Kinetic Reaction Estimation of Bifidus Milk

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Abstract:

The quantitative data of colony forming unit (cfu) in Bifidus milk as plain and with dibis are scarce mainly because of the lack of suitable methods to quantify the degree of changes in it. These calculations were obtained for Bifidus milk (plain) and with 5% dibis kept at 5, 10 and 15°C for fresh, 3, 6, 9, 12, 15, 18 and 21 days. The order of the reaction was found in the second order kinetics during storage at the studied temperature range (5-15°C). The velocity constants (K) were decreased with increasing of storage temperature up to 10°C and then increased at 15°C. While, the mean of k values is lower in Bifidus milk samples with dibis than that in plain samples. The Dϑ values in all Bifidus milk samples were increased with increasing storage temperature up to 10°C and then decreased at 15°C. While, the mean value of Dϑ was higher in Bifidus milk samples with dibis than that in plain samples. The Bifidus milk samples with dibis had higher values of Ea than that of plain samples. While, the Bifidus milk samples with dibis needed higher values of Ea than that of plain samples to complete the reaction. The Z-values illustrated an increase trend with increasing of storage temperature in all Bifidus milk samples. The Q_{10}-values of all Bifidus milk samples were decreased with increasing of storage temperatures in all treatments.

Keywords: Bifidus milk–Velocity Constant–Dibis–Storage Temperature.

Introduction:

The response to changes in storage temperature was expected to be different for the colony forming unit (cfu) of Bifidus milk. The storage temperature dependency of the death rate constant can best be expressed by the Arrhenius equation **(Burton, 1982)**. The reaction rate constant value (k), activation energy (Ea) and other thermodynamic parameters are very useful to evaluate the performance of Bifidus milk. The quantitative data on cfu in Bifidus milk as plain and with dibis are, however, scarce mainly because of the lack of suitable methods to quantify the degree of changes in it.

Hence, this study was undertaken to estimate kinetic reaction of probiotic bacteria in Bifidus milk with or without dibis kept at 5, 10 and 15°C for fresh, 3, 6, 9, 12, 15, 18 and 21 days, respectively.

Materials and Methods:

Milk source:

Bulk buffalo's milk used in the present study were obtained from the fresh morning bulk milk of herds of the Animal Production Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch).

Bifidobacterium bifidum

It was provided by the Chr. Hansen Company.

Siwi Date, Skim Milk Powder and Gelatin:

They were obtained from local market.

Preparation of Date Syrup (Dibis):

The pulp of Siwi date was separated from kernel, weighted, washed twice and extracted by water 1:2 (pulp: water) at 70°C for two hours with stirring at intervals. Mixture was placed in molds and then pressing. The

resulting juice was filtered using cheese clothes (double layer), concentrated using water bath at 70°C until total soluble solids reached 72% to avoid spoilage of the date syrup during storage. The date syrup was sterilized by tyndallization and kept at room temperature until used.

Manufacture of Bifidus Milk:

Buffalo's milk (6% fat) was mixed with 1.5% skim milk powder and 0.4% gelatin. The mixture was divided into two equal portions. The two portions were heated to $90\pm1^{\circ}$C for 15 min., and directly cooled to 42-44°C. The dibis was added as following:

Control: Plain Bifidus milk.   T1: Adding 5% dibis (w/w).

Each part was inoculated with 10% active growing culture of *Bifidobacterium bifidum* according to **Ahmed (2009)**. The inoculated batches were incubated at 42-44°C until coagulation, after which they were kept at $6\pm2^{\circ}$C over night, then were stirred and bottled according to **El-Sonbaty *et al.* (2008)**. The products were kept at 5, 10 and 15°C and analyzed as fresh, 3, 6, 9, 12, 15, 18 and 21 days.

Bifidobacteria Count:

Bifidobacteria count was enumerated according to **Dave and Shah (1996)** using modified MRS agar medium (m-MRS), supplemented with 0.05% L-Cysteine HCL and 0.3% lithium chloride. The plates were incubated at 37°C for 48 hours under anaerobic condition.

Modified MRS (m-MRS) Medium:

This medium consists of: MRS medium (54 gm), L-cystein hydrochloride (0.5 gm), lithium chloride (3 gm), agar (15 gm) and distilled water (1000 ml). Ingredients were dissolved in distilled water and pH was adjusted to 6.5 before sterilization at 121°C for 20 min.

Kinetic Reaction Estimation of Bifidus Milk:

The kinetic reaction estimation of Bifidus Milk was calculated as described by **Anap *et al*. (1987)** and **Mansour (1999)**.

Results and Discussion:

Reaction order:

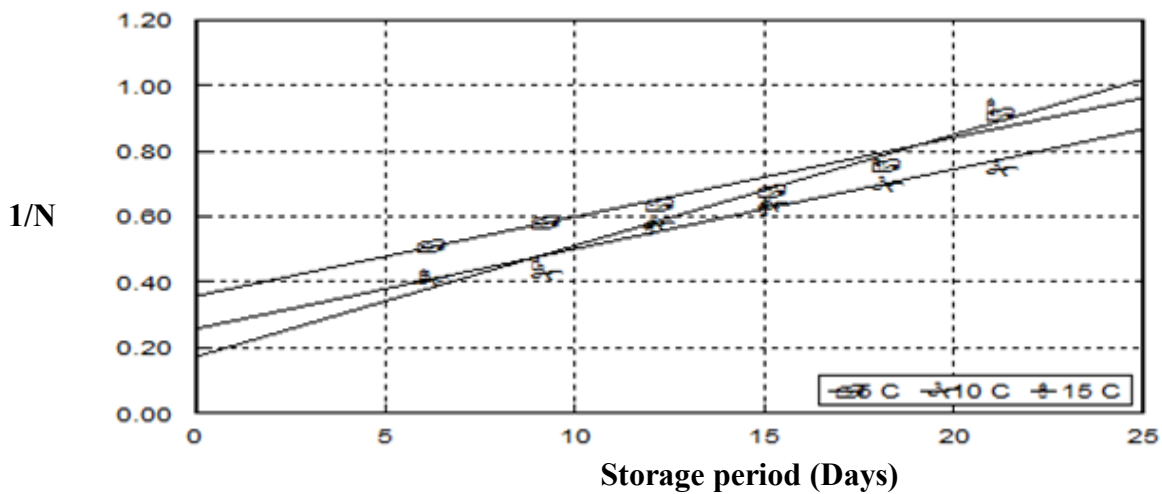The order of the reaction is that which, in this method of representation, yields a straight line plot.

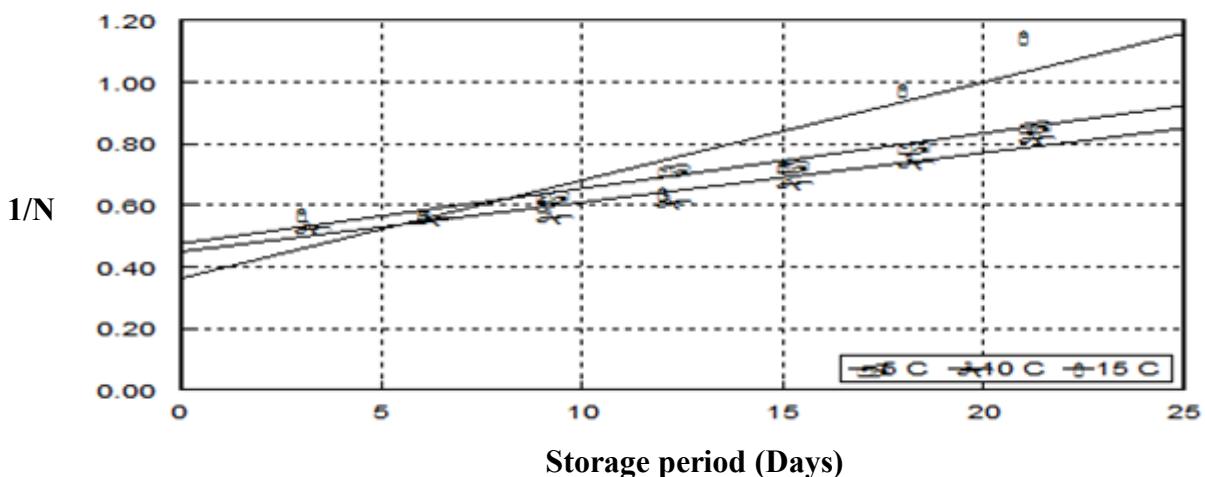Figure 1: Plot according to 2nd order reaction of cfu of Bifidus milk samples (Plain).

Figure 2: Plot according to 2nd order reaction of cfu of Bifidus milk samples (5% dibis).

From plotted data of the different Bifidus milk samples (Figures 1& 2); it is found that the reaction could adequately be described by second order reaction during storage at the tested temperature range (5-15°C). This was varied than the findings obtained by **Mansour *et al*. (2010)**, who found that the order reaction of shelf-life in milk was first order reaction during storage of milk samples.

Velocity Constants (K):

The calculated values of the velocity constants (k) in day^{-1} of this reaction throughout the storage periods of Bifidus milk samples at different temperatures are presented in Table 1 and Fig 3. As expected, it can be declared that the velocity constants (K) decreased with increasing of storage temperature up to 10°C and then increased at 15°C. On the other hand, the mean value of k values was lower in Bifidus milk samples with 5% dibis than that in plain samples.

Table 1: Values of velocity constants (k) and decimal increase times (Dϑ) of cfu of Bifidus milk samples kept at different temperatures.

Storage temperature ($^{\circ}$C)	K (day^{-1})		Dϑ	
	Plain	5% dibis	Plain	5% dibis
5	0.2933	0.2429	7.85	9.48
10	0.2857	0.2062	8.06	11.17
15	0.3850	0.4068	5.98	5.66
Mean	0.3213	0.2853	7.30	8.77

Decimal Increase Times (Dϑ):

The values of decimal increase times (Dϑ) of all studied Bifidus milk samples are presented in Table 1 and Fig 4.
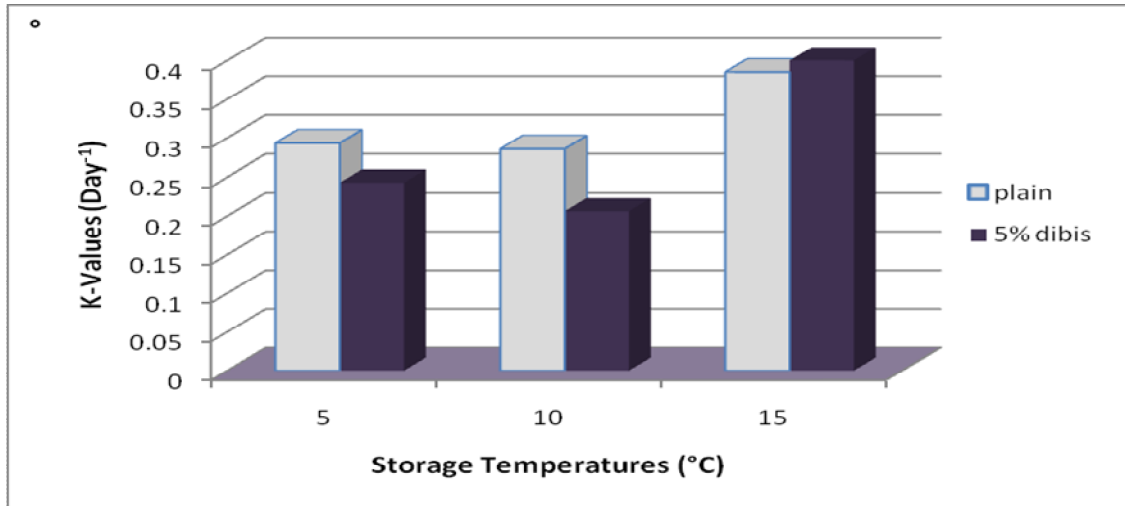

Figure 3: Changes of velocity constants (k) for plain Bifidus milk and with 5% dibis at different storage temperatures.

From these data, it is found that the values of decimal increase time (Dϑ) for the values of cfu in Bifidus milk samples increased with increasing of storage temperature up to 10°C and then decreased at 15°C. On the other hand, the mean value of Dϑ is higher in Bifidus milk samples with 5% dibis than that in plain samples.
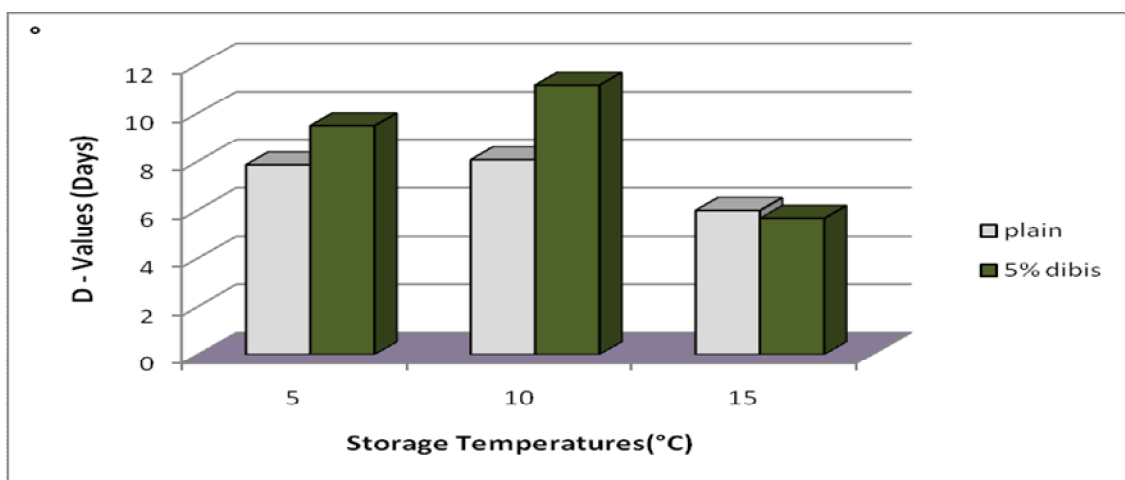

Figure 4: Decimal increase times for plain Bifidus milk and with 5% dibis at different storage temperatures.

Activation Energy (Ea):

Data in Table 2 indicated that the Bifidus milk samples with 5% dibis had higher value of Ea than that of plain samples. Additionally, the Bifidus milk samples with 5% dibis needed higher values of Ea than that of plain samples to complete the reaction.

Table 2: Activation energy (Ea) value of cfu of Bifidus milk samples held at different temperatures (Plain and with 5% dibis).

Activation energy Ea (KJ/mol.K)	
Plain	**5% dibis**
17.379	32.942

Thermal Coefficient (Z):

The calculated Z-values for the reaction of cfu in Bifidus milk samples induced throughout storage periods were cleared in Table 3 and Fig 5. From the presented data, the thermal coefficient values ranged from 86.57 to 89.68°C (with mean value of 88.13°C) at the tested temperature range for Bifidus milk samples without dibis and from 45.67 to 47.31°C (with mean value of 46.49°C) at the tested temperature range for Bifidus milk samples with 5% dibis.

Table 3: Thermal coefficient (Z) and temperature coefficient (Q10) values of cfu of Bifidus milk samples held at different temperatures.

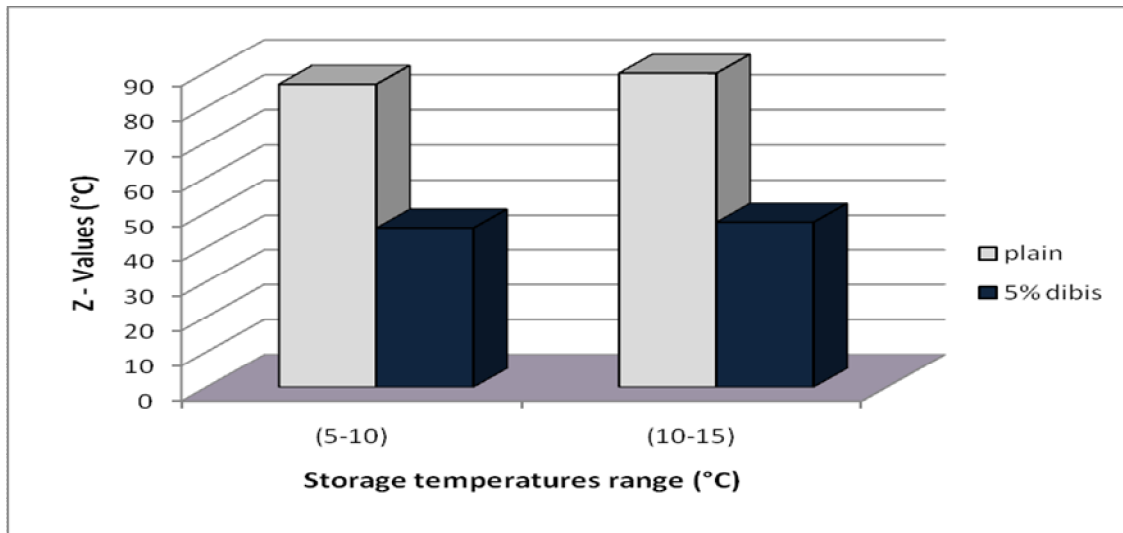| Temperature range ($^\circ$C)	Z-value ($^\circ$C)		Q_{10}-value	
	Plain	**5% dibis**	**Plain**	**5% dibis**
5-10	86.57	45.67	1.30	1.66
10-15	89.68	47.31	1.29	1.63
Mean	88.13	46.49	1.30	1.65

Figure 5: Z-values of plain Bifidus milk and with 5% dibis at different storage temperatures.

Generally, the values of thermal coefficient illustrated an increase trend with increasing of storage temperature in all Bifidus milk samples. These results are in agreement with those reported by **Mansour et al. (2010)**.

Temperature Coefficient (Q_{10} value):

The calculated data of Q_{10}-values of the cfu in Bifidus milk samples after storage periods under the temperature ranges are cleared in Table 3 and Fig 6.

On the other hand, the results of Q_{10}-values for cfu in Bifidus milk samples under the storage temperature ranges are varied from 1.29 to 1.30 (with mean value of 1.30) in the tested temperature range for plain Bifidus milk samples and from 1.63 to 1.66 (with mean value of 1.65) at the tested temperature range for Bifidus milk samples with 5% dibis.
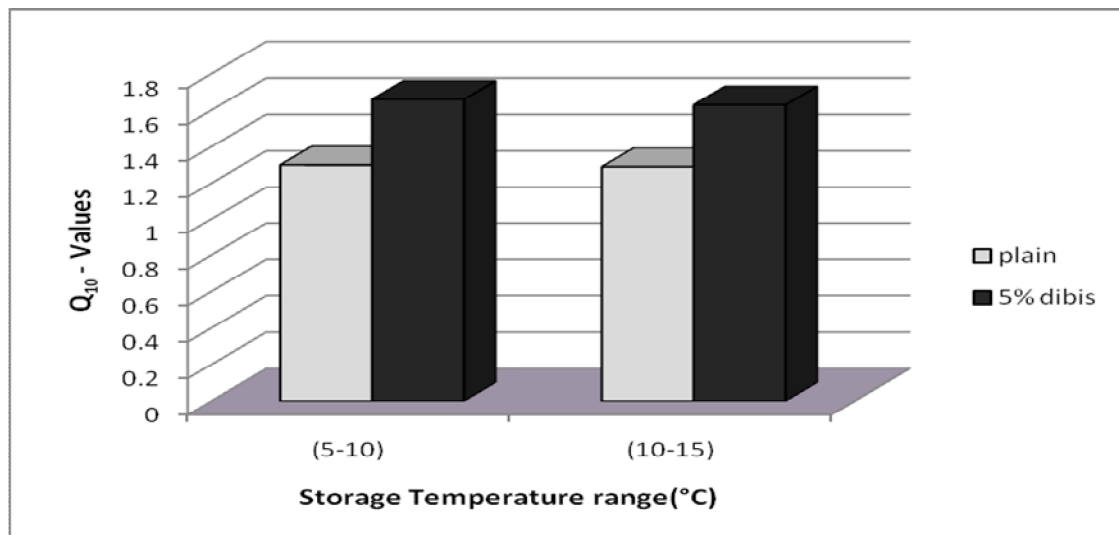
Figure 6: Q_{10}-values of plain Bifidus milk and with 5% dibis at different storage temperatures.

Additionally, the Q_{10}-value of Bifidus milk samples were decreased with increasing the storage temperature in both of plain and examined samples. These results are in harmony with those reported by **Mansour *et al*. (2010)**.

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تقدير حركية التفاعل في لبن البفيدس

على إبراهيم منصور[1] ، خالد حمدي سلمان[1] ، عادل علي تمام[2] ، فتحي السيد الجزار[2]

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تم إستخدام اللبن الجاموسى كامل الدسم (6% دهن) فى إعداد لبن البفيدس وذلك باضافة 1.5% لبن فرز مجفف و 0.4% جيلاتين، وتم تقسيم المخلوط الى جزئين (عينة مقارنة- عينة بها 5% دبس) ، ثم التسخين على درجة حـرارة 90 ْم لمدة 15 دقيقـة ، ثم التبريد الى درجة حرارة 42-44 ْم ، ثم إضافة الدبـس بنسبة 5% الى المعاملة الثانية، ثم إضافة بادئ *Bifidobacterium bifidum* بنسبة 10% ، والتحضين على هذه الدرجة (42-44 ْم) ، ثم التخزيـن على ثلاث درجات حرارة مختلفة (5 ، 10 ، 15 ْم) لمدة 21 يوم ، ويشـير التقدير الكمي لحركية التفاعل إلى ما يلـي :-

- أمكن أولاً معرفة رتبة التفاعل من خلال التمثيل البيانى لقيم أعداد البكتريا باستخدام المعادلات القياسية المعروفة ، والتى تستخدم لتقدير حركية التفاعل . أوضحت النتائج المتحصل عليها أن التغيرات التى تحدث فى أعداد البكتريا أثناء التخزين على مدى درجات الحرارة 5-15 ْم تتبع التفاعلات من الرتبة الثانية .

- أمكن أيضاً تقدير ثابت سرعة التفاعل (K) من خلال حساب ميل التمثيل البيانى ، الذى تم منه معرفة رتبة التفاعل . وقد أوضحت النتائج الحسابية تناقص ثابت سرعة التفاعل في عينات

البفيدس المقارنة ، والمحتوية على 5% دبس بزيادة درجة حرارة التخزين حتى 10 ْم ، ثم تزداد عند 15 ْم ، بينما كان متوسط ثابت سرعة التفاعل أقل في العينات المحتوية على 5% دبس عنها في عينات البفيدس السادة .

- وقت الزيادة العشرى (D9) ، فقد تم تتبعها أثناء فترة التخزين باستخدام التمثيل البيانى للعدد البكتيري مقابل مدة التخزين على تدريج نصف لوغاريتمي. وقد أمكن حساب هذه القيم (D9) من خلال ميل العلاقة الناتجة . وقد أوضحت النتائج الحسابية زيادة الـ D9 في عينات بفيدس المقارنة، والمحتوية على 5% دبس بزيادة درجة حرارة التخزين حتى 10 ْم ، ثم تتناقص عند 15 ْم ، بينما كان متوسط القيم أعلى في العينات المحتوية على 5% دبس ، عنها في عينات البفيدس السادة . بإستخدام معادلة أرهينيوس الشهيرة أمكن عمل العلاقة البيانية الخطية التى يمكن من الميل حساب طاقة التنشيط لهذه التغيرات التى تحدث أثناء التخزين ، وهذه الطاقة تعبر بصورة مباشرة عن تأثير درجة حرارة التخزين على سرعة التفاعل (K) ، وقد أوضحت النتائج الحسابية أن عينات لبن البفيدس المحتوية على 5% دبس تحتاج إلى طاقة تنشيط أعلى من عينات المقارنة لاستكمال التفاعل.

- أمكن أيضاً تقدير قيم المعامل الحرارى (Z) للتغير فى العدد البكتيري لعينات اللبن البفيدس فى أثناء مدة التخزين ، وقد أوضحت النتائج زيادة فى هذه القيم مع الزيادة فى درجة الحرارة في جميع المعاملات .

- أمكن أيضا تقدير معامل التعجيل أو الإسراع Q_{10} ، وقد أوضحت قيم Q_{10} انخفاضاً بالارتفاع في درجة حرارة التخزين .