Effect of Chemical Composition on the Buffering Capacity of Selected Dairy Products

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ABSTRACT

Eight locally-produced dairy products were used in the study: yoghurt, labneh, Nabulsi cheese, dried and liquid jameed, acidic whey, cream and cow's milk. For each product, the *in vitro* acid-buffering capacity (BC) was determined, using a 5% (dry matter) aqueous suspension titrated with 0.1N HCl until the pH reach 1.5 and then back-titrated to pH of 10.0 by addition of 0.1N NaOH. BC values were calculated by dividing the titratable acidity of each sample by the change in pH units. The resultant BC values were highest for whey, liquid jameed, yoghurt and cream (2.45 – 4.86), intermediate for milk, labneh, dried jameed and the commercial antacid (1.50 – 2.25) and lowest for Nabulsi cheese (0.57). These values showed a varying degree of correlation with the content of protein, ash, Na, K and P, and alkalinity of the ash (R = 0.99, 0.67, 0.72, 0.60, 0.59 and 0.42 respectively), while the weakest correlation was with aspartic acid content (R = 0.20). In general, the BC values obtained appeared to be largely due to differences in the studied dairy products chemical composition. Possible reasons are discussed for observed variations in pH among the products tested, when an acid or alkali was added.

Keywords: Buffering Capacity, Chemical Composition, Dairy Products, Yoghurt, Nabulsi Cheese, Jameed, Cow's Milk, Acidic Whey.

INTRODUCTION

Dairy products have long been recognised as an important source of nutrients and they are often consumed as part of a healthy, balanced diet. As well as helping to build strong bones, such products can have a part in reducing the risk of hypertension and certain cancers, and thus play a role in maintaining the health of people of all ages (Park, 1991; Kampman *et al*., 1994; Gambelli *et al*., 1999; Huth *et al*., 2006).

Some foods can act as a buffer against gastric acid, although the capability depends on the nature and amount of ingested protein and the activity of intestinal hormones (McArthur *et al.*, 1988; Park 1991; Joseph *et al*., 2003; Al-dabbas *et al*., 2010). Different dairy products, especially milk and fermented products, such as yoghurt and cheese, are among the foods that have been reported to exhibit buffering activity, which is related to the sum of the individual activities of different acid-base groups in substances such as phosphate, citrate, lactate, carbonate, propionate, acetate, amino acids and proteins (Walstra and Jenness, 1984; Banon and Hardy, 1992; Le Graet and Brule, 1993; Lucey *et al*., 1993a; Kailasapathy *et al*., 1996; Salaun *et al.*, 2005). When milk is acidified, the numerous H^+ ions added become bound to amino groups in the side chains of amino acids, forming NH_3^+ ions. With the addition of alkali, on the other hand, H^+ ions are released from COOH groups, leading to the formation of COO^- ions

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(Alfa Laval, 1995). Since dairy products have the ability to bind or release ions, any changes in pH upon addition of acid or alkali will tend to be small

Chemical antacids, whether mild alkalis or their salts, are widely used to prevent the burning sensation that most people experience occasionally after consuming a meal. While generally effective against mild, acute episodes of 'heartburn', more potent chemical remedies are required for severe, chronic cases. However, antacids reported to have side-effects, like most other drugs. For example, aluminium hydroxide can cause constipation, reduce the absorption of vitamins A and D, inactivate thiamine and lead to phosphate depletion (Cooke *et al*., 1978; Maton and Burton, 1999). Antacids containing calcium may cause constipation and kidney stones due to excessive levels of calcium in the body (Maton and Burton, 1999; Tytgat *et al*., 2003). Moreover, antacids have been reported to induce vomiting, alkalosis, arterial hypertension, heart failure and renal disease (Maton and Burton, 1999; Gabriely *et al*., 2008).

The aim of this study was to determine the antacid potential of certain dairy products that are commonly consumed in Jordan. Thus, tests were carried out *in vitro* to assess the influence of their chemical composition on buffering capacity in response to the addition of acid and then alkali under standard conditions.

MATERIALS AND METHODS

Sample Preparation

Eight locally- produced diary products made from pasteurised cow's milk obtained from the pilot milk plant (University of Jordan, Amman) were used in the study. The products include: yoghurt, Labneh, dried and liquid Jameed, acidic whey, Nabulsi cheese, cream and cow's milk.

Chemical Analysis

Moisture, total ash and protein content were determined by methods described by the Association of Official Analytical Chemists (AOAC, 1990). To determine the Ca, K and Na content, each sample was prepared according to AOAC (1990) and examined by emission spectroscopy, using Thermo atomic absorption spectrophotometer model S-1 (Thermo, MA, USA). Phosphorus content was determined by spectroscopy (AOAC, 1995) at 650 nm, using a UVD – 2950 spectrophotometer (Labomed, California, USA). Aspartic and glutamic amino acids analyses were performed on studied dairy products after acid hydrolysis by means of an automatic amino acid analyser model S433 (Sykam GmbH, Germany) using the method described by Spackman *et al*. (1958). Briefly, 10 ml of 6N HCl were added to 0.1 g of each sample in a test tube. The contents of the tubes were hydrolysed for 20 h at 110^{o}C with continuous stirring, before being allowed to cool to room temperature. After adding distilled water, the hydrolyzate was filtered to remove any sediment and evaporated to dryness under vacuum at 65^{o}C. The resultant material was dissolved in 1 ml of citrate buffer (pH 2.2) and 100 µl was injected into the amino acid analyser by an autosampler maintained at 12^{o}C.

Titrations

An amount equivalent to 5.0 g on dry weight bases from each sample was either used directly for titration or suspended in distilled water and stirred continuously with a magnetic stirrer to yield a homogenised suspension. For comparative purposes, a commercial antacid tablet (680 mg $CaCO_3$ and 80 mg $MgCO_3$) was dissolved in 100 ml of distilled water. Initial pH values of individual dairy products and antacid drug solutions were determined.

A forward titration was carried out on each of the test materials suspension by gradual addition of 0.5 ml standard 0.1N HCl until the pH value reduced to 1.5, the

normal pH of the stomach. Then, a back titration was performed by gradual addition of 0.5 ml standard 0.1N NaOH until the pH reach value of 10.0, the initial pH of the commercial antacid.

All measurements were made with a microprocessor pH meter (model 211, Hanna Instruments, North Carolina, USA) adjusted to operate at room temperature. For both forward and back titrations, the total volumes of acid and alkali added to each sample were recorded separately after an equilibration period of 1 min followed the addition of acid or alkali. The alkalinity of the dry ash (% NaOH) and titrable acidity of each product (% lactic acid) were determined according to AOAC (1995).

Assay for Acid-Buffering Capacity (BC)

For each sample, the BC value was determined mathematically by dividing the titratable acidity over the total changes in pH units as the initial pH was reduced to pH 1.5 using the buffering intensity formula given by Van Slyke (1922):

$$\frac{\Delta B}{\Delta \text{pH}} = \frac{\text{(total volume of acid or base added) x (normality of acid or base)}}{\text{pH unit change produced}}$$

It was assumed that the higher the BC value obtained, the greater was the buffering capacity of the test material.

Statistical Analysis

Data obtained in triplicate were analysed by the Statistical Analysis System (SAS, 1997) and significant differences between means were determined. Differences at $P < 0.05$ were considered to be significant. Correlation coefficients (R values) were determined by MS Excel software, 2007.

RESULT AND DISCUSSION

Chemical Analyses and Titration Curves

Table 1 shows the content of moisture, ash, crude protein, aspartic and glutamic amino acids, Na, K, Ca, P and the alkalinity of ash based on dry weight. Figure 1 and 2 present the forward and back titration curves for each sample when was acidified from its initial pH to 1.5 and then returned to its initial pH by adding alkali. A clear difference (hysteresis) between the two curves was observed for antacid, milk, cream, Labneh, cheese and dried Jameed (Fig. 2). The different hysteresis patterns obtained with these products are likely to reflect interactions that occurred between food components and the added acid or alkali which lead to the ionization and solubilization of different acid-base constituents. The variation in each sample's protein resulted from different denaturation methods and the environment of protein which makes some ionizabel groups become accessible for titration within a protein after a change in pH or denaturation, also contributes in different hysteresis curves (Singh *et al*., 1997). The hysteresis pattern of cow's milk is resulted from the differences in solubilization properties of colloidal calcium phosphate and to the acidic amino acids present in caseins and whey proteins upon pH changes resulted from acid or base addition. Marked hysteresis between curves can be seen for Nabulsi cheese, this may due to the added rennet enzyme which affects the protein spatial structure resulted from enzymatic denaturation and to the possible minerals reactions such as Na, K, Ca and P which reacted with acid-base groups, thereby changing the spatial structure of the protein and its acid-base equilibrium and influencing proton exchange during titration. A further contributory factor could have been the protonation of carboxyl groups in certain amino acids, such as aspartic and glutamic acids (Al-Dabbas *et al*., 2010)

Table (1): Chemical composition of the studied dairy products on dry matter basis[1].

Product	Moisture (%)	Protein (%)	Ash (%)	Na (mg/100g)	K (mg/100g)	Ca (mg/100g)	P (mg/100g)	Aspartic acid (mg/100g)[2]	Glutamic acid (mg/100g)[2]	Alkalinity of ash (%)
Milk	87.9 ± 1.2[b]	29.2± 0.9[d]	5.8± 0.2[e]	390.0± 7.3[g]	766.7 ± 8.3[c]	1017.5± 5.7[d]	1378.3±6.4[b]	2033.3±8.8[c]	5916.7±10.3[f]	3.3±0.1[d]
Yoghurt	86.1 ± 1.5[b]	23.7± 1.3[e]	5.7± 0.1[e]	515.0± 7.6[f]	953.6 ± 9.1[b]	888.6± 9.2[c]	1270.7±8.1[d]	1642.9±4.6[g]	4607.1±7.1[g]	10.7±0.1[b]
Labneh	74.3 ± 0.8[c]	29.6± 1.3[d]	4.3± 0.3[f]	952.5± 8.5[c]	436.6 ± 10.3[d]	442.8± 5.7[g]	958.0±3.9[e]	1735.4±7.1[f]	7237.4±8.6[c]	10.1±0.2[c]
Nabulsi cheese	61.1 ± 1.1[d]	35.0± 1.3[c]	15.4± 0.6[c]	3534.1±15.1[c]	304.0 ± 5.8[g]	1432.5± 6.2[b]	822.1±8.5[f]	2120.8±9.5[d]	7429.3±11.4[d]	1.0±0.2[e]
Dried Jameed	28.2 ± 2.0[f]	67.5± 0.7[b]	13.4± 0.2[d]	2118.8± 9.0[d]	215.9 ± 7.9[h]	1434.8± 2.0[b]	230.4±5.3[g]	4710..3±7.4[b]	13690.8±12.3[b]	1.3±0.2[e]
Liquid Jameed	88.6 ± 1.0[b]	71.1± 1.2[a]	26.2± 0.5[b]	8468.9±8.0[a]	407.6 ± 6.2[e]	804.2± 7.3[f]	2913.7± 7.1[a]	4956.1±9.6[a]	14385.9±15.4[a]	13.0±0.3[a]
Whey (acid)	93.4 ± 2.0[a]	7.9 ± 0.4[f]	28.8± 0.3[a]	7878.8±12.4[b]	2197.0± 11.0[a]	1666.7± 9.1[a]	1348.5±5.3[c]	737.9±8.3[h]	1439.4±9.6[h]	ND
Cream	57.4 ± 0.7[c]	37.1± 1.1[c]	3.5± 0.1[g]	230.8± 6.0[h]	361.7 ± 7.4[f]	1231.2± 8.3[c]	183.1±7.8[h]	3075.1±5.2[c]	9096.2±8.6[c]	ND

[1] Results are means of three replicate determinations ± SD. Values in the same columns followed by different letters are significantly different ($P < 0.05$)

[2] Aspartic and glutamic amino acids content are calculated for 100 gram of dried matter of sample. ND, not detected

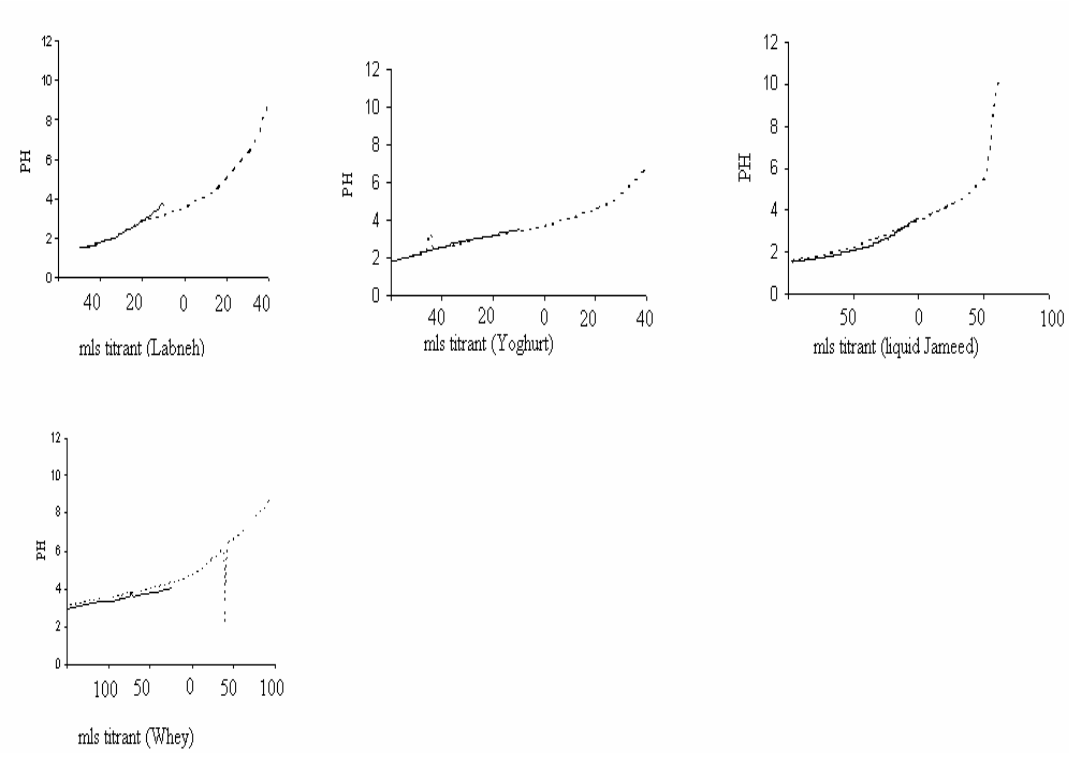

Fig. (1): Forward (—— 0.1N HCl) and backward (------0.1N NaOH) titration curves of 5% suspension of whey, Labneh, yoghurt and liquid Jameed.

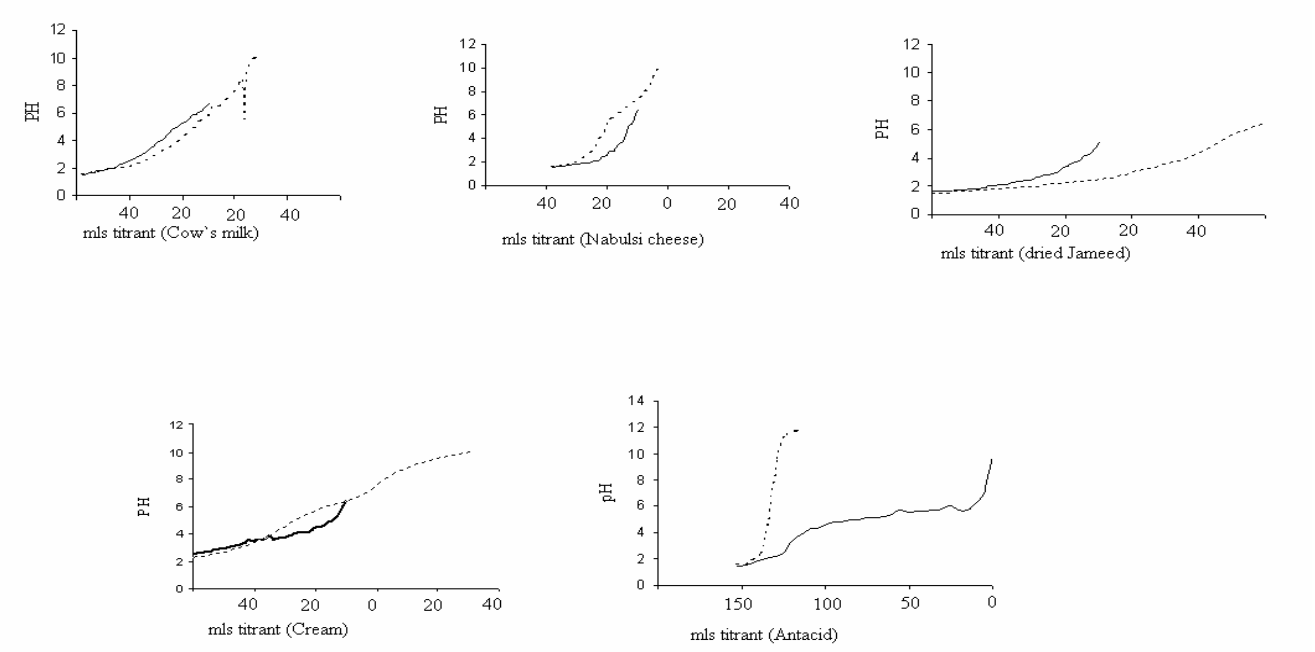

Fig. (2): Forward (—— 0.1N HCl) and backward (------0.1N NaOH) titration curves of 5% suspension of cow's milk, Nabulsi cheese, dried Jameed, cream and antacid tablet.

Locally produced cream is obtained by skimming the coagulated layer of milk from the surface of an open and flat stainless steel tray that heated to about 70°C until the milk colour turned to brown. The collected creams is usually frozen and used as cream filler for some traditional sweets. Several different biochemical changes occur during this heat treatment and affect the titration curves like; changes in calcium-phosphate precipitation, changes in the calcium phosphate composition, degradation of lactose, release of inorganic phosphate (Van Boekel, 1999) and casein degradation (Gaucheron *et al*., 2001).

Dried Jameed and Labneh are fermented dairy products with low moisture and high lactic acid contents which contribute in buffering capacity. The hysteresis curves of these products may be related to the high protein content resulted from formation of casein network due to excessive draining of water from such products. Other contribution factor is the existence of carbonate and bicarbonate resulted from the heat treatment of their milk which affects the acid-base equilibrium.

The lack of any marked hysteresis in case of whey, liquid Jameed and yoghurt (Fig. 1) were related to other features. Although the initial pH was relatively low in each case (pH 3.50 – 4.03), and it was expected that less acid would be needed to achieve a final pH value of 1.5, more acid was required in practice. This phenomenon may have been due to the formation of lactic acid in these fermented products, to the solubilization of minerals such as calcium and to the solubilization and denaturation of protein. These changes that occurred may have increased the availability of chemical groups capable of reacting with added acid, thereby enhancing BC.It has been reported that the pH titration curve of bovine serum albumin is affected by proton exchanges

during titration with acid due to the presence of calcium and to the structural changes occurred in the calcium binding protein, calmodulin (Harmsen *et al*. 1971; Nemirovskiy *et al*.1999)

Buffering Capacity

According to the BC values obtained (Table 2), the studied products could be separated into three groups, of which the first included those with values of 2.45 – 4.86. This group comprised the fermented products (whey, liquid Jameed and yoghurt) and cream. The proportional chemical composition within this group varied widely (Table 1). Liquid Jameed and cream were characterized by relatively high protein content 71.1% and 37% respectively, and high proportions of aspartic and glutamic acids, lactic acid, as well as of Na, Ca or P. The high protein content improves the formation of the casein network in Jameed, which leads to curd firmness, decreases the loss of dry matter and subsequently increases the buffering capacity. The high heat treatments of milk for long time to produce the cream and thermal degradation of milk affect the acid-base equilibrium of carbonate and bicarbonate and induces an increase in buffering capacity of cream (Lucey *et al.*, 1993a). In contrast, yoghurt and acidic whey were lower in protein, but they have considerable amounts of lactic acid, caseins and inorganic phosphate which increase their buffering capacity. Acidic whey, in particular, had high proportions of lactic acid, alkaline ash, Na, K, Ca, and inorganic phosphate. In addition, whey protein has a unique composition, with predominantly acidic amino acids, and its addition to yoghurt has been shown to enhance the buffering capacity of yoghurt under both acidic and alkaline conditions (Kailasapathy *et al*., 1996). The degradation of milk urea by urease enzyme into CO_2 and NH_3 enhances the acid-base equilibrium with carbonate and bicarbonate in yoghurt and also increases the yoghurt buffering capacity (Salaun *et al*., 2005).

Table (2): Initial pH, titrable acidity and alkalinity, acid –buffering capacity and acidity of studied dairy products [1].

Food items	pH	Titratable acidity (ml)	Titratable alkalinity (ml)	Acid-buffering capacity	Acidity as lactic acid (%)[2]
Milk	6.64 ± 0.10[b]	98.1 ± 0.6[d]	62.3 ± 0.5[d]	1.93 ± 0.07[f]	0.142 ± 0.005[f]
Labneh	3.63 ± 0.08[f]	39.5 ± 0.7[h]	52.0 ± 0.9[e]	1.85 ± 0.03[g]	0.45 ± 0.008[e]
Nabulsi cheese	6.45 ± 0.09[cb]	28.5 ± 0.4[i]	24.0 ± 0.4[f]	0.57 ± 0.09[i]	0.041 ± 0.005[g]
Whey (acid)	4.03 ± 0.05[e]	123.0 ± 0.3[b]	114.0 ± 0.3[a]	4.86 ± 0.04[a]	11.23 ± 0.003[a]
Yoghurt	3.53 ± 0.06[f]	62.5 ± 0.1[e]	70.0 ± 0.5[c]	3.08 ± 0.01[c]	0.65 ± 0.003[d]
Dried Jameed	5.03 ± 0.03[d]	53.0 ± 0.7[f]	91.1 ± 0.5[b]	1.5 ± 0.08[h]	0.63 ± 0.005[d]
Liquid Jameed	3.60 ± 0.04[f]	97.0 ± 0.9[d]	93.2 ± 0.7[b]	4.62 ± 0.10[b]	1.48 ± 0.009[c]
Cream	6.40 ± 0.02[c]	120.0 ± 0.3[c]	120.3 ± 0.4[a]	2.45 ± 0.04[d]	2.22 ± 0.006[b]

Food items	pH	Titratable acidity (ml)	Titratable alkalinity (ml)	Acid-buffering capacity	Acidity as lactic acid (%)[2]
Antacid tablet	9.82 ± 0.1 [a]	185.4 ± 0.1 [a]	30.3 ± 0.01[f]	2.23 ± 0.67[e]	0.00 ± 0.00 [h]

[1] Results are means of three replicate determinations ± SD. Values in the same columns followed by different letters are significantly different ($P < 0.05$)

[2] Acidity calculated on dry matter basis of products.

Although the initial pH of the above products were low (pH 4.03- 3.5), and the expected amount of acid added to reach a final pH 1.5 was low, these products consume higher amount of acid to make drastic changes in pH; as a result the titrable acidity (numerator) is being large in respect to unit of pH change (denominator), leading to a high BC.

An intermediate buffering capability was observed for the commercial antacid drug, milk, Labneh and dried Jameed, with BC values of 1.50 – 2.25 (Table 2). The antacid showed the highest buffering capacity against added acid, which was effectively neutralized by the basic salts present. This would involve an increase in pH from the formation of either alkaline bicarbonate (HCO_3^-, *p*ka 6.36) or carbonic acid (H_2CO_3, *p*ka 10.25), which are key components of the buffering system, and neutralization required a relatively large addition of acid. Cow's milk also showed good acid-buffering capability and again required a large amount of acid to lower the pH by one unit.It has been reported that several compositional factors affect the buffering capacity of milk, including the proteins present and more minor constituents, such as inorganic phosphate, organic acids, salts, solubilization of colloidal calcium phosphate and to the acidic amino acids present in casein and whey proteins (Krichmeier, 1980; Lucey *et al*., 1993 a, b; Lucey *et al*., 1996; Salaun *et al*., 2005). These substances contribute to the buffering effect of cow's milk over a wide pH range.

The BC values for dried Jameed and Labneh may be partly attributable to the protein content 67.5% and 29.6%, respectively, and to the formation of casein network which trapped the dried buffering matter and also to the relatively high levels of aspartic and glutamic amino acids, lactic acids, together with varying proportions of alkaline ash and the inorganic elements (Table 1 and 2). The relatively high amount of salt added to the dried Jameed and subsequent sun drying induces the loss of some buffering materials in its whey, leading to a lower buffering capacity than liquid Jameed.

The lowest BC value (0.57) was obtained from Nabulsi cheese, despite its high content of protein, aspartic and glutamic acids, and high initial pH (Tables 1 and 2). Such a low BC value related to different factors that affect curd formation (coagulation) such as, type of splitting enzymes, renneting pH, draining, salting and heat treatment of milk before cheese making. Syneresis and firmness of the curd resulted from pressing or NaCl addition or a decrease in pH during renneting will increase the loss of buffering matter in the whey during cheese draining and lower the buffering capacity of cheese. In addition, the pasteurization of cheese milk causes denaturation of whey protein and insolubilization of

calcium phosphate that will be lost during pressing and the buffering capacity is decreased (Salaun *et al*., 2005). The hydrophobicity of the cheese protein resulted from the addition of rennet to cow's milk in the manufacturing process create a protein surface with few amino acids which tend to lower the buffering capacity of cheese.

For the products studied here, there was a good correlation between the BC values obtained and the content of protein, ash, Na, K, P and alkalinity of the ash (R = 0.99, 0.67, 0.72, 0.60, 0.59 and 0.42 respectively) and a weak correlation with the aspartic acid content (R = 0.2).

With dairy products, the high protein content ensures a relatively high buffering capacity, due to the buffering effect of amino groups present and to the formation of the casein network which reduces dry matter loss during draining. Protein molecules usually carry an electric charge, except at the pH corresponding to the isoelectric point. At a low pH, below the isoelectric point, the terminal lysyl, histidyl and arginyl groups can be positively charged (Hidvegi and Lasztity, 2003). Any of these groups can form a complex directly with a negatively-charged phosphate anion and alter the acid demand. As a result, the titratable acidity value is large in relation to a one-unit change in pH and a high BC value is obtained.

At intermediate pH values, only the lysyl and arginyl groups are positively charged and can form a complex with phosphate ions so that less acid is required to produce a one-unit change in pH. The protonation of carboxyl groups in acidic amino acids, such as aspartic and glutamic acids (*p*ka 4.6), that are present in the protein may also contribute to the acid-base equilibrium, leading to a buffering capability. On the other hand, organic acids present in dairy products, such as citric, lactic and carbonic acids are characterised by the possession of one or more carboxyl groups. These will be in equilibrium with their salts and act as a buffer that provides a high BC value.

CONCLUSION

The results of the present study support the view that differences in BC among the dairy products were largely due to differences in chemical composition. High protein content, in particular, appears to be associated with a high initial pH and BC, which could be attributed to the buffering effect of the amino groups present and protonation of carboxyl groups in acidic amino acids. Other compositional factors that are likely to play a part in the buffering capability of dairy products are the content of phosphate, aspartic and glutamic amino acids, ash, small amounts of organic acids and the alkalinity of the ash.

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أثر التركيب الكيميائي لبعض منتجات الألبان في القدرة على تنظيم الحموضة

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ملخـص

تمت دراسة مقدرة تأثير التركيب الكيميائي لبعض منتجات الألبان المصنعة محليا من حليب الأبقار في تنظيم درجة الحموضة في ظروف المختبر (in vitro) عند الإضافة التدريجية لمحلول حمض الهيدروكلوريك المخفف (0.1 N) للوصول إلى درجة حموضة 1.5 ثم الإضافة التدريجية لمحلول هيدروكسيد الصوديوم المخفف (0.1 N) لنفس العينة المدروسة للوصول إلى درجة حموضة 10.0 ومقارنتها بفاعلية قرص دواء يستخدم لتنظيم الحموضة في المعدة. أشارت النتائج إلى أن القدرة التنظيمية للحموضة كانت عالية (2.45- 4.86) لكل من اللبن، شرش اللبن، الجميد السائل والقشدة وكانت متوسطة (1.5- 2.25) لكل من الحليب واللبنة والجميد الصلب وقرص تنظيم الحموضة ، بينما كانت القدرة التنظيمية منخفضة (0.57) للجبنة البيضاء المبسترة. بينت الدراسة أن هناك معامل ارتباط قوي بين القدرة التنظيمية لهذه المنتجات ومحتواها من البروتين (R= 0.99)، والمعادن (R=0.67)، والصوديوم (R= 0.72)، والبوتاسيوم (R= 0.60)، والفسفور (R= 0.59)، بينما كان معامل الارتباط قليل مع المحتوى من حمض الأسبارتيك (R= 0.42). تؤكد هذه الدراسة على أهمية استخدام منتجات الألبان للتخفيف من حرقة المعدة بدلاً من تناول الأدوية.

الكلمات الدالة: القدرة التنظيمية للحموضة، التركيب الكيميائي، منتجات الألبان، لبن رايب، لبنة، جبنة نابلسية، جميد، حليب وقشدة بقرية، مصل حامضي.

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