SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
http://dergipark.gov.tr/akademik-gida

Akademik Gıda 18(2) (2020) 105-115, DOI: 10.24323/akademik-gida.758806

Research Paper / Araştırma Makalesi

Effect of Storage Conditions on Stability of Low Glycemic Index Noodles with Enzymatically Modified Ingredients

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Received (Geliş Tarihi): 05.11.2019, Accepted (Kabul Tarihi): 12.04.2020
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ABSTRACT

Stability of a food product mainly depends on its ingredients. Stable food products have a huge consumer market. In this study, noodles were prepared using enzymatically modified ingredients. The shelf stability of noodles was determined under two different conditions, ambient (27°C, 65% RH) and accelerated (37°C, 92% RH). Samples were withdrawn at particular intervals and analysed for their physico-chemical, *in-vitro* and *in-vivo* properties. The properties of stored products were compared with those of initial products. Noodles with enzymatically modified ingredients showed reduced glycemic index (GI) compared to their native forms. Noodles with enzymatically modified ingredients can be stored up to 60 days at an ambient condition without any negative effect on their quality. As the noodles with enzymatically modified ingredient showed promising results with reference to their quality characteristics, it can be beneficial in maintaining the health of the individuals with diabetes mellitus, if supported by *in-vivo* studies.

Keywords: Low glycemic index, Noodles, Shelf-life, Modified ingredients

Saklama Koşullarının Enzimatik Olarak Değiştirilmiş Düşük Glisemik İndeksli Eriştelerin Stabilitesi Üzerine Etkisi

ÖZ

Bir ürünün stabilitesi esas olarak preparatta kullanılan bileşenlere bağlıdır. Kararlı ürünler büyük tüketici pazarına sahiptir. Bu çalışmada erişte enzimatik olarak modifiye edilmiş bileşenler kullanılarak hazırlanmıştır. Hazırlanan eriştele, normal (27°C, 65% RH) ve hızlandırılmış (37°C, 92% RH) olmak üzere iki farklı koşul altında raf ömrü stabilitesi açısından incelenmiştir. Örnekler belirli aralıklarla alınmış ve fiziko-kimyasal, *in vitro* ve *in vivo* özellikleri için analiz edilmiştir. Depolanan ürünlerin bulguları başlangıçtaki ürünlerle karşılaştırılmıştır. Enzimatik olarak modifiye edilmiş bileşenler içeren eriştele, doğal formlarına kıyasla azalmış glisemik indeks (GI) göstermiştir. Enzimatik olarak modifiye edilmiş erişte numunelerin, kalitesini etkilemeden ortam koşullarında 60 gün boyunca saklanabildiği bulunmuştur. Enzimatik olarak modifiye edilmiş bileşenli eriştele, kalite özelliklerinde umut verici sonuçlar gösterdiğinden, *in vivo* çalışmalarla desteklendiğinde diyabetli (diyabetes mellitustan) bireylerin sağlığının korunmasında faydalı olabilir.

Anahtar Kelimeler: Düşük glisemik indeks, Erişte, Raf ömrü, Modifiye bileşenler

INTRODUCTION

Wheat is one of the major grains used as staple food across the World. Starch is the major constituent of wheat endosperm and this is the important factor deciding the final product quality. Starch from different grains were used as a raw material for most of the food industries, which includes noodles, pasta, sauce, ketchup and so on in its native and modified forms [1]. It is evident that different processing techniques alters starch properties differently. Modification using chemicals and enzymes being more prevalent among all the process. Starch modification using chemicals is well established. Whereas, relatively few works have been carried out on the enzymatic modification of starch. Modification of starch through enzymes affects its physico-chemical properties. This technique can be utilized for the modification of flour samples, as the earlier studies on this aspect are absent. This approach of modification of ingredients may be beneficial for whole foods in the food industry. Few studies emphasised on the formation of resistant starches during enzyme modification using pullulanase by debranching the banana starch [2]. A carbohydrate-active enzyme known as Amylomaltase is involved in transferring glucan units from one unit to another. This was used to modify potato starch, resulted in disappearing of amylose fractions and formation of amylopectin fractions in starch. These starch samples showed a thermo reversible gelling property, resembling the property of gelatin [3]. An investigation on starch by partial α-amylase treatment to develop low glycemic index starch was carried out earlier [4], as a result, molecular size of amylose and amylopectin reduced rapidly and also reduced postprandial glycemic response in rats was observed. On the basis of all the earlier studies, work was planned to modify the flour samples to make the low glycemic index flours. The objectives of the study were to develop enzymatically modified common flours used in noodle processing and formulation of low glycemic index noodles with modified ingredients. Stability of noodles and its validation through physico-chemical and nutritional analysis.

MATERIALS AND METHODS

Procurement and Pre-Processing of Raw Materials

Ingredients such as *Triticum aestivum* and *Triticum dicoccum* were procured from local market. These raw materials were cleaned to remove the foreign particles and were ground in local chakki mill to suitable particle size flours. Flours obtained were sieved (Mesh size-100µm) and stored in air-tight packages until the end of the study. Enzymes such as pepsin, invertase, amyloglucosidase, α-amylase inhibitor (from *Triticum aestivum*, Type III, lyophilized powder, 5-100 inhibitor units/mg protein using porcine pancreatic α-amylase) and pancreatic α-amylase were procured from Sigma Chemicals, USA. Branching enzyme (Enzyme Commission No. 2.4.1.18; Source organism: *Bacillus subtilis*, Biological function: Transfer a segment of a (1->4)- α-d-glucan chain to a primary hydroxyl group in a similar glucan chain) was procured from Prozomix, UK. All other chemicals used for the study were of analytical grade unless and otherwise mentioned.

Modification of Ingredients and Noodle Formulation

Modification of ingredients was carried out according to preliminary studies on modified ingredients [5, 6]. In brief, noodle dough was prepared with the addition of α-amylase inhibitor and the pH of the dough was maintained at 6±0.5. For the addition of branching enzyme, flour suspension was treated with β-amylase (0.64% starch basis) and branching enzyme (0.6% starch basis) and the obtained modified flour mixture was freeze dried and used in noodle preparation.

Noodle Formulation and Optimization

On the basis of the preliminary analysis carried out on noodles with modified ingredients, three types of noodles (including control) were taken for the shelf-life study and analysed for different quality parameters at selected withdrawal period. Noodles were prepared using Lab Scale Noodle making machine (Imperia Restaurant-RM 220, Italy) with the capacity of 1 kg per batch, according to previously followed method [7]. In brief, modified flours were mixed with lukewarm water to form firm dough. The obtained dough was rested for 10-15min. Later dough was sheeted by passing between two rollers of the noodle making machine to reduce the thickness to 5 mm and cut to appropriate length in the noodle cutter. These noodles were dried at 55-60°C for 1-1.5 h. Later these dried noodles were used for shelf life studies.

Shelf-Life Study

Prepared noodles were packed in HDPE bags and stored under two different conditions namely, ambient (27°C, 65% RH) and accelerated (37°C, 92% RH) conditions. Samples stored at these conditions were analysed for its physico-chemical, sensory and nutritional characteristics to apprehend the influence of storage on the samples. Samples (placed at both ambient and accelerated) were withdrawn at 30 days interval to perform the above mentioned analyses. Details of the withdrawal period and selected analysis parameters were given in Table 1.

Shelf Stability

Noodles were withdrawn according to the schedule planned and were analysed for the planned analytical parameters soon after withdrawal. Analysis was completed within 3-4 days of withdrawal, in the order accelerated samples and ambient samples.

Cooking Quality of Noodles

Cooking quality characteristics of noodles were analysed for each withdrawal of all the samples to determine the influence of storage on the cooking behaviour and cooking loss. Noodles were subjected to cooking quality evaluation adopting the standard method from AACC (66-50) [8]. Noodles were cut into approximately 5cm in length. 25 g of the sample is weighed and placed in 250 mL boiling water. Noodles were examined at 30 sec interval to check the doneness by placing between two petri plates. Once the white core portion disappears indicates complete cooking of noodles. Time was recorded and noodles were strained. The obtained gruel was taken for cooking loss analysis. Cooked weight of the noodles was recorded.

Table 1. Details of the withdrawal period and analysis parameters during shelf-life study for all the samples

Withdrawal period	Analysis parameters
0 day (Initial)	▪ Chemical composition ▪ Cooking quality ▪ Colour and Texture analysis ▪ Sensory analysis ▪ *In vitro* analysis of starch digestibility, starch fractions ▪ *In vitro* estimated glycemic index ▪ *In vivo* glycemic index analysis
30 days	▪ Cooking quality ▪ Colour and Texture analysis ▪ Sensory analysis ▪ *In vitro* analysis of starch digestibility, starch fractions ▪ *In vitro* estimated glycemic index ▪ *In vivo* glycemic index analysis (Accelerated)
60 days	▪ Cooking quality ▪ Colour and Texture analysis ▪ Sensory analysis ▪ *In vitro* analysis of starch digestibility, starch fractions ▪ *In vitro* estimated glycemic index ▪ *In vivo* glycemic index analysis (Ambient)

Instrumental Colour Measurement of Noodles

Noodles were analysed for colour parameters using Lab scan-XE (Reston, USA). This instrument was equipped with D-65 illuminant with a 2° angle view and with a 2 mm width slit adopting the method used by Bharath Kumar and Prabhasankar [7]. For the analysis of PCI, L* (a lightness indicator) and b* (a yellowness indicator) were taken to get the standard colour index of the pasta. When the pasta incorporated with other ingredients (modified or unmodified) the Modified Pasta Colour Index (PCI_m) and Whiteness Index (WI) were calculated using the values L*, a* and b* as explained [9, 10] using the below mentioned formulae:

For pasta colour index: $$\mathrm{PCI} = \sqrt{\mathrm{L}^{*2} + \mathrm{b}^{*2}}$$

For modified pasta colour index: $$\mathrm{PCIm} = \sqrt{\mathrm{L}^{*2} + \mathrm{b}^{*2} + \mathrm{a}^{*2}}$$

For whiteness Index: $$\mathrm{WI} = 100 - \sqrt{(100 - \mathrm{L}^{*})^2 + a^{*2} + \mathrm{b}^{*2}}$$

Instrumental Firmness of Noodles

Firmness of the noodles (both fresh and dried) was measured using the Texture analyser TA-XDi (Stable Micro Systems, UK) equipped with Warner Bratzler blade for shear [7]. The data thus obtained were analyzed statistically using Duncan's Multiple Range Test (DMRT) [11].

Sensory Analysis

Prepared noodles were evaluated for its quality characteristics and for its acceptance. Panelists who regularly participate in evaluating the noodles were selected (Male and Female; 10-15). Quantitative descriptive analysis (QDA) method to analyze the product was used for the study [12]. Sensory attributes used for the analysis were in terms of;

Cream colour – Appearance of noodle surface colour light to dark

Distinct strands – Strand quality sticky/individual/non-sticky
Firmness – Fragile/brittle/hard
Chewiness – Rubbery/long lasting
Cooked cereal – Typical cooked cereal aroma
Starchy – Mouthfeel very starchy/powdery after taste
Sweetness – Pleasant taste of typical cereal sweetness. [13]

In Vitro Starch Digestibility

Starch Profile Analysis

Analysis of *in vitro* rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and total starch (TS) were analysed and also free glucose (FG) and total glucose (TS) were determined using the method of Englyst [14]. Conversion factor used was 0.9 to convert glucose to starch. Each sample was analysed in triplicates. Schematic representation of analytical method is given in Fig.1.

RDS, SDS, RS and TS were calculated using the below mentioned equations;

$RDS = (G_{20}\text{-}FG) \times 0.9$

$SDS = (G_{120}\text{-}G_{20}) \times 0.9$

$RS = TS\text{-}(RDS + SDS)$

$TS = (TG\text{-}FG) \times 0.9$

Where, G20 is the value of glucose hydrolysed during the first 20 min of *in vitro* digestion, G120 is the value of glucose hydrolysed after 120 min of *in vitro* digestion.

Estimated Glycemic Index (EGI)

In vitro glycemic index was analysed using the method of Englyst et al*.* [14]. 50 mg of sample was cooked in 5 ml of distilled water for 3 to 5 min. Ten mL of hydrochloric acid and potassium chloride (HCl-KCl) buffer (pH 1.5) was added to the cooked sample with 0.2 mL of pepsin solution (1 g pepsin in 10 mL HCl-KCl buffer). This was incubated at 40°C for 1 h at the shaking water bath. After incubation the mixture was made up to 25 mL with Tris-Maleate buffer (pH 6.5) with the enzyme solution α-amylase (5 µL - 2.6 U). The reaction mixture was incubated at 37°C in a shaking water bath maintaining the constant shaking for the continuous reaction. During incubation an aliquot of 1 mL in duplicates were withdrawn from the mixture at 30, 60, 90, 120, 150 and 180 min time intervals into different tubes. These tubes were immediately kept in boiling water bath (100°C) for 5 min to inactivate the enzyme activity and stored in refrigerator until the end of the incubation time (180 min). At 180 min the tube was removed from the shaking water bath and the enzyme was inactivated. Later all the tubes from the refrigerator were removed and equilibrated to 60°C at the water bath set to the same temperature. To the tubes 3 mL of sodium-acetate buffer (0.4 M, pH 4.7) was added with 60 µL of amyloglucosidase enzyme solution. These tubes were incubated at 60°C for 45 min at shaking water bath. Later volume of the reaction mixture was adjusted to 10 mL with distilled water. An aliquot of 0.5 ml was incubated with GOD-POD reagent to estimate the glucose content in each withdrawals as explained earlier study [12].

The values obtained for the starch hydrolysis were plotted against the time interval. The hydrolysis index (HI) was calculated considering the area under the hydrolysis curve expressed as a percentage ratio of area under the curve for the test food and standard food (white bread).

$$HI = \frac{\text{Area under the hydrolysis curve for the test food}}{\text{Area under the hydrolysis curve for the standard food}} \times 100$$

Estimated glycemic index (EGI) was calculated using the values obtained for HI and substituting in the following equation stated by Goni et al. [15]:

$$EGI = 39.71 + (0.549 \times HI)$$

Glycemic Index Analysis of Noodles Involving Healthy Volunteers

Selection of Participants for the Study

For the purpose of GI analysis, twenty healthy subjects (10 Men, 10 Women) were selected, considering their previous health history. They were non-smokers, not using any medicines, not on any therapeutic diet, no recent weight loss or gain (>2kg) over the previous three months, had regular eating habits (morning breakfast, afternoon lunch and night dinner), fasting blood glucose levels were between 4-5.9 mmol/L. Prior to study Institutional Human Ethical clearance was taken from the University of Mysore, Mysore. Consent was obtained from all the subjects before the study, by explaining the importance of the study on their health. Characteristics of the participated subjects were given in Table 2. For each withdrawal cycle of stored noodles, same participants were chosen for the test throughout the study to eliminate any variations in the results.

Test Meal

As white bread GI considered to be 100, this was served as a reference food in identifying GI of the noodles. *Durum* noodles (control) and other two low GI noodles were prepared freshly on the day of test, next day of withdrawal from ambient and accelerated conditions. To improve and enhance the noodle palatability known amount of salt and non-starch spice mix were added during the preparation of noodles. There was no major diet modification except for the exclusion of some high carbohydrate and fat foods during the study. Experiment was conducted on four non-successive days with white bread, *durum* noodles (control) and other two low GI

noodles respectively. Each portion of the diet consisting of 50 g carbohydrates equivalent. Finger prick blood glucose analysis was carried out, according to earlier study [12]. In brief; the subjects were given food after a 12 h fasting and blood glucose levels before food (fasting) and after food (15, 30, 45, 60, 90 and 120 min) were checked using Accu-Chek Active (Roche Diagnostics India Pvt. Ltd.). Subjects were allowed to drink minimum quantity of water during the study. To identify the GI of the noodles results were compared with 50 g white bread values and calculated using the equations below:

$$\mathrm{GI} = \frac{\text{Incremental area under the blood glucose response curve of a test food}}{\text{Incremental area under the blood glucose response curve of a standard food}} \mathrm{X}\ 100$$

Table 2. Characteristics of participated healthy subjects

Subjects	Sex*	Age (Years)	Body Mass Index (Kg/m^2)	Fasting blood glucose (mmol/L)
1	M	25	21.5	5.1
2	M	25	23.4	4.2
3	M	30	22.2	4.9
4	M	24	22.2	4.1
5	M	28	22.5	4.5
6	M	21	24.0	5.1
7	M	26	21.2	4.3
8	M	24	23.2	5.1
9	M	28	21.2	5.2
10	M	24	24.1	4.9
11	F	23	23.8	4.1
12	F	21	21.1	4.6
13	F	29	23.9	4.5
14	F	31	24.1	5.5
15	F	24	20.9	5.1
16	F	23	24.0	4.2
17	F	24	23.8	4.9
18	F	21	21.8	5.0
19	F	23	22.2	4.1
20	F	22	22.8	4.8

*: M-Male, F-Female

Statistical Analysis

The results are expressed as mean±SD unless otherwise stated. Analysis of variance (ANOVA) was used to determine the statistical significance of difference between the samples. To simplify the expression of the results, only significant differences were systematically recorded in the text and tables incorporating the method used by Winer [16] and Duncan [11].

RESULTS AND DISCUSSION

Analysis of Initial Sample (0 day)

Cooking quality of noodles analysed at 0 day (Table 3) were within the acceptable limits, with highest cooking time (9.23 min) in case of DI (a) noodles as the structure became firm due to modification process. Cooking loss was within the acceptable limit (8%), with highest in DI (a) 4.9%, which can be correlated with highest cooking time. Instrumental colour analysis reported highest L* value of 72.14 for TA (b), as it contained *T. aestivum* flour, followed by control (68.84) and DI (a) (56.22). Apart from L* value PCI_m and WI also reported highest for TA (b), which is 71.9 and 69.61 respectively. Firmness values were 1.47, 3.25 and 1.32 N/mm for control, TA (b) and DI (a) samples respectively ($p \leq 0.05$). *In vitro* analyses of 0 day samples indicated highest IVSD for control sample (74.3%) followed by TA (b) and DI (a) with 55.5% and 50.1% respectively. Lowest RDS was observed in TA (b) with 34% and highest in case of DI (a) with 60% compared to control (77%). Simultaneously SDS was higher in TA (b) compared to DI (a) and control samples. EGI of TA (b) was lowest (50.2) among all other samples, which indicates the effect of branching enzyme on the product. Glycemic index results revealed that samples DI (a) and TA (b) falls under low GI category with GI of 50 and 54 respectively. Sensory analysis of the modified noodles indicated that with modification there was a significant change in firmness and chewiness of the noodles. The results revealed that modification of ingredients improved some of the quality attributes of noodles and are acceptable even after the modification. All the samples were acceptable after modification, with overall quality of all the samples was above 8.0 on 15 cm QDA scale.

Influence of Storage on Cooking Quality of Noodles

Samples prepared with enzymatically modified ingredients stored at ambient and accelerated condition were analysed for the cooking quality changes during

storage. Results were given in Table 3. Samples stored at ambient condition (Table 3a) showed decrease in cooking time for TA (b) and DI (a) from 7.2 min and 9.2 min to 6.2 min and 7.1 min respectively. The reduction of cooking time may be due to the enzyme hydrolysis process during modification. Along with the cooking time reduction, there was reduction in cooked weight of the sample from 70.2g and 62.4g to 67.4g and 60.2g. This may be due to less water uptake by the sample due to polymerization process occurred by the use of branching enzyme to convert amylose into amylopectin [17]. Cooking loss of the samples reduced in at 30 days analysis for the sample TA (b), as the polymeric and crystalline structure avoids the leaching out of solid into the gruel during cooking. Due to storage the sample structure still became tougher to disintegrate. Cooking loss reduced from 3.2% (0 day) to 2.9% (30 days). Whereas, for the sample DI (a) cooking loss increased from 4.6% to 4.9%. At 60 days analysis cooking time increased, but the cooked weight decreased with higher solid leach out during cooking. This may be due to the disintegration of the sample during cooking, subjected to elevated temperature and boiling. Cooking loss increased to 4% (TA-b) and 5.1% (DI-a) samples. The samples started disintegrating during cooking and were not analysed further.

Samples stored at accelerated condition (Table 3b) were analysed for the cooking quality characteristics, results indicated that at the end of 30 days samples showed reduced cooking time and cooked weight. Due to the high humidity and elevated temperature samples lost its integrity and while cooking strands got disintegrated to some extent. Due to this reason solid leach out increased significantly, this was above 5% for both of the samples. Because of the quality loss the samples were not continued further for the storage study. From this it can be inferred that noodles with enzymatically modified ingredients can be stored at ambient condition for 60 days and accelerated condition for 30 days.

Table 3a. Influence of Ambient condition on quality characteristics of the noodles*

		0 Day			Ambient 30 Days			Ambient 60 Days		
		CON	TA (b)	DI (a)	CON	TA (b)	DI (a)	CON	TA (b)	DI (a)
Cooking time (min)		9.02^{b}±0.15	7.25^{q}±0.10	9.23^{y}±0.18	7.39^{a}±0.19	6.21^{p}±0.15	7.22^{x}±0.14	7.43^{a}±0.12	6.24^{p}±0.24	7.51^{x}±0.34
Cooking loss (%)		3.9^{a}±0.7	3.2^{q}±0.3	4.6^{x}±0.1	3.9^{a}±0.7	2.9^{p}±0.2	4.9^{xy}±0.3	3.9^{a}±1.1	4.0^{r}±0.2	5.1^{xy}±0.4
Colour	L*	68.84^{b}±0.21	72.14^{p}±0.16	56.22^{x}±0.24	65.48^{a}±0.11	71.47^{p}±0.22	57.56^{x}±0.22	65.48^{a}±0.28	71.22^{p}±0.19	59.24^{y}±0.24
	a*	0.97^{a}±0.11	0.12^{p}±0.08	6.97^{x}±0.89	0.95^{a}±0.12	0.12^{p}±0.18	6.57^{x}±0.59	0.91^{a}±0.24	0.11^{p}±0.27	6.44^{x}±0.27
	b*	10.22^{ab}±0.98	9.75^{q}±0.14	18.14^{y}±0.21	10.01^{ab}±0.05	9.57^{q}±0.10	17.95^{y}±0.28	9.90^{a}±0.14	9.10^{p}±0.22	16.99^{x}±0.24
PCI_m		69.59^{b}±1.10	71.90^{p}±0.09	60.01^{x}±0.12	66.24^{a}±0.15	72.11^{p}±0.24	60.65^{x}±1.19	66.22^{a}±0.48	71.80^{p}±0.48	61.96^{xy}±1.11
WI		67.25^{a}±1.24	69.61^{p}±0.98	53.06^{x}±0.88	64.05^{a}±0.85	69.91^{p}±0.99	53.45^{x}±1.18	64.08^{a}±1.17	69.82^{p}±1.11	55.37^{y}±1.98
Firmness (N/mm)		1.47^{a}±0.10	3.25^{p}±0.25	1.32^{x}±0.57	4.09^{c}±0.68	4.01^{q}±0.15	3.25^{z}±0.47	3.31^{b}±0.22	3.47^{p}±0.18	2.11^{y}±0.19

*: PCI was calculated for CON sample. CON-Control noodles with 100% *T. durum*, TA(b)-*T. aestivum* flour (wheat flour) modified using branching enzyme, DI(a)-*T. dicoccum* flour modified using α-amylase inhibitor. Mean values in the same row with different alphabets differ significantly (p≤0.05) (a,b,c for CON, p,q,r for TA(b) and x,y,z for DI(a))

Table 3b. Influence of Accelerated condition on quality characteristics of the noodles*

		0 Day			Accelerated 30 Days		
		CON	TA (b)	DI (a)	CON	TA (b)	DI (a)
Cooking time (min)		9.02^{b}±0.15	7.25^{q}±0.10	9.23^{y}±0.18	7.33^{a}±0.09	6.21^{p}±0.19	8.22^{x}±0.15
Cooking loss (%)		3.9^{b}±0.7	3.2^{p}±0.3	4.6^{x}±0.1	3.3^{a}±0.1	5.9^{q}±0.3	5.2^{y}±0.2
Colour	L*	68.84^{b}±0.21	72.14^{p}±0.16	56.22^{y}±0.24	61.70^{a}±0.10	71.11^{p}±0.29	53.25^{x}±0.25
	a*	0.97^{a}±0.11	0.12^{p}±0.08	6.97^{x}±0.89	0.88^{a}±0.11	0.10^{p}±0.08	7.09^{y}±0.21
	b*	10.22^{ab}±0.98	9.75^{q}±0.14	18.14^{y}±0.21	9.50^{a}±0.14	9.10^{p}±0.17	15.22^{x}±0.09
PCI_m		69.59^{b}±1.10	71.90^{p}±0.09	60.01^{y}±0.12	62.43^{a}±0.98	71.69^{p}±0.17	55.83^{x}±0.14
WI		67.25^{b}±1.24	69.61^{p}±0.98	53.06^{xy}±0.88	60.53^{a}±1.89	69.71^{p}±1.18	50.33^{x}±1.99
Firmness (N/mm)		1.47^{a}±0.10	3.25^{p}±0.25	1.32^{x}±0.57	3.30^{b}±0.09	3.59^{p}±0.98	2.29^{y}±0.29

*: PCI was calculated for CON sample. CON-Control noodles with 100% T. durum, TA(b)-*T. aestivum* flour (wheat flour) modified using branching enzyme, DI(a)-*T. dicoccum* flour modified using α-amylase inhibitor. Mean values in the same row with different alphabets differ significantly (p≤0.05) (a,b,c for CON, p,q,r for TA(b) and x,y,z for DI(a))

Influence of Storage on Colour and Firmness of Noodles

Samples stored at ambient condition did not show any significant change in the colour values (L*, a* and b*). There was slight reduction in L* value from 72.14 to 71.47 (30 days), to 71.22 (60 days) for TA (b). In case of DI (a) sample, which already has some brown pigments, its L* value increased from 56.22 to 57.56 (30 days), to 59.24 (60 days) (Table 3a). This may be due to the surface reaction of moisture uptake leading to make the surface of the noodles dull in case of TA (b) and in case of DI (a) sample surface dark pigments disappears and makes the surface look brighter. In case of a* value there was significant changes in TA (b) samples and values were almost similar in case of DI (a) samples. PCI_m of samples stored at ambient condition changed from 72.11 to 71.80 and from 60.65 to 61.96 for TA (b)

and DI (a) samples respectively. Same trend was followed in case of whiteness index. Samples stored at accelerated condition showed reduced L* value for both the samples from 72.14 to 71.11 (TA-b) and from 56.22 to 53.25 (DI-a). This reduction may be due to loss of surface moisture content in the stored noodles [18]. a* and b* values of the samples indicated that with storage there was significant reduction in case of DI (a) sample and significant increase in case of TA (b) samples. Whiteness index reduced significantly to 50.33 in case of DI (a).

Samples stored under ambient condition (Table 3b) were stable till 60 days of storage. Firmness values were significantly increased during storage of the samples. TA (b) sample showed increase from 3.2N/mm to 4.0N/mm (30 days), to 3.4N/mm (60 days). This may be due to the increased strength by the formation of amylopectin in the sample. In case of DI (b) sample firmness values increased from 1.3N/mm to 3.2N/mm (30 days), to 2.1N/mm (60 days). Samples stored at accelerated condition also showed slight increase in the firmness values of the noodles. Noodle firmness increased from 3.2N/mm to 3.5N/mm (TA-b) and from 1.3N/mm to 2.2N/mm (DI-a). Here also increase in the firmness can be attributed to the loss of moisture during storage of the sample. This directly affects the moisture uptake during cooking by that affecting the firmness of the noodles.

Influence of Storage on *In Vitro* Starch Digestibility and Starch Profile of Noodles

In vitro starch digestibility for the samples stored at ambient and accelerated condition were represented graphically in Fig.1. Results of samples stored under ambient condition revealed that IVSD did not change till 30 days of storage. At 60 days analysis IVSD increased significantly from 55.5% to 56.2% and 50.1% to 52.1% in TA (b) and DI (a) samples respectively. This may be due to the compact structure formed in TA (b) was unable to hydrolyse in the beginning stage. But as the storage period proceeds the compactness of the sample is lost the hydrolysis was occurred by increasing the IVSD. In the case of sample DI (a), as the time proceeds the inhibitory activity of the enzyme retards, the hydrolysis enzyme starts its reaction to increase the release of starch from the matrix. In case of samples stored at accelerated condition, the IVSD did not change till the end of 30 days. As further the analysis was not carried out due to noodle quality deterioration.

Starch profile analysis results indicated that (Fig.1) samples stored under ambient condition, here an interesting result was obtained as the RDS of the sample (TA-b) reduced at 30 days analysis from 34.3% to 30.2%, which was an important factor to make the product low GI. Eventually SDS content increased from 28.4% to 30.2% and RS content also increased from 3.2% to 3.8%. Later during 60 days analysis RDS increased and SDS reduced for the sample. In case of DI (a) sample RDS increased from 60.7% to 61.5% at the end of 30 days. At 60 days RDS increased to 62.5% and SDS reduced to 27.2%. Resistant starch content of the samples increased in both the samples from 3.2% to 3.8% (TA-b) and 2.7% to 3.2% (DI-a). This is also a positive indicator to make the noodles health beneficial.

Samples stored at accelerated condition (Fig. 1) showed reduced RDS from 34.3% to 33.2% for TA (b) sample and from 60.7% to 59.2% for DI (a) sample. SDS content of the sample also showed increasing trend with 28.4% and 26.7% at 0 day and 29.2% and 27.2% at 30 days analysis for TA (b) and DI (a) samples respectively. Resistant starch content of the sample increased in case of TA (b) from 3.2% to 3.8% and slight decrease was reported in DI (a) sample from 2.7% to 2.2%. Further analysis was terminated due to sample deterioration.

Influence of Storage on Estimated Glycemic Index and Glycemic Index of Noodles

Results of the study (Fig.1) revealed that EGI value of the noodle samples stored at ambient condition increased significantly at 30 days of storage. EGI increase was from 50.2 to 52.9 (TA-b) and from 51.5 to 53.2 (DI-a) samples. Further increase was observed at 60 days analysis to 53.2 (TA-b) and 54.9 (DI-a). This may be due to enzyme inactivity and also dissolution of the polymeric structure during storage process. In case of accelerated samples EGI value increased to 52 (TA-b) and 53.5 (DI-a) at 30 days analysis. This may be due to the exposure to the high humidity conditions and elevated temperature during storage condition. Further analysis of the samples stored at accelerated condition was not carried out due to the increased disintegration of the sample.

To understand the low glycemic effect of the modified noodles on storage, glycemic index involving volunteers was studied. Results revealed that (Fig.2), on storage at ambient condition for 60 days there was no significant increase in GI for Control and DI(a) samples with marginal increase of GI. In case of TA(b), GI increased from 54.1 to 57.2, even then it is acceptable as the GI is still with in medium GI range. Noodles stored at accelerated condition for 30 days showed increase in GI indicating the quality deterioration of the product. GI of the Control, TA(b) and DI(a) increased from 80.4, 54.1 and 50.4 to 89.6, 65.1 and 62 respectively. Results of this can be correlated with cooking properties of the noodles, where at 30 days accelerated condition product quality deteriorated and was unacceptable for further analysis.

Influence of Storage on Sensory Properties of Noodles with Enzymatically Modified Ingredients

Sensory analysis for the samples stored at ambient condition was carried out. Results were given in Fig. 3. Results indicated that on storage surface colour characteristics reduced drastically in both the samples.

Strands were distinct and no difference in firmness was observed for all the samples at 30 days. At 60 days analysis strands were disintegrated and making the sample less acceptable by the panellists. Overall quality score was significantly reduced from 11.1 to 7.5 for TA (b) and from 9.9 to 7.2 for DI (a) on a 15 cm QDA scale. As the overall quality score was below and around 7.5 the analysis was terminated at the end of 60 days of storage. Samples stored at accelerated condition showed less surface colour and completely disintegrated noodle strands and unfit for further analysis of the sample. The overall quality score for the samples reduced drastically from 11.1 to 7.0 (TA-b) and from 9.9 to 6.5 (DI-a). These results made the samples not analyse further. From this it can be confirmed that enzymatically modified samples can be stored at ambient condition for 60 days and accelerated condition for less than 30 days.

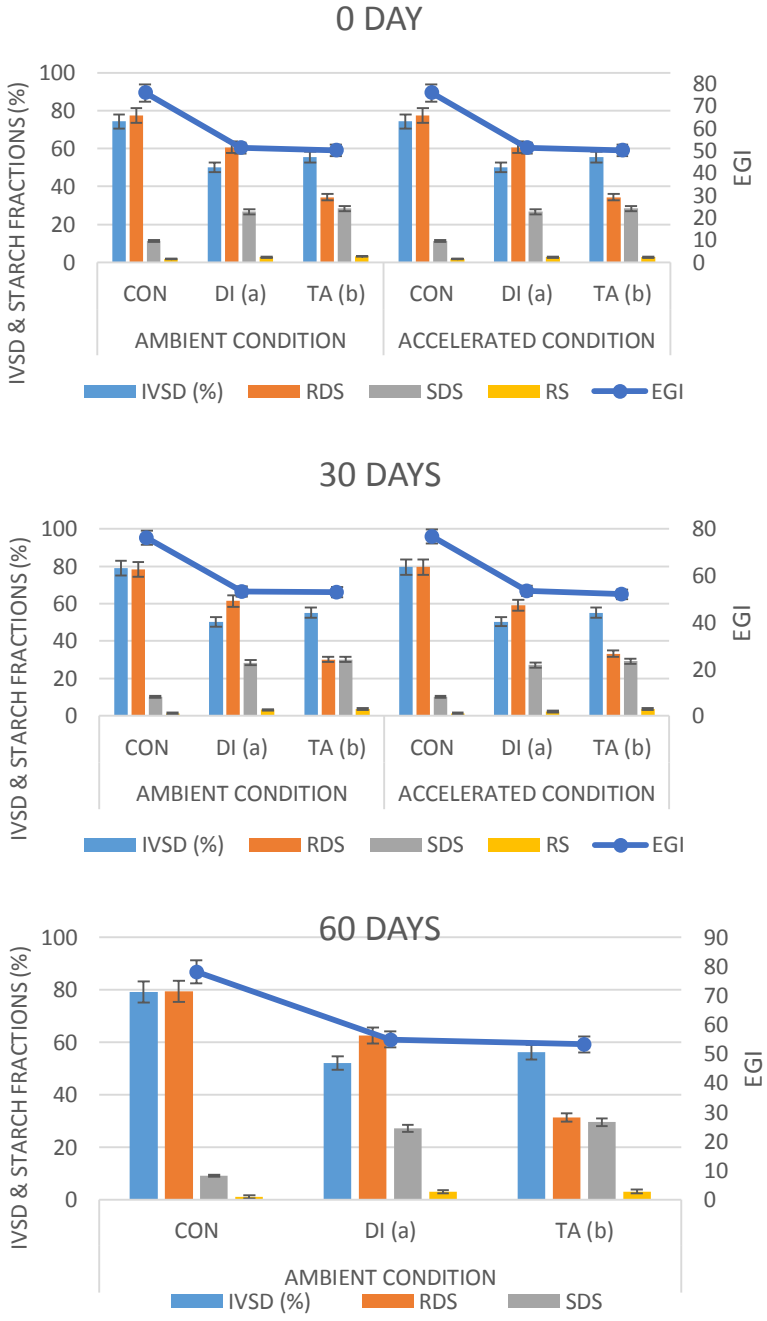
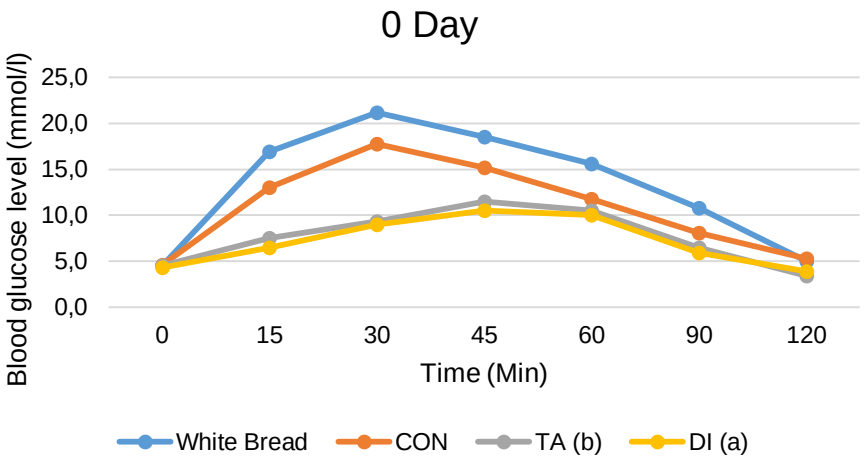
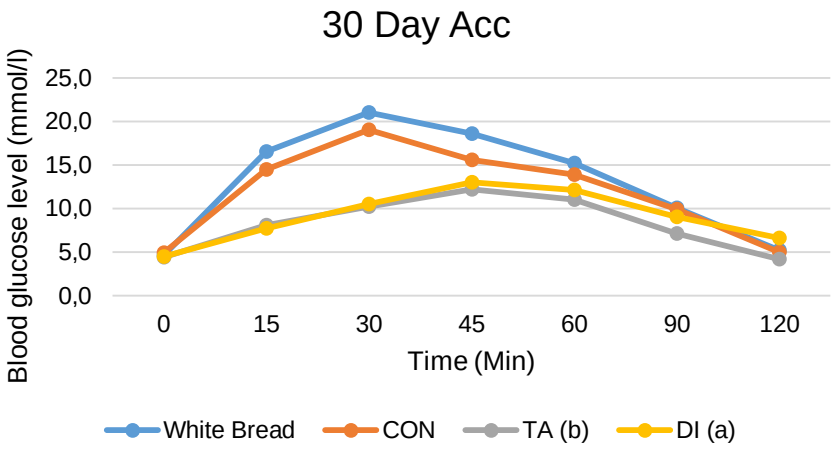


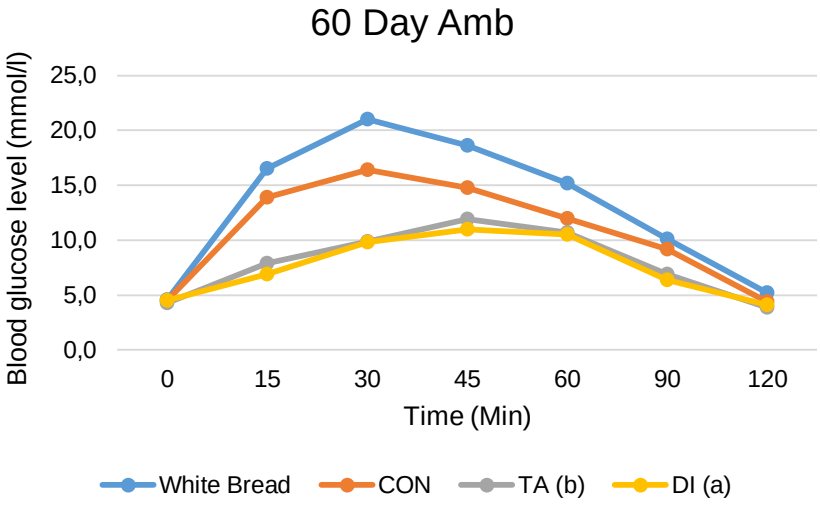
Figure1. Influence of storage at different conditions on IVSD, Starch Profile and EGI of noodles with modified ingredients (IVSD-*In vitro* starch digestibility, RDS-Rapidly digestible starch, SDS-Slowly digestible starch, RS-Resistant starch, EGI-Estimated glycemic index; Sample abbreviations as per Table 3)



CON-80.4	TA(b)-54.1	DI(a)-50.4



CON-89.3	TA(b)-65.1	DI(a)-62.0



CON-80.9	TA(b)-57.2	DI(a)-52.2

Figure 2. Blood glucose response for the low glycemic index noodles at 0 day, 30 days (Accelerated condition) and 60 days (Ambient condition) in healthy individuals (*Per 50g carbohydrates portion; White bread as reference sample (GI=100). Sample abbreviations as per Table 3)

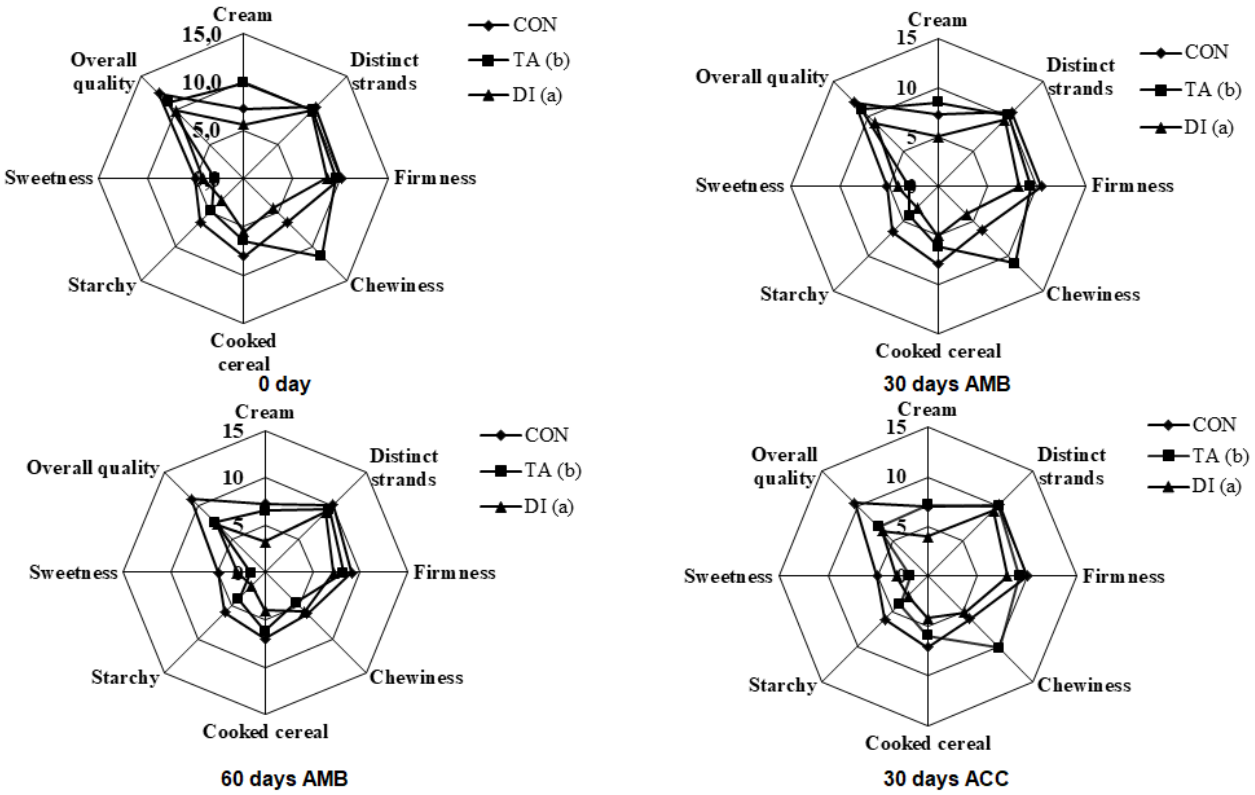


Figure 3. Sensory profiles of samples stored at ambient condition (Enzymatically modified) (CON-Control noodles with 100% *T. durum*, TA(b)-*T. aestivum* flour (wheat flour) modified using branching enzyme, DI(a)-*T. dicoccum* flour modified using α-amylase inhibitor. AMB- Ambient condition, ACC- Accelerated condition)

CONCLUSION

From the present study, it can be concluded that storage study is essential for a product to understand the physical and chemical properties of the product during its storage. Samples stored at the ambient condition can be stored for longer time than in the accelerated condition with high humidity and elevated temperature. Noodles with enzymatically modified ingredients can be stored for 60 days at the ambient condition and 30 days at the accelerated condition. During the storage there will be reduction in the product quality due to disruption of noodle strands during cooking, thereby increasing the cooking loss. This infers that upon storage of noodles beyond 60 days at ambient and 30 days in accelerated conditions, noodles will disintegrate faster and releases glucose faster when digested. Hence the GI of the product increases drastically upon storage under these conditions. Thus, it can be concluded that enzymatically modified samples can be stored up to 60 days at the ambient condition with minimal product quality deterioration. More such studies to be carried out in the days to come to understand the usefulness of the modification process to be utilized in noodle industries and thereby benefiting the population in need.

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