventional foods. They are consumed as part of routine diet due to their association with established health benefits to fulfill the needs of vulnerable masses. (Rajasekaran and Kalaivani 2013).

There are strong evidences supporting the presence of phytoceutics in spices and their inclusion in diet improves hedonic response. Actually, spices are seasonings with aromatic profile and strong antioxidant potential, widely used in the traditional culinary (Baselga-Escudero *et al.,* 2017; Yashin *et al.,* 2017). Spices like turmeric encompass good nutritional profile and proven to be a promising source of health promoting compounds. It imparts desirable aroma, color & flavor as well as improves antioxidant status. Curcumin is the principle turmeric bioactive moiety exhibiting 100 times more antioxidant potential than vitamin C & E (Sharifi-Rad *et al.,* 2020; Bagchi 2012).The chemical identity of curcumin is1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione with $C_{21}H_{20}O_6$ molecular formula. Whilst, the structural configuration showed a pair of aromatic rings displaying ortho-methoxy chemical moiety coupled with unsaturated β-diketon of hepta-carbons. Physically, curcuminoids are deep orange-yellow crystalline powder and insoluble in aqueous medium (Nurjanah and Saepudin, 2019; Nelson *et al.,* 2017).

Unfortunately, bioavailability and stability of curcumin is very low owing to its rapid degradation into various metabolites, limited solubility, sensitivity to pH variations and instability to high temperature (Martinez-Guerra *et al.,* 2019; Liu *et al.,* 2017). For the purpose, various techniques have been probed out to enhance bioavailability as well as ease out food applications of curcumin (Fang and Bhandari, 2010). Among latest intrusions, microencapsulation under freeze dried conditions using sugar, protein, lipid, gums, native & modified polysaccharides and synthetic polymers as coatings is gaining popularity. Foregoing explorations

have assured high encapsulating efficiency and shelf life of microencapsulated curcuminoids (Malacrida *et al.* 2011). Recently, Guo *et al.* (2020) studied the effect of encapsulating techniques (spray drying and freeze drying) on enrobing efficiency and stability of curcumin using maltodextrin, gelatin and modified corn starch in various combinations as coating material. The freeze dried micro-particles of curcumin showed better encapsulation efficiency while spray dried curcumin microcapsules have fine particle size with smooth surface. However, both methods improved the stability and solubility of curcumin.

Product development is a pre-requisite tool to carry bioactive components to the targeted population. Purposely, bakery products are mostly relished for their ready to eat nature, low cost, availability in different shapes & flavor, good keeping quality and long shelf life (Ajila *et al.* 2008). One of the oldest functional foods is bread, utilized as mean for the intrusion of various phytochemicals. In terms of industrial production and consumer utilization, it is given special importance among bakery products (Hathorn *et al.* 2008). However, industrialists and food scientist are trying to improve the bread quality, microbial safety and storage. The main cardinal attribute relevant to bread quality is rheological properties that deal with its volume and texture. For the purpose, farinograph analysis is one of promising tools to evaluate rheological parameters of bread dough. These parameters are influenced by incorporation of turmeric powder and its bioactive ingredients (Okoko and Ogbomo 2010). Keeping in view, current study was designed to analyze effect of turmeric powder and microencapsulated curcumin on physicochemical and sensory properties of bread.

MATERIALS AND METHODS

The current research was carried out in the Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad and accomplished within a year (2018).Turmeric variety (Kasur) was procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. The washing of raw material was done to remove the adhered dust, dirt and other foreign matter for further analysis.

Preparation of Conventional and Supercritical Extracts: In conventional method, aqueous ethanol extract (50:50) of turmeric was prepared in orbital shaker for 65 min at constant temperature of 50ºC following the prescribed procedures of Ashraf *et al.* (2016) and Bagchi *et al*. (2012). Afterwards, solvent extract was filtered and concentrated through Rotary Evaporator (Eyela, Japan).

Supercritical fluid extract of dried turmeric rhizome was acquired through SFT-150 system using 99.8% pure CO_2 at 50 ºC. After the placement of sample in 100 mL extraction vessel, CO_2 was liquefied by keeping time (150 min) and pressure (4500 psi)constant to accelerate the solvation & mass transfer of the desired analyte using the guidelines of Wakte *et al.* (2011).

Microencapsulation of Curcumin: Curcumin was encapsulated using homogenous emulsions comprised of various proportions of maltodextrin (15 & 20 g) as well as gelatin (2, 4 & 6 g) as mentioned in Table 1. For the purpose, gelatin was dissolved in warm distilled water and mixed with maltodextrin solution already prepared in ethanol to make different ratios of maltodextrin and gelatin. Now, conventionally and supercritical extracted curcumin was added at concentration of 10% depending on weight of encapsulating material. The mixture was homogenized at 15,000 rpm for 10 min. Secondly, the prepared emulsions were frozen at -35ºC for 24 hrs following the freeze drying at -30 °C according to prescribed method of Malacrida and Telis (2011). The resultant material was finely ground and stored for further analysis.

Encapsulation Efficiency (EE): The prepared microcapsules were tested for their entrapment capacity depending on the total curcumin contents following the protocol of Malacrida and Telis (2011). Purposely, freeze dried curcumin microcapsules were taken in a 25 mL standard volumetric flask and the volume was made using ethanol. Prepared solutions were subjected to centrifugation (3500 rpm) after homogenizing samples in vortex for 5 min. Afterwards, collected supernatant as well as curcumin emulsion were used to measure absorbance at 245 nm. The curcumin content was calculated using the standard curve.

Encapsulation efficiency (EE%) was expressed as:

$EE\% = (C_E/C_0) \times 100$

C_E= curcumin content in the freeze dried powder

C_0= curcumin content in emulsion

Table 1. Treatments for microencapsulation of curcumin.

Treatments	Maltodextrin (g)	Gelatin (g)
T_{1CSE}	15	2
T_{1SFE}		
T_{2CSE}	15	4
T_{2SFE}		
T_{3CSE}	15	6
T_{3SFE}		
T_{4CSE}	20	2
T_{4SFE}		
T_{5CSE}	20	4
T_{5SFE}		
T_{6CSE}	20	6
T_{6SFE}		

CSE=Conventional Solvent Extract
SFE=Supercritical Fluid Extraction

Product Development: Prior to bread development, proximate analysis of the flour was carried out. Moreover, rheological properties of dough was measured by Brabender Farinograph (Brabender GmbH & Co. KG, Germany) employing the procedure of AACC, (2000).

Bread Preparation: The bread was made in the Bakery Section of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. After weighing all ingredients (flour, yeast, salt, sugar, fat and water)precisely, mixing is performed to hydrate and blend the flour until smooth, shiny and viscoelastic dough is prepared. For the development of functional/nutraceutical bread prototypes, inclusion of turmeric powder, microencapsulated nutraceutical$_{CSE}$ and nutraceutical$_{SFE}$ were made in following treatments *i.e.* T_1, T_2 and T_3 @ 3, 1 and 0.5%, respectively with T_0 as control (without turmeric). Now the precisely mixed dough was kept under fermentation conditions of 30-35ºC temperature and 85% relative humidity unless dough volume doubles after entrapment of gases produced by yeast in gluten network. The dough was punched in between the fermentation duration to increase its gas retention capacity. The fermented dough was scaled, sealed and moulded to get desired baked bread following panning in baking pans. Afterwards, moulded dough was kept for proofing at a temperature of 30-35ºC and 85% relative humidity for 60min.Lastly, it was baked at 230 ºC for 30min to achieve desirable crust color, smooth crumb and symmetry. Subsequently, bread was left for cooling for about 1 hr.

Physicochemical Analysis: The prepared bread samples were evaluated for the variations in color, texture (hardness) and antioxidant potential (TPC) during the storage period. The color and texture of bread were measured following the procedure of Mohamed *et al.* (2010). All the product treatments were analyzed for their antioxidant activity by measuring TPC as described by Himesh *et al.* (2011).

Color: The resultant bread formulations were analyzed for their color using CIE-Lab Color Meter (CIELAB SPACE, Color Tech-PCM, USA) using the method of Mohamed *et al.* (2010). For the purpose, product surface was exposed and respective color values as L^* (lightness), a^* (–a greenness; +a redness), and b^*(–b blueness; +b yellowness) were measured.

Texture: Texture of bread was analyzed by triple beam snap (three-point break) technique of Texture Analyzer (TA-HDi, Stable Microsystems, UK). A 50 kg load cell having crosshead speed of 10 mm/min was used. The required force to compress bread loaves was noted and the average value was computed following to protocol described by Mohamed *et al.* (2010).

Volume: Bread volume was determined as mentioned by Amir *et al.* (2013) through rapeseed displacement method. Purposely, volume of rapeseed filled container was measured. Afterwards, bread was positioned in container following refilling container with rapeseed. The volume of refilled rapeseed was noted. The volume of rapeseed displaced corresponds to bread volume.

Total Polyphenols: Total Phenol Content (TPC) in functional/nutraceutical bread samples was calculated using Folin-Ciocalteu method based on the reduction of phosphotungstic acid to phosphotungstic blue (Himesh *et al.* 2011). Purposely, 50 µL of extract was mixed with 250 µL of Folin-Ciocalteu's reagent followed by the addition of 750 µL 20% Na_2CO_3 solution. The total volume of the solution was marked up to 5 mL with distilled water. Absorbance was recorded after 2 hrs at 765 nm with UV/visible light Spectrophotometer (CECIL, CE7200) against control, having all reaction reagents except sample extract. Total polyphenols were estimated and values were expressed as gallic acid equivalent (mg gallic acid/g).

$$C=c\times V/\ m$$

C = Total phenolic contents (mg/g plant extract, in GAE)
c = Concentration of gallic acid (mg/mL)
V = Volume of extract (mL)
m = Weight of turmeric (g)

Sensory Evaluation: The sensory evaluation of functional/nutraceutical bread was carried out by the procedure of Meilgaard *et al.* (2007) using 9-point hedonic scale. Slices of turmeric bread were provided to semi-trained panelist with random codes at different time intervals; 0, 24, 48, 72 and 96 hrs to evaluate effect of treatments and storage intervals on external (volume, crust color, symmetry, evenness of bake, crust character etc.) as well as internal (grain, crumb color, aroma, taste, texture etc.) sensory parameters.

Statistical Analyses: Furthermore, Microsoft Excel (version 2013) was used to handle and summarize the data. Three replicates were taken for each test except for sensory response (n = 10). For composition, rheological properties of flour and encapsulation efficiency of curcumin microcapsule, one-way analysis of variance (ANOVA) under completely randomized design (CRD) was executed. While, two way ANOVA under CRD was applied for physico-sensorial parameters and TPC as storage was also involved in addition to treatments. The data for each attribute was subjected to statistical analysist o investigate the significant effect of treatments using statistical software Cohort version 6.1 (Costat-2003). Later, Tukey's honest significant difference (HSD) test was used for means differences at $p<0.05$ (Mason *et al*., 2003).

RESULTS AND DISCUSSION

Proximate Composition: The proximate analysis of flour explicated moisture 11.50±0.39%, crude protein 10.56±0.42%, fat 0.87±0.04%, fiber 0.48±0.02%, ash 0.52±0.01% and NFE 76.07±2.74%.The current results are in agreement with Lim *et al.* (2011), reported moisture, crude protein, fat, fiber, ash and Nitrogen Free Extract (NFE) as 14.02, 12.83, 1.52, 2.10, 0.43 and 69.10%, respectively. Currently, Nisar *et al.* (2020) determined moisture (8.64%), crude protein (8.90%), crude fat (2.29%), crude fiber (1.44%), ash (1.6%) and NFE (76.92%) contents of whole wheat flour. Similarly, Chukwu *et al*., (2020) confirmed moisture 10.55%, crude protein 11.30%, fat 9.70%, fiber1.53%, ash 3.34% and carbohydrate 50.13%. Previously, Kumar *et al.* (2011) recorded protein 11.5%, fat 1.4% and carbohydrate 75.3% in bread flour. One of the peers, Lim *et al.* (2011), reported moisture, protein, fat, fiber, ash and NFE as 14.02, 12.83, 1.52, 2.10, 0.43 and 69.10%, respectively.

HPLC quantification of curcumin: HPLC analysis of both extracts is a mandatory tool for quantification of bioactive components. Furthermore, accurate information about exact quantity of curcumin in both extracts is important to assess its effective dose in designer bread formulations. Statistical analysis regarding HPLC quantification for turmeric bioactive moiety indicated momentous changes in curcumin content, extracted using supercritical fluid (CO_2) & conventional solvent (ethanol) as function of treatments. Means for the effect of extraction conditions on curcumin content (Figure 1) elucidated highest yield (52.41±2.38 mg/g) in extract obtained using supercritical fluid trailed by ethanol with total curcumin content as 31.48±1.35 mg/g. The instant results are in line with research work of Chao *et al.* (2018), investigated a validated protocol for determination of curcumin I, II & III and other volatile components of turmeric. They found that %recovery of three variants of curcuminoids could be increased by enhancing column temperature and absorption wavelength upto 35°Cand 430nm, respectively that would improve resolution power and chromatographic conditions. Presently, Vidal-Casanella *et al.* (2020) characterized different turmeric varieties and related curry powder for curcuminoids using HPLC chromatography equipped with diode array detection (DAD). They employed two HPLC module under reverse phase condition and analyzed better compositional fingerprints of three curcuminoids analogues at 420nm depending on their origin. It may be concluded that curcumin content not only changed as a function of variety, geographical location, environmental factors but the extraction conditions and method of characterization also significantly have effect on its total yield.

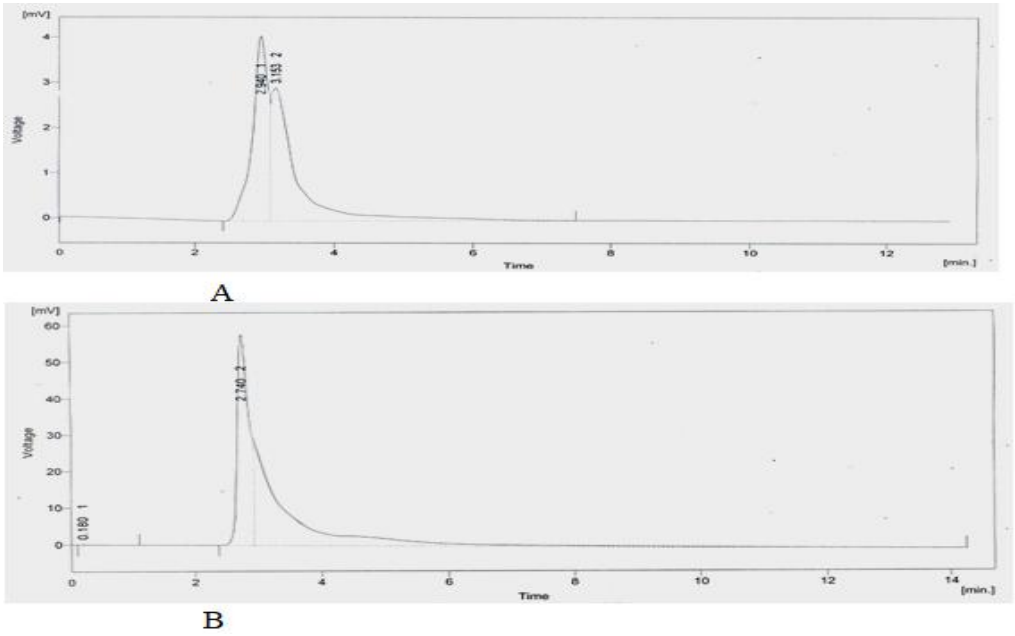


Figure 1: chromatograms of curcumin (a) ethanolic extract (b) supercritical extract at 150 min

Encapsulation efficiency: Statistical analysis for the effect of various ratios of wall material (maltodextrin and gelatin) on encapsulation efficiency of curcumin extracted under supercritical condition (Table 2) illuminated highest value (73.58±3.16%) for T_{SFE6} obtained using maltodextrin and gelatin at ratio of 20:6 trailed by T_{SFE3}, T_{SFE5}, T_{SFE2}, T_{SFE4} and T_{SFE1} as 71.39±2.21, 65.23±2.73, 62.84±2.32, 59.42±2.07 and 57.75±1.96% employing encapsulating wall material (maltodextrin and gelatin) at concentration of 10:6, 20:4,

10:4, 20:2 and 10:2, respectively. Amongst conventionally obtained extracts, the observed values for curcumin encapsulation was 69.32±2.83 (T_{CSE6}), 62.54±2.72 (T_{CSE3}), 61.85±2.42 (T_{CSE5}), 55.37±2.39 (T_{CSE2}), 54.91±1.86 (T_{CSE4}) and 48.29±1.73% (T_{CSE1}).

The current findings are in collaboration with Malacrida and Telis (2011), evaluated effect of different combinations of gelatin (0.5, 0.9, 1.8, 2.6, 3 and 6%) and maltodextrin (12, 18, 19, 20.6, 24.4, 28.1 and 29.7%) on encapsulation efficiency of curcumin. They noticed variations in this trait from 50.8 to 81.1%. Accordingly, maximum core retention was observed up to 81.1±0.3% by using emulsions containing 18% maltodextrin and 6% gelatin as wall material. On the other hand, formulations containing higher amount of maltodextrin rapidly hydrate within 5 min of agitation whereas, increased gelatin content lowered the solubility of curcumin microcapsules. Later on, Delfiya *et al.* (2015) elaborated influence of different contents of guar arabic and maltodextrin (from 0:100 to 100:0) as coating matrix on encapsulation power of turmeric oleoresins. They assessed that encapsulation efficiency improves by increasing concentration of gum arabic in micro emulsion due to its more viscosity that enrobes micro-particles and prevents coalescence. Recently, Guo *et al.* (2020) studied the effect of encapsulating techniques (spray drying and freeze drying) on enrobing efficiency and stability of curcumin using maltodextrin, gelatin and modified corn starch in various combinations as coating material. The freeze-dried curcumin microcapsules have encapsulation efficiency above 70% as compared to spray dried curcumin micro-particles.

Table 2. Effect of treatments on encapsulation efficiency of curcumin.

Treatments	Encapsulation Efficiency(EE%)
Microencapsulated Curcumin$_{SFE}$	
T_{SFE1}	57.75±1.96c
T_{SFE2}	62.84±2.32bc
T_{SFE3}	71.39±2.21ab
T_{SFE4}	59.42±2.07c
T_{SFE5}	65.23±2.73b
T_{SFE6}	73.58±3.16a
Microencapsulated Curcumin$_{CSE}$	
T_{CSE1}	48.29±1.73c
T_{CSE2}	55.37±2.39b
T_{CSE3}	62.54±2.72ab
T_{CSE4}	54.91±1.86b
T_{CSE5}	61.85±2.42ab
T_{CSE6}	69.32±2.83a

Values are expressed as means ± standard deviation. Means within columns with different letter are significantly different ($P< 0.05$);

SFE=Supercritical Fluid Extract

T_{SFE1}=Matodextrin:gelatin (15:2)

T_{SFE2}= Matodextrin:gelatin (15:4)

T_{SFE3}= Matodextrin:gelatin (15:6)

T_{SFE4}= Matodextrin:gelatin (20:2)

T_{SFE5}= Matodextrin:gelatin (20:4)

T_{SFE6}= Matodextrin:gelatin (20:6)

CSE=Conventional Solvent Extract

T_{CSE1}= Matodextrin:gelatin (15:2)

T_{CSE2}= Matodextrin:gelatin (15:4)

T_{CSE3}= Matodextrin:gelatin (15:6)

T_{CSE4}= Matodextrin:gelatin (20:2)

T_{CSE5}= Matodextrin:gelatin (20:4)

T_{CSE6}=Matodextrin:gelatin(20:6)

It was analyzed that increase in maltodextrin did not affect appreciably curcumin retention due to limited emulsification capacity. The encapsulation power was dependent on gelatin content. During emulsification, gelatin reduces interfacial surface tension as well as rate of coalescence.

Farinographic Parameters: The momentous changes were observed in farinographic properties of flour; water absorption, development time and stability of dough (Table 3). It was inferred that flour substituted with turmeric powder (T_1)has maximum water absorption of 64.26±2.89%while values for this trait were 62.50±2.18, 62.35±2.15 and 61.80±2.42% for T_0, T_3 and T_2, respectively. Similarly, time needed for dough development increased (3.2±0.06 min) in T_1 as compared to T_0(2.8±0.09 min), T_2 (2.9±0.13 min) and T_3 (3.0±0.11 min). Conversely, inclusion of turmeric powder (T_1) decreased dough stability (7.70±0.22 min) that increased to 8.30±0.32, 8.54±0.37 and 8.62±0.25 min for T_0, T_2 and T_3, correspondingly.

Outcomes concerning dough rheological attributes are supported by Park *et al.* (2012), evaluated effect of various turmeric levels on farinographic properties. The study revealed that water absorption increased from 49.6 to 69.4% with addition of turmeric powder (0-8%). This was related to fiber portion of turmeric rhizome that allows more water to bind hydroxyl group (OH) by hydrogen bonding. According to Goldstein *et al.* (2010), fiber weakens dough by disrupting the gluten network. However, addition of polyphenols enhances stability of dough by making hydrogen linkages between OH group of phenol and carbonyl moiety of protein thus strengthening gluten network (Sivam*et al.* 2010).

Physicochemical Analysis: Means pertaining to L^* value (Table 4) for control (T_0), functional (T_1), microencapsulated nutraceutical$_{CSE}$ (T_2) and microencapsulated nutraceutical$_{SFE}$ (T_3) supplemented bread delineated the highest score for T_0 (40.39±1.97) followed by T_3 (40.35±1.38), T_2 (39.04±1.32) and T_1 (36.16±1.04). However, momentous reduction from 40.70±1.92 to 36.70±1.28 in L^* value was noticed during 96 hr storage. Regarding the trait a^*(Table 4), value

increased towards yellow-orange with addition of turmeric. The means for a^* indicated T_0, T_1, T_2 and T_3 as 17.09±0.73, 20.86±0.82, 18.51±0.76 and 18.72±0.82, accordingly. During the course of time, a^* values declined from 19.93±0.83 to 17.43±0.76 till the end of storage.

Table 3. Effect of treatments on rheological properties of flour samples.

Treatments	Water Absorption(%)	Dough Development Time(min)	Dough Stability(min)
T_0	62.50±2.18ab	2.8±0.09ab	8.30±0.32b
T_1	64.26±2.89a	3.2±0.06b	7.70±0.22c
T_2	61.80±2.42b	2.9±0.13a	8.54±0.37ab
T_3	62.35±2.15ab	3.0±0.11a	8.62±0.25a

Values are expressed as means ± standard deviation. Means within columns with different letter are significantly different (P< 0.05); T_0= Control, T_1=Flour containing 3% turmeric powder, T_2=Flour containing 1% microencapsulated nutraceutical$_{CSE}$, T_3=Flour containing 0.5% microencapsulated nutraceutical$_{SFE}$

The recorded values for b^*(Table 4) revealed significant differences as function of treatments; 24.68±0.97 in T_0, 28.78±1.51 in T_1, 25.72±1.29 in T_2 and 25.95±1.05 in T_3. During storage, momentous increase in b^* value was detected from 24.68±1.17 to 28.13±1.31 at 0 and 96 hr intervals, correspondingly. The means relating to chroma value of designer bread are presented in Table 5. The chroma values for T_0, T_1, T_2 and T_3 were 30.90±1.18, 35.48±1.69, 31.22±1.24 and 31.71±1.38, respectively. Besides, the storage showed non-significant decline for this trait at 0, 24, 48, 72 and 96 hr *i.e.*31.76±1.32, 31.99±1.47, 32.47±1.52, 32.73±1.38 and 33.30±1.55, correspondingly. For hue angle, non-substantial changes were observed in T_0,T_1,T_2 andT_3 by 0.32±0.009, 0.30±0.014, 0.32±0.012 and 0.30±0.005, respectively as a function of active ingredient supplementation(Table 5). Likewise, storage did not impart any significant variations on hue angle.

Table 4. Effect of treatments and storage on l^*, a^* and b^* value of bread.

Treatments	Hours						Means
	Characteristics	0	24	48	72	96	
T_0	**L^***	42.86±1.37	41.75±1.50	40.62±1.84	39.44±1.34	37.32±1.22	40.39±1.97^a
	a^*	18.56±0.76	17.78±0.72	17.05±0.69	16.64±0.68	15.42±0.58	17.09±0.73^b
	b^*	22.20±0.88	23.66±0.95	24.78±0.93	25.34±1.12	27.45±1.10	24.68±0.97^b
T_1	**L^***	38.14±1.25	37.66±0.98	36.04±1.03	35.55±1.09	33.42±1.18	36.16±1.04^b
	a^*	21.70±0.89	21.38±0.91	20.95±0.85	20.48±0.81	19.78±0.79	20.86±0.82^a
	b^*	27.45±0.85	27.86±1.12	28.44±0.92	29.52±1.35	30.61±1.04	28.78±1.51^a
T_2	**L^***	40.02±1.24	39.84±1.31	39.25±1.35	38.62±1.28	37.48±1.25	39.04±1.32^{ab}
	a^*	19.62±0.79	19.14±0.74	18.75±0.81	17.98±0.77	17.06±0.66	18.51±0.76^{ab}
	b^*	24.12±1.23	24.63±0.83	25.98±1.05	26.55±1.14	27.36±1.31	25.72±1.29^{ab}
T_3	**L^***	41.78±1.31	41.24±1.28	40.82±1.25	39.35±1.32	38.56±1.29	40.35±1.38^a
	a^*	19.85±0.89	19.24±0.91	18.92±0.85	18.16±0.81	17.45±0.79	18.72±0.82^{ab}
	b^*	24.97±1.09	25.26±1.03	25.69±1.12	26.73±0.94	27.12±1.10	25.95±1.05^{ab}
Mean	**L^***	40.70±1.92^a	40.12±1.55^a	39.18±1.27^{ab}	38.24±1.39^{ab}	36.70±1.28^b	
	a^*	19.93±0.83	19.38±0.89	18.92±0.77	18.32±0.84	17.43±0.76	
	b^*	24.68±1.17b	25.35±0.82^b	26.22±1.15^{ab}	27.03±1.24^{ab}	28.13±1.31^a	

Values are expressed as means ± standard deviation. Means within column and row with different letter are significantly different (P< 0.05); T_0= Control, T_1=Flour containing 3% turmeric powder, T_2=Flour containing 1% microencapsulated nutraceutical$_{CSE}$, T_3=Flour containing 0.5% microencapsulated nutraceutical$_{SFE}$

Table 5. Effect of treatments and storage on chroma and hue angle of bread.

Treatments	Hours						Means
	Characteristics	0	24	48	72	96	
T_0	**Chroma**	29.71±1.13	30.40±1.17	30.90±1.19	31.15±1.15	32.36±1.21	30.90±1.18b
	Hue angle	0.32±0.005	0.32±0.010	0.32±0.003	0.31±0.008	0.32±0.013	0.32±0.009
T_1	**Chroma**	34.90±1.65	35.03±1.71	35.23±1.70	35.85±1.67	36.38±1.75	35.48±1.69a
	Hue angle	0.30±0.013	0.29±0.015	0.30±0.007	0.30±0.011	0.30±0.002	0.30±0.014
T_2	**Chroma**	31.09±1.12	31.18±1.28	32.04±1.07	32.06±1.35	32.24±1.41	31.22±1.24b

T_3	**Hue angle**	0.32±0.010	0.32±0.012	0.31±0.008	0.32±0.015	0.32±0.007	0.32±0.012
	Chroma	31.34±1.35	31.38±1.42	31.74±1.28	31.89±1.52	32.24±1.39	31.71±1.38b
Mean	**Hue angle**	0.31±0.006	0.31±0.011	0.31±0.010	0.29±0.013	0.30±0.009	0.30±0.005
	Chroma	31.76±1.32	31.99±1.47	32.47±1.52	32.73±1.38	33.30±1.55	
	Hue angle	0.31±0.010	0.31±0.007	0.31±0.015	0.31±0.011	0.31±0.006	

Values are expressed as means ± standard deviation. Means within column with different letter are significantly different ($P< 0.05$);T_0= Control, T_1=Flour containing 3% turmeric powder, T_2=Flour containing 1% microencapsulated nutraceutical$_{CSE,}$ T_3=Flour containing 0.5% microencapsulated nutraceutical$_{SFE}$

The recent findings are corroborated by Sikkhamondhol *et al.* (2009), investigated influence of various turmeric contents (0.10-0.25%) on color of bread. They deduced that L^* value decreased from 80.15±1.58 to 78.23±2.23 while a^* and b^* value increased from 3.57±0.39 to 5.71±0.24 and43.12±1.23 to 53.09±1.91, respectively. Furthermore, Lim *et al*., (2011) inferred the effect of turmeric powder on color tonality of bread and observed significant decline in L^* while rise in a^* and b^* values, ensuing darker and yellower bread crumb. In fact, this color variation is attributed to turmeric phenolics "curcuminoids" that confer yellowish tint to product. The colorimeter results by Seo *et al.* (2010) indicated change in color parameter of cake by alteration in turmeric ratio. The turmeric inclusion from 0.5 to 5% remarkably transformed lightness (L^*), yellowness (b^*) and redness (a^*) from 86.1 to 64.3, 29.9 to 38.1 and 2.0 to 4.8, respectively. Likewise, Wahanik *et al*., (2018) assessed the changes in color tonality of pasta having various content of turmeric powder (10 to 50 g/Kg flour) in different pasta formulations. They verified that color did not change significantly in raw pasta however, the brightness (L^*) of cooked pasta changed to redness due to increase in a^* and b^* value.

The means related to volume depicted significant variations as function of treatments; 686.20±8.25, 572.60±7.56, 650.16±9.75 and 679.32±10.06 cm^3 for T_0, T_1, T_2 and T_3, correspondingly (Figure 2). However, volume elucidated non-substantial decline during storage from 655.25±8.43 to 653.50±7.50, 648.20±8.83, 642.36±9.07 and 635.48±8.38 cm^3 at 0, 24, 48, 72 and 96 hrs, respectively (Figure 2). Means for hardness in T_0, T_1, T_2 and T_3 were 2.97±0.11, 3.36±0.10, 3.05±0.09 and 3.14±0.11 kg/cm^2, respectively (Figure 3). During storage, minimum value for hardness was observed at 0 hr *i.e.* 2.93±0.13 that significantly increases to 3.37±0.11 kg/cm^2 at 96 hr (Figure 3). On the other hand, maximum value of TPC 81.12±3.48 mg GAE/100g was noticed in bread supplemented with microencapsulated supercritical fluid extract of turmeric (T_3) whilst, the values for T_2, T_1 and T_0 were 76.19±3.25, 68.65±2.81 and 50.22±1.70 mg GAE/100g, accordingly. Nonetheless, phenolics decreased momentously during 0 to 96 hrs storage interval from 70.82±3.69 to 66.92±3.01 mg GAE/100g (Figure 4).

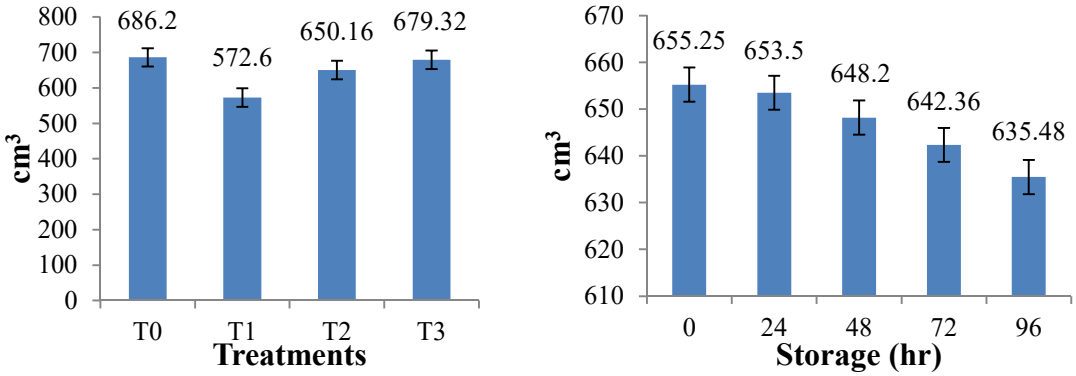
The current results are supported by Lim *et al.* (2011), evaluated effect of 2, 4, 6 and 8% turmeric powder on hardness, volume and phenolics of breads. They assessed a significant reduction in bread volume with an increment in turmeric powder due to low content of wheat gluten responsible for development of network structure. However, hardness changed from 213.1 ± 13.9 to 299.8 ± 14.8 g/cm^2owing to increase in bread density. Nevertheless, turmeric powder has improved total phenolics from 30.9 to 150.5 mg GAE/100g. Although phenolics recorded in turmeric powder were 2195 mg GAE/100g yet baking resulted in 32 to 54% losses. Nonetheless, phenolics in turmeric supplemented breads are more as compared to control group. They recommended daily intake of two slices of 4% turmeric supplemented bread providing 40.12 mg GAE/100g phenolics containing 4.6 mg curcumin.

Conclusively, addition of curcumin microcapsules extracted under supercritical conditions in bread is a stable entity as an antioxidant as well as it did not impart any adverse impact on physiochemical attributes of the developed designer bread. It is interesting to mention that during physiochemical analysis; all values were within the acceptable range and showed better evaluator's response. On the other hand, bread having turmeric powder in its formulation have reduced volume and poor gluten network making it harder than other counterparts. Curcumin microcapsules prepared using conventional solvent were not as pure as curcumin microcapsules obtained under supercritical conditions thus lowing total phenolics of bread.

Sensory Analysis: A semi-trained taste panel (semi-trained) was assigned with the task to evaluate the functional/nutraceutical breads by senses in order to find the effects on external characteristics such as, color, volume, symmetry, evenness of bake along with crust character. Likewise, the effects on internal characteristics *i.e.* bread grain, taste, aroma, texture and crumb color were also investigated.

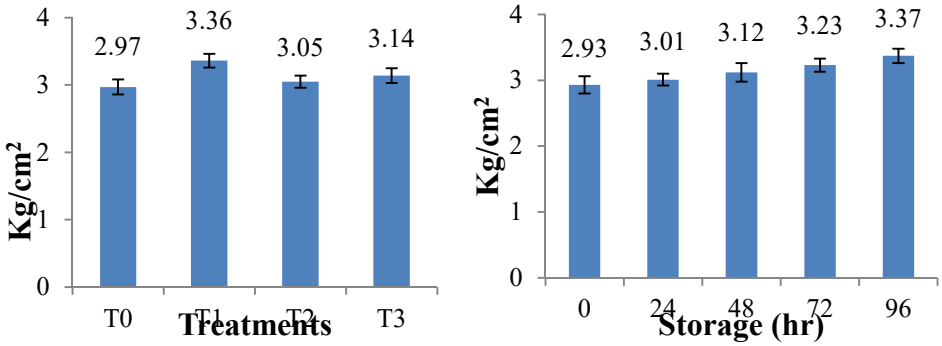
External Characteristics: Means for volume (Table 6) differed significantly from 6.95±0.24 to 8.01±0.39 in T_1 and T_0, respectively. However, storage imparted non-momentous decline in volume from 7.83±0.26 at initiation to 7.37±0.28 at the termination of the study. The maximum scores for crust color were assigned to T_2 (7.04±0.21) trailed by T_3 (6.85±0.23), T_0 (6.54±0.25) and T_1 (5.94±0.19). Likewise, storage also resulted decline in color scores *i.e.* 6.86±0.20 (0 hr), 6.78±0.27 (24 hr),

6.67±0.21 (48 hr), 6.38±0.32 (72 hr) and 6.29±0.25 (96 hr).



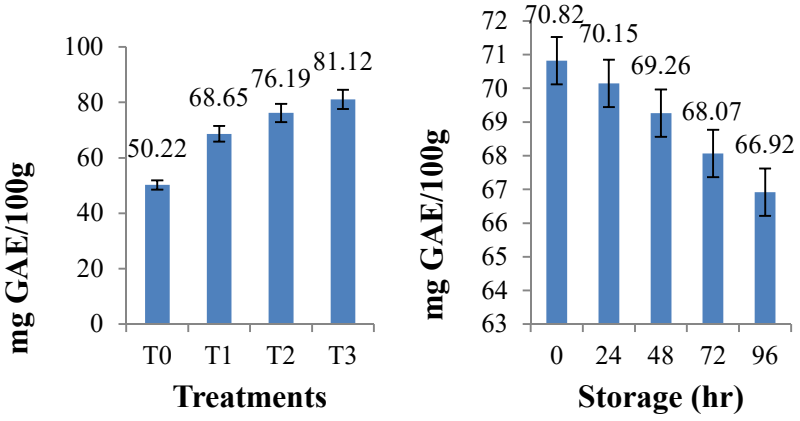
T_0= Control; T_1=Bread containing turmeric powder; T_2=Bread containing microencapsulated nutraceutical$_{CSE;}$ T_3=Bread containing microencapsulated nutraceutical$_{SFE}$

FIGURE 2. EFFECT OF TREATMENTS AND STORAGE ON VOLUME (CM^3) OF BREAD



T_0= Control; T_1=Bread containing turmeric powder; T_2=Bread containing microencapsulated nutraceutical$_{CSE;}$ T_3=Bread containing microencapsulated nutraceutical$_{SFE}$

FIGURE 3. EFFECT OF TREATMENTS AND STORAGE ON HARDNESS (kg/cm^2) OF BREAD



T_0= Control; T_1=Bread containing turmeric powder; T_2=Bread containing microencapsulated nutraceutical$_{CSE;}$ T_3=Bread containing microencapsulated nutraceutical$_{SFE}$

FIGURE 4. EFFECT OF TREATMENTS AND STORAGE ON TPC (mg GAE/100g) OF BREAD

Statistical analysis for bread symmetry revealed non-momentous differences during storage and treatments. The scores for this trait were 3.83±0.16, 3.54±0.12, 3.61±0.18 and 3.69±0.11 for T_0, T_1, T_2 and T_3, respectively. Means related to evenness of bake for bread explicated non-momentous change due to treatments. The

recorded scores for T_0, T_1, T_2 and T_3 were 1.94±0.05, 1.63±0.08, 1.71±0.06 and 1.92±0.07, respectively. On the other hand, storage from 0 to 96 hr resulted in scores variation from 2.03±0.09 to 1.58±0.04, respectively. The means for crust characteristic were 2.72±0.11 (T_0), 2.50±0.08 (T_1), 2.61±0.09 (T_2) and 2.97±0.10 (T_3) whereas, storage resulted in slight decline in rating from initiation to termination of the study *i.e.* 2.92±0.09 to 2.45±0.11.

Internal Characteristics: The scores for grain (Table 7) assigned to T_0, T_1, T_2 and T_3 were 12.23±0.56, 12.14±0.54, 12.32±0.38 and 12.25±0.52, correspondingly. Likewise, storage did not change this attribute from 0 to 96 hrs. It was observed that treatments and storage depicted significant effect on color of crumb, the respective scores varied from 8.18±0.26 to 7.26±0.35 (T_0), 8.35±0.40 to 7.85±0.36 (T_1), 8.24±0.29 to 7.82±0.25 (T_2) and 7.98±0.32 to 7.42±0.25 (T_3) during 0 to 96 hr.

Treatments have substantial effect on aroma and the maximum scores were reported in T_3 8.05±0.26 whilst, minimum in T_1 7.34±0.23. Likewise, T_3 (16.41±0.61) has shown the highest scores for taste that differed significantly for T_2 (16.08±0.52), T_0 (15.91±0.69) and T_1 (15.25±0.72). Finally, the scores for texture indicated significant variations among the treatments. The scores assigned to T_0, T_1,T_2 and T_3 were 12.33±0.46, 11.64±0.41, 12.45±0.55 and 13.02±0.52, respectively. Similarly, momentous declining trend was observed for aroma, taste and texture and at 0 hr recorded scores were 8.01±0.31, 16.27±0.53 and 12.87±0.45 that reduced to 7.22±0.33, 15.49±0.65 and 11.78±0.50, respectively at 96 hr of storage.

Table 6. Effect of treatments and storage on external characteristics of designer bread.

Treatments		Hours				
	Characteristics	**0**	**24**	**48**	**72**	**96**
	Volume	8.22±0.35	8.15±0.40	8.04±0.24	7.92±0.28	7.76±0.36
	Crust color	6.84±0.21	6.75±0.28	6.62±0.19	6.45±0.32	6.16±0.27
T_0	**Symmetry**	4.02±0.13	3.95±0.19	3.86±0.14	3.74±0.11	3.62±0.18
	Evenness of bake	2.15±0.09	2.06±0.07	1.95±0.09	1.82±0.05	1.70±0.08
	crust character	2.94±0.09	2.85±0.12	2.70±0.08	2.58±0.12	2.46±0.11
	Volume	7.18±0.21	7.06±0.32	6.98±0.25	6.85±0.38	6.72±0.29
	Crust color	6.36±0.19	6.28±0.23	6.15±0.29	5.92±0.17	5.02±0.21
T_1	**Symmetry**	3.75±0.17	3.68±0.15	3.54±0.09	3.42±0.13	3.30±0.14
	Evenness of bake	1.82±0.05	1.75±0.06	1.64±0.03	1.52±0.07	1.48±0.06
	crust character	2.72±0.11	2.64±0.08	2.52±0.09	2.38±0.11	2.26±0.10
	Volume	7.85±0.23	7.78±0.29	7.66±0.38	7.54±0.25	7.40±0.34
	Crust color	7.22±0.35	7.15±0.32	7.06±0.24	6.95±0.28	6.82±0.19
T_2	**Symmetry**	3.82±0.15	3.75±0.12	3.62±0.11	3.50±0.18	3.38±0.16
	Evenness of bake	1.96±0.07	1.84±0.09	1.72±0.04	1.58±0.06	1.45±0.05
	crust character	2.86±0.10	2.75±0.09	2.62±0.11	2.50±0.08	2.38±0.12
	Volume	8.10±0.26	8.02±0.34	7.88±0.22	7.75±0.18	7.62±0.25
	Crust color	7.02±0.23	6.96±0.30	6.88±0.18	6.76±0.31	6.65±0.29
T_3	**Symmetry**	3.96±0.20	3.84±0.17	3.70±0.11	3.58±0.13	3.42±0.12
	Evenness of bake	2.08±0.06	2.02±0.04	1.94±0.05	1.85±0.08	1.72±0.07
	crust character	3.18±0.14	3.10±0.12	2.98±0.09	2.86±0.08	2.74±0.10

Values are expressed as means ± standard deviation. Means within column and row with different letter are significantly different ($P< 0.05$); T_0= Control, T_1=Flour containing 3% turmeric powder, T_2=Flour containing 1% microencapsulated nutraceutical$_{CSE}$, T_3=Flour containing 0.5% microencapsulated nutraceutical$_{SFE}$

Table 7. Effect of treatments and storage on internal characteristics of designer bread.

Treatments		Hours				
	Characteristics	**0**	**24**	**48**	**72**	**96**
	Grain	12.88±0.59	12.64±0.42	12.18±0.61	11.86±0.38	11.60±0.46
	Crumb color	8.18±0.26	8.04±0.39	7.82±0.31	7.55±0.27	7.26±0.35
T_0	**Aroma**	8.15±0.31	8.02±0.23	7.94±0.27	7.66±0.38	7.45±0.26
	Taste	15.74±0.50	15.58±0.64	15.25±0.51	14.96±0.59	14.72±0.68
	Texture	13.06±0.42	12.74±0.59	12.35±0.40	11.98±0.48	11.52±0.53
T_1	**Grain**	12.72±0.45	12.58±0.38	12.14±0.55	11.76±0.58	11.52±0.46

	Crumb color	8.35±0.40	8.28±0.32	8.20±0.24	8.06±0.28	7.85±0.36
	Aroma	7.72±0.36	7.58±0.32	7.35±0.28	7.12±0.19	6.96±0.24
	Taste	16.24±0.48	16.12±0.53	15.96±0.67	15.75±0.51	15.52±0.76
	Texture	12.18±0.47	11.96±0.50	11.70±0.42	11.36±0.37	11.02±0.51
	Grain	12.64±0.53	12.52±0.60	12.34±0.34	12.18±0.45	11.96±0.39
	Crumb color	8.24±0.29	8.18±0.21	8.12±0.34	7.98±0.22	7.82±0.25
T_2	**Aroma**	7.85±0.35	7.76±0.22	7.64±0.33	7.28±0.27	6.86±0.18
	Taste	16.34±0.49	16.25±0.53	16.12±0.61	15.96±0.75	15.75±0.67
	Texture	12.85±0.46	12.68±0.57	12.45±0.42	12.22±0.61	12.08±0.55
	Grain	12.45±0.37	12.32±0.43	12.18±0.46	11.96±0.39	11.80±0.41
	Crumb color	7.98±0.32	7.86±0.28	7.72±0.30	7.58±0.36	7.42±0.25
T_3	**Aroma**	8.35±0.35	8.26±0.32	8.12±0.24	7.88±0.28	7.64±0.21
	Taste	16.76±0.52	16.62±0.68	16.45±0.57	16.24±0.71	15.98±0.56
	Texture	13.42±0.48	13.25±0.41	13.10±0.53	12.85±0.58	12.55±0.41

Values are expressed as means ± standard deviation. Means within column and rows with different letter are significantly different ($P< 0.05$); T_0= Control, T_1=Flour containing 3% turmeric powder, T_2=Flour containing 1% microencapsulated nutraceutical$_{CSE,}$ T_3=Flour containing 0.5% microencapsulated nutraceutical$_{SFE}$

Natural dietary agents like spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated therapeutic ability. Currently, Karić *et al.* (2020) evaluated the effect of turmeric, olives and sunflower seeds incorporation upto 10% on nutritional and sensorial parameters of bread. Accordingly, the addition of spices in baked goods is nutritionally a very reasonable substitute to white bread. The addition of spices to bread not only improves its organoleptic parameters but also increased nutritional value. Previously, Muhammad *et al.* (2014) evaluated variations in overall acceptability of bread incorporated with turmeric powder (3 & 4%). They noted maximum liking scores for bread supplemented with 3% turmeric during storage period of 96 hours owing to better color and flavor. In the same way, supplementation of various levels of cinnamon (0, 1, 2, 3 and 4%) on organoleptic properties of breads showed significant difference in color, appearance and overall acceptability with the highest scores (8.44) for bread containing 2% cinnamon powder in contrast to control (7.90). Earlier, Lim *et al.* (2011) assessed liking scores for breads supplemented at different ratios (2, 4, 6 and 8%) of turmeric. The bread crumb color due to turmeric powder addition at 8%, had the lowest acceptability as it interfere with natural color of bread. The maximum hedonic response was noted at 2% substitution level having optimum color, flavor, aroma and texture. Increase in turmeric content also enhanced phenolics and volatiles that negatively impact taste of bread.

Conclusions: Globally, escalating trend of junk food and prevailing poor dietary practices lead towards various life threatening maladies. The current issue motivates health care professionals to probe food based strategies that are cost effective and cheap. In this respect, designer foods are attaining public interest owing to a great number of therapeutic applications. Turmeric (*Curcuma longa*)holds an array of phytonutrients; curcuminoids accountable for its antioxidant potential. In limelight of product development, designer bread was prepared using turmeric powder, microencapsulated conventional & supercritical fluid extract. Physicochemical attributes of bread samples containing supercritical microencapsulated curcumin showed better results as compared to turmeric powder. Conclusively, microencapsulation is a promising tool to protect bioactive moieties from adverse effect of heat, light and air thus enhancing sensory attributes and shelf life of resultant prototypes.

PRACTICAL APPLICATIONS

The bioactive entities of spices are worth considering regarding their antioxidant power. Moreover, it also enhances acceptability of product by upgrading their quality parameters. Turmeric constitutes plethora of over hundred secondary metabolites; curcumin is one of them. Unfortunately, it exhibits poor stability and systemic bioavailability. To counteract this issue, microencapsulation was employed as a promising tool to magnify both functional and therapeutic potential of curcumin in designer foods.

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