Tropical Journal of Pharmaceutical Research July 2025; 24 (7): 913-919
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
https://dx.doi.org/10.4314/tjpr.v24i7.8

Original Research Article

Study of two lactases for the manufacture of lactose-free yogurt by K-Lolac enzymatic method

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Sent for review: 11 April 2025 *Revised accepted: 21 July 2025*

Abstract

Purpose: *To investigate the efficiency of two lactase enzymes in producing lactose-free yogurt.*
Methods: *Two lactase enzymes (NOLA™5500 fit and Ha-lactase™ 5200 – CH HANSEN) were used in pasteurized cow's milk at 1000 µL under 4 °C for 18 h. The hydrolysis rate was determined using the K-Lolac kit (Megazyme©). Fermented milk was prepared from hydrolyzed milk, and its pH and syneresis were analyzed. Protein enrichment was applied to improve its organoleptic quality.*
Results: *The initial raw milk contained 42.15 g/L of lactose. There was a significant decrease in the amount of lactose following treatment with the two enzymes ($p < 0.05$). There was no significant difference in residual lactose level and hydrolysis rate of Ha-lactase compared to NOLA ($p > 0.05$). Lactase-treated samples showed significantly higher syneresis rate compared to control ($p < 0.05$). Fermented milk made from hydrolyzed milk showed increased syneresis compared to hydrolyzed milk, necessitating protein enrichment, which improved its rheological properties and sensory quality.*
Conclusion: *Ha-lactase significantly improves lactose hydrolysis compared to NOLA fit. Protein enrichment further significantly enhances the texture and overall quality of fermented milk, making it a suitable alternative for lactose-intolerant consumers.*

Keywords: *Lactase, NOLA fit, Ha-lactase, K-Lolac, Lactose-free yogurt*

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Milk and dairy products are essential products in the Algerian consumption model. They occupy a fundamental place in the food chain of the population, especially younger groups, due to their nutritional, organoleptic, and specific qualities. They are recommended for all ages corresponding to different needs, possess high calcium content, and have become an excellent source of proteins and vitamins [1]. Almost 75 % of the Algerian population is lactose intolerant; meanwhile, lactose represents the main carbohydrate fraction of mammalian milk [2]. Lactose intolerance is the discomfort associated with lactose consumption that exceeds the body's capacity for digestion and tolerance. While some people are indeed lactose intolerant, others mistakenly think they are lactose intolerant and impose on themselves an unnecessary diet that does not provide enough calcium, which is, among other things, a risk factor for osteoporosis [3].

For specific technological and nutritional purposes, today's dairy industry also modulates lactose composition of milk through the use of adapted technologies and thus offers lactose-intolerant consumers reduced lactose content with sufficient calcium and protein intake. Lactose is hydrolyzed using enzymes (either microbial or fungal origin) into glucose and galactose, the latter being produced from strains of *Kluyveromyces lactis, Kluyveromyces fragilis, Bacillus circulans, Aspergillus oryzae* and *Aspergillus niger* [4]. It is therefore important to quantify lactose accurately in milk and milk products [5].

A considerable number of methods for determining carbohydrates in milk have been developed, including gravimetric, polarimetric spectrophotometry, high liquid performance chromatography –RI (HPLC-RI), HPLC-PAD analysis and enzymatic method which was used in this study.

To achieve this, the effect of two industrial enzymatic preparations, β-galactosidase derived from *Bifidobacterium bifidum* and *Kluyveromyces lactis*, was evaluated on lactose hydrolysis. The resulting preparation was used to produce natural yogurt using the enzymatically treated milk.

METHODS

Raw materials and products

Local raw milk samples were collected fresh from the farm at Agri-Mazagran University of Mostaganem, Algeria. The raw milk was collected from cows of black magpie breed in April 2019 in compliance with good sampling practices, hygiene, and cold transport rules. Two commercial enzyme preparations of β-galactosidase were used to prepare lactose-reduced milk for comparative purposes (NOLA™ Fit5500 and HA-Lactase 5200 (CHR-HANSEN)).

Preparation of lactose-free milk

After pasteurization at 80 °C for 5 min, the pasteurized milk was hydrolyzed following previous method [6]. A dose of 1000 µL/L of each neutral lactase sample (NOLA™ Fit5500 and Ha-lactase) was collected under class II biological safety cabinet (BSC II) and introduced separately into the milk samples. The mixture was incubated for 18 h at 6 °C with slow stirring to prevent microbial growth. A deproteinization step was carried out using concentrated solutions of Carrez II and Carrez I (Merck) according to established protocol [7].

Yoghurt preparation

Several tests and recipes were carried out before the final results were obtained, and certain modifications and enrichments to the milk were suggested. The modifications and enrichments were represented by the following batches: Control yogurt (T) made from pasteurized cow's milk without any additives, Yoghurt H based on milk treated with the enzyme Ha-Lactase 5200 without the addition of protein concentrates (Cp; EPIPROT 60 UL from EPI INGREDIENT-France), Yoghurt (H+) based on milk treated with Ha-Lactase 5200 and enriched with Cp (10g/L), Yoghurt (N-) based on milk treated with NOLA™ Fit5500 without the addition of Cp, Yoghurt (N+) based on pasteurized cow's milk and treated with NOLA™ Fit5500. Protein amendment was carried out at 50 °C at a dose of 1 %. The yogurt batches were prepared following previous method [6] with few modifications which include direct inoculation of the lactic ferment (CHR HANSEN YC-X16) at 0.05 % with homogenization for 20 min. After fermentation, yogurt samples were stored in a refrigerator at 4 °C for 21 days. Thereafter, physicochemical and sensory analysis were carried out. Yogurt batches T, H, and N were subjected to lactose determination.

Lactose determination

Lactose concentration tests were carried out in triplicate for each test using K-LOLAC Lactose Enzyme Dosage Kit and following instructions described by the producer (Megazyme©). K-Lolac measures the absorbance of NADPH at a wavelength of 340 nm, a product of several chain reactions from glucose. The latter was derived from lactose hydrolysis by β-galactosidase kit [7]. The amount of lactose as a function of the absorbance of the NADPH (ΔA) was calculated using Eq 1.

$$C = (V*MW)/(\varepsilon*d*v*2)F*\Delta A \quad \ldots\ldots\ldots (1)$$

Where: V is final volume, MW is the molecular weight of D-glucose or lactose (g/mol), E is extinction coefficient of NADPH at 340 nm (6,300 ($1\ mol^{-1}cm^{-1}$), D is the path of light (cm), V is sample volume, 2 is 2 moles of NADPH produced for each mole of D-glucose or lactose, F is Dilution factor (5.4) for all standard liquid milk preparations. In the case of lactose, the value obtained was:

$$C = (1.17 \times 180.6) / (6300 \times 1.0 \times 0.1 \times 2) \times 5.4 \times \Delta A \ldots\ldots (2)$$

Therefore; $C = 0.3233 \times 5.4 \times \Delta A$

pH measurement

The pH of coagulation and post-acidification was monitored every seven days (days 1, 7, 15, and 21) using an electronic pH meter (ADWA-AD 1200). After calibrating, the electrode was directly immersed in the product to be analyzed (yogurt), and the pH displayed was recorded. Determinations were done in triplicate.

Syneresis measurement

After stirring the yogurts, the gels obtained were centrifuged at 6000 rpm at 10 °C for 10 min. The supernatant in each tube was collected and weighed. Determinations were done in triplicate every 7 days. Syneresis was determined using Eq 3 [8].

Syneresis (%) = (Supernatant weight/initial weight)100 …… (3)

Sensory analysis

Sensory analysis was done following previous procedure [9]. For this assessment, various yogurt batches were presented to a panel of 16 evaluators aged between 22 and 73 years.

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS version 22.0, IBM, Armonk, NY, USA). Results of the physicochemical and sensory analyses were statistically processed by total randomization bi-factorial analysis of variance, followed by a comparison of the means using the Newman-Keuls test (Stat Box). $P < 0.05$ was statistically significant.

RESULTS

Lactose content in milk

There was a significant decrease in the amount of lactose for the samples treated with both enzymes ($p < 0.05$). Furthermore, there was no significant difference in percent lactose hydrolysis following treatment with both enzymes ($p > 0.05$; Figure 1).

pH during yogurt fermentation

There was decrease in pH values until a pH of 4.6 was reached at the end of fermentation. In this study, the control sample required a minimum of 3 h 40 min to reach pH 4.6, while the H+ sample took a maximum of 4 h 30 min. Samples of hydrolyzed milk took longer to reach pH 4.6 compared to control. Specifically, samples treated with HA-lactase took 30 min longer, and those treated with NOLA™ Fit took 20 min longer. Also, samples fortified with protein concentrate showed a longer maturation time compared to non-fortified samples (Figure 2).

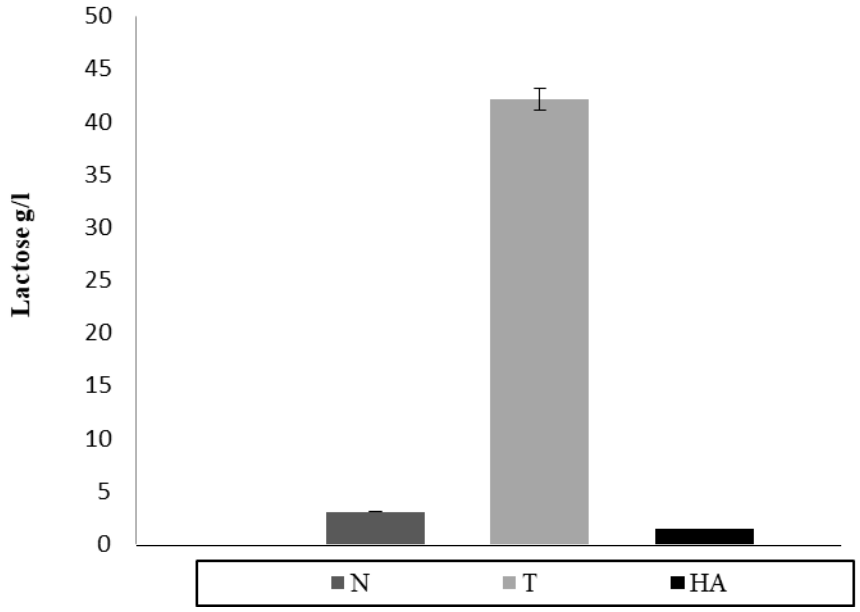

Figure 1: Residual lactose in different batches of milk. N: milk batch treated with NOLA Fit; HA: milk batch treated with Ha-lactase; T: control batch without enzyme treatment

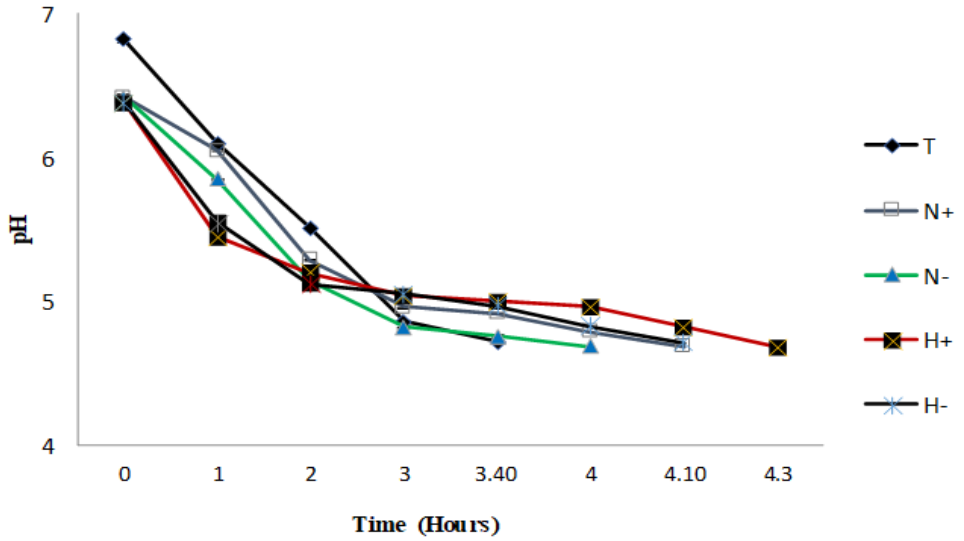

Figure 2: pH profile during yogurt maturation. T: control yogurt (no enzyme treatment); H+: yogurt made with milk treated with Ha-lactase and fortified with protein concentrate; H−: yogurt made with milk treated with Ha-lactase without protein fortification; N+: yogurt made with milk treated with NOLA Fit and fortified with protein concentrate; N−: yogurt made with milk treated with NOLA Fit without protein fortification

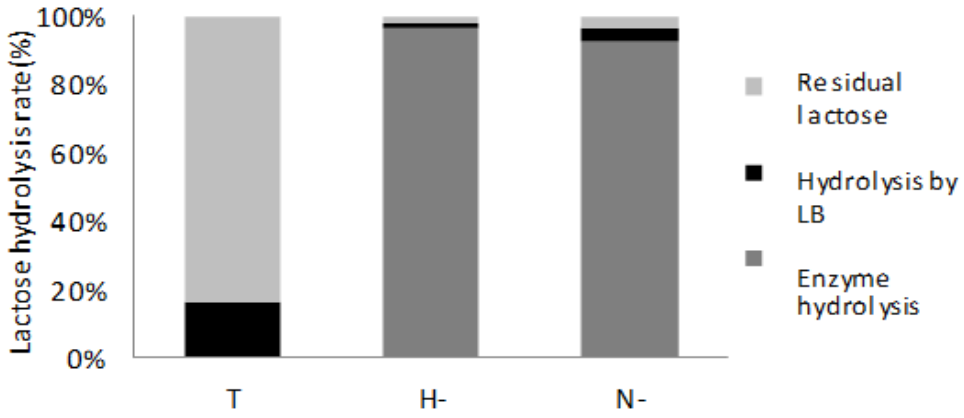

Figure 3: Rate of lactose hydrolysis in yogurt samples. T: control yogurt (no enzyme treatment); H−: yogurt made with milk treated with Ha-lactase without protein fortification; N−: yogurt made with milk treated with NOLA Fit without protein fortification

Lactose content in yogurt

Enzyme-treated samples showed significantly lower lactose content compared to control (p > 0.05). Furthermore, there was no significant difference in lactose content between enzyme-treated samples without protein fortification (p > 0.05; Table 1).

pH profile of yogurt during post-acidification period

During post-acidification phase, pH of the fermented milk continued to decrease, reaching a value of 4.1 for control (T) and 4.35 for H+ sample on Day 21. Enzymatic treatment without protein enrichment resulted in significantly higher pH compared to control and unenriched samples (p < 0.05). Protein enrichment resulted in significant increase in acidity of the yogurts compared to unenriched samples (p < 0.05; Table 2).

Syneresis during post-acidification period

Yogurt samples without enzymatic treatment (T) showed the lowest percentage of syneresis during post-acidification period, with an average of 55.59 %, compared to the samples treated with Nola Fit (> 59 %) and Ha-Lactase (> 68 %). There was significant difference in syneresis rate between lactase-treated samples and controls (p < 0.05). Furthermore, enriched samples showed lower percentage of syneresis compared to unenriched samples during post-acidification (Figure 4).

Table 1: Residual lactose content in the three yogurt samples (mean ± SD; n = 3)

Sample	T	N-	H-
Lactose (g/L)	35.17±1.15[a]	1.47 ±0.59[b,c]	0.87±0.12[c]

T: control yogurt (no enzyme treatment); H−: yogurt made with milk treated with Ha-lactase without protein fortification; N−: yogurt made with milk treated with NOLA Fit without protein fortification. Different uppercase letters in superscripts indicate significant difference at $p < 0.05$

Table 2: pH profile during storage (mean ± SD; n = 3)

Sample	Day 1	Day 7	Day 15	Day 21	Mean±SD
T	4.693±0.002	4.297±0.005	4.19± 0.01	4.100± 0.02	4.320±0.260[e]
N^+	4.673±0.006	4.417±0.006	4.343±0.015	4.320± 0.01	4.330±0.162[d]
N^-	4.667±0.007	4.430± 0.010	4.403±0.005	4.393±0.006	4.470± 0.130[c]
H^+	4.657±0.006	4.433±0.013	4.380± 0.01	4.357±0.004	4.460±0.140[b]
H^-	4.687±0.012	4.501±0.006	4.453±0.006	4.280± 0.01	4.480±0.170[a]

T: control yogurt (no enzyme treatment); H+: yogurt made with milk treated with Ha-lactase and fortified with protein concentrate; H−: yogurt made with milk treated with Ha-lactase without protein fortification; N+: yogurt made with milk treated with NOLA Fit and fortified with protein concentrate; N−: yogurt made with milk treated with NOLA Fit without protein fortification. [a]$P < 0.05$ vs H+ and control, [c]$p < 0.05$ vs N+ and control

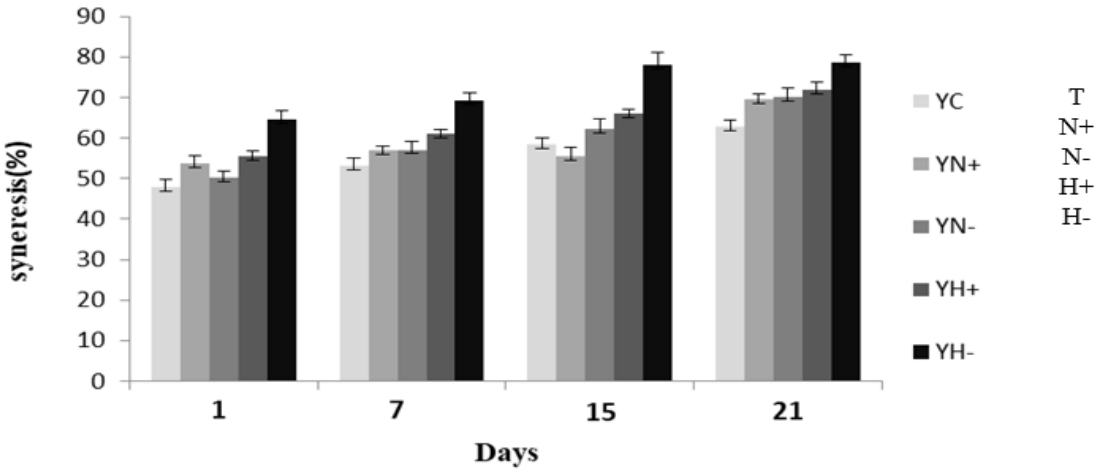

Figure 4: Rate of syneresis of yogurt batches during post-acidification period. T: control yogurt (no enzyme treatment); H+: yogurt made with milk treated with Ha-lactase and fortified with protein concentrate; H−: yogurt made with milk treated with Ha-lactase without protein fortification; N+: yogurt made with milk treated with NOLA Fit and fortified with protein concentrate; N−: yogurt made with milk treated with NOLA Fit without protein fortification

Sensory attributes

Sensory attributes, including colour, odour, consistency, texture, taste, and acidity, were evaluated. There was no significant difference in colour and odour among the samples. The panel perceived the yogurt samples hydrolyzed with Ha-Lactase as having a more pronounced sweet taste compared to Nola Fit–hydrolyzed sample ($p > 0.05$). Yoghurt N was classified as the least acidic, without any significant difference ($p > 0.05$) from other samples. Protein enrichment had a significant effect ($p < 0.05$) on the consistency and texture of yogurts hydrolyzed by Nola Fit. In contrast, protein enrichment did not have significant effect on the consistency of yogurts hydrolyzed by Ha-Lactase. However, a significant difference in preference was observed between yogurts hydrolyzed by the two types of enzymes, both with and without protein enrichment (Table 3).

DISCUSSION

These results demonstrate the effect of the lactases that hydrolyze lactose into glucose and galactose [10]. The hydrolysis rate achieved by HA-lactase exceeds the 90 % rate reported in a previous study [11] for lactases from *Kluyveromyces lactis*. Differences between HA-lactase and NOLA™ Fit may be due to the composition of metallic ions. Potassium (K^+) and magnesium (Mg^{2+}) act as activators, while calcium (Ca^{2+}) and sodium (Na^+) act as inhibitors for enzymes derived from *Kluyveromyces lactis* (HA-lactase).

Table 3: Descriptive analysis of sensory evaluation (mean ± SD, n = 3)

Sample	N+	N-	H+	H-	Effect
Color	3.000 ±0.894	2.938±0.854	2.750±1.065	2.500±1.095	NS
Odor	1.813±0.655	2.000±0.816	1.938±0.854	2.313±0.602	NS
Consistency	3.063±0.772^{a}	2.313±0.873^{b}	2.625±0.885^{a}	1.688±0.793^{b}	*
Texture	3.500±0.730^{a}	1.625±0.806^{b}	2.125±0.975^{b}	1.625±0.719^{b}	*
Sweet taste	1.375±0.500^{b}	1.313±0.602^{b}	1.875±0.719^{a}	1.938±0.854^{a}	*
Acidity	1.688±0.793	1.563±0.727	1.813±1.074	1.625±0.619	NS
Preferences	7.250±1.252^{a}	6.983±1.573^{a,b}	5.750±1.653^{b}	5.565±2.047^{b}	*

T: control yogurt (no enzyme treatment); H+: yogurt made with milk treated with Ha-lactase and fortified with protein concentrate; H−: yogurt made with milk treated with Ha-lactase without protein fortification; N+: yogurt made with milk treated with NOLA Fit and fortified with protein concentrate; N−: yogurt made with milk treated with NOLA Fit without protein fortification. $^{ab}P < 0.05$, $^{*}p < 0.05$, NS: non-significant ($p > 0.05$)

For enzymes from *Bifidobacterium bifidum* (NOLA™ Fit), Ca^{2+}, Mg^{2+}, and Mn^{2+} are activators, whereas Cu^{2+}, Zn^{2+}, and Fe^{2+} inhibit enzyme activity. Treated samples (especially those with HA-lactase) showed lower lactose levels (below 1.5 g/L), which falls within the lactose-free range. Delay in reaching the final pH of 4.6 in samples treated with lactase enzymes suggests that lactose hydrolysis influences the kinetics of yogurt fermentation, which is in tandem with previous studies [12].

Slower acidification observed in hydrolyzed milk may be attributed to the carbohydrate preference of the starter cultures, particularly *Streptococcus thermophilus*, which metabolizes lactose more efficiently than its monosaccharide components [13]. Also, the further delay in samples fortified with protein concentrate may be due to increased buffering capacity of the milk matrix, which slows down pH reduction during fermentation. These findings highlight the combined influence of substrate composition and enzymatic treatment on fermentation dynamics.

Limited lactose reduction during fermentation, as seen in the control (T) was similar to findings from other studies [3,14]. This may help explain why symptoms still appear in lactose-intolerant individuals when consuming regular yogurt. However, this risk is much lower for fermented milk in which the lactose is already hydrolyzed. This study reported greater lactose breakdown in enzymatically pretreated samples. Furthermore, there is negative correlation between the initial percentage of lactose hydrolysis and the amount degraded by lactic acid bacteria. This may be attributed to reduced availability of lactose as a substrate, which impacts metabolic activity of bacteria. Lactic acid bacteria are less efficient in the absence of lactose and struggle to adapt their metabolism to switch from lactose to glucose [6].

Syneresis is the phenomenon whereby yogurt gel separates, and it is often considered as a defect affecting both texture and consumer acceptability. In this study, syneresis was used as an indicator of structural stability and quality of yogurt following enzymatic treatment and protein enrichment. The results showed that higher lactose hydrolysis rates were associated with greater syneresis. This correlation has been reported in other studies, and it was attributed to reduction in the production of exopolysaccharides (EPS) by lactic acid bacteria or degradation of EPS by glycoside hydrolases during extended fermentation [15]. Protein enrichment, on the other hand, was associated with reduced syneresis, likely due to improved water retention and gel structure. These findings underline the importance of balancing enzymatic treatment and protein enrichment to maintain desirable yogurt texture and minimize separation [16].

Sensory analysis demonstrates the impact of lactose hydrolysis and protein enrichment on several organoleptic qualities of yogurt. The absence of significant changes in colour and odour in all samples was in tandem with prior findings [17], which reported that protein enrichment does not affect these attributes. The higher perceived sweetness in yogurts treated with Ha-Lactase corresponded with their higher lactose hydrolysis rate. This supported previous reports [18], indicating that glucose and galactose (the products of lactose hydrolysis) are sweeter than lactose, contributing to a stronger sweet taste in these yogurts. Although no significant difference in acidity was detected, yogurt N was perceived as the least acidic.

Protein enrichment significantly enhanced consistency and texture in yogurts hydrolyzed by Nola Fit, likely due to increased protein-protein interactions and gel stability. However, this effect was not observed in yogurts treated with Ha-Lactase, which may be related to differences in enzymatic action or sugar composition influencing gel formation. Preference scores favored the N+ yogurt, indicating a positive

consumer response to the combined effect of Nola Fit hydrolysis and protein enrichment. Significant difference in acceptability between yogurts treated with different enzymes suggests that the type of lactase used, as well as protein enrichment, play a crucial role in consumer perception and product quality.

CONCLUSION

Hydrolysis of lactose with the two enzyme preparations (Ha-lactase and Nola fit) significantly reduces lactose content in milk and yoghurt, increases rate of lactose hydrolysis, higher pH, and reduces syneresis during post-acidification period. Protein enrichment significantly improves the rheological properties of the yogurt, resulting in better organoleptic quality.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and the authors will bear all liabilities about claims relating to the content of this article.

Bouasria Benbouziane conceived, designed the study, reviewed and edited the manuscript. Amina Chadli contributed to the methodology, formal analysis, and original draft writing, Mohamed Cherif Bentahar collected and analyzed the data, Djilali Benabdelmoumene contributed to the methodology, revision, and review. All authors read and approved the manuscript for publication.

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