Acid-Base Buffering Properties of Five Legumes and Selected Food *in vitro*

[1]Maher M. Al-Dabbas, [1]Khalid Al-Ismail, [1]Ruba Abu Taleb and [2]Salam Ibrahim
[1]Department of Nutrition and Food Technology, Faculty of Agriculture,
University of Jordan, Amman 11942, Jordan
[2]Department of Family and Consumer Sciences, Faculty of Food and Nutritional Sciences,
North Carolina A and T State University, Greensboro, NC 27411-1064

Abstract: Problem statement: *In vitro* acid-Buffering Capacity (BC) values of 5% (dry matter) aqueous homogenized suspension of five legumes (broad bean, lentils, chickpea, kidney bean and lupine) and of selected antacid home preparations (cow's milk, almond, peanut, licorice, carob and lettuce stem) were investigated within and among samples from their respective initial pH until pH was decreased to 1.5. BC was the highest for cow's milk, carob, licorice and lettuce stem (BC values 1.65-1.97), intermediate for almond and peanut (BC values, 1.37-1.64) and the lowest for selected legumes (0.84-1.36). **Approach:** The purpose of this study was to measure *in vitro* the buffering capacity potential of legumes and other foods commonly used in Jordan as heartburn remedies to determine the ability of these products to de-acidify, neutralize acid, or increase pH levels of an acid and a base solution. **Results:** BC of the studied legumes showed positive and strong correlations, with protein, aspartic and glutamic amino acids contents (R = 0.95, 0.94, 0.89, respectively) and relatively weak correlation with phosphorus content (R = 0.38). **Conclusion/Recommendations:** The differences in BC within and among studied samples were largely due to the differences in their chemical compositions. Protein, fiber, ash, organic acids and aspartic and glutamic acids contents and alkalinity of ashes showed significant BC, while high fat content in almond and peanut failed to show considerable BC.

Key words: Buffering capacity, antacid home preparations, legumes, pH

INTRODUCTION

Most foods possess a chemical property called buffering capacity, which allows them to resist changes in pH. For example, in animal tissues, lactic acid, phosphate salts, amino acids and proteins are involved with the maintenance of pH values. In plant tissues, pH values are dependent on the presence of poly-carboxylic acids, phosphate salts, fiber and proteins. In dairy products, particularly milk and cheese, buffering capacity is related to individual amino acids as well as protein, phosphate, citrate, lactate, carbonate, propionate and acetate (Le Graet and Brule, 1993; Banon and Hardy, 1992; Lucey *et al*., 1993a; Walstra and Jenness, 1984; Salaun *et al*., 2005).

Acid-base neutrality in the digestive tract is continually challenged by the production of hydrogen ions (H^+) from normal body processes. The digestive system is quite robust and generally is able to maintain an equitable acid to base balance. However, under a variety of conditions such as the ingestion of food which contains acidic substances, excessive stress, consumption of processed foods, chemical pollutants, erratic eating patterns and the end products of digestion and metabolism, the concentration level of H^+ sometimes becomes unbalanced and the individual experiences effects of excess acid causing acid indigestion (heart burn) and in extreme cases stomach ulceration (Chalupa and Kronfeld, 1983).

Antacids (base or basic salt) are widely used to prevent the "burning feeling" or acid indigestion in response to an acidic unbalance of stomach acid. While generally effective against mild cases of "heartburn" in more severe and chronic cases, more potent chemical remedies are sometimes needed. Moreover, like any other drug antacids can have side effects. For example, aluminum hydroxide can cause constipation and produce decreases in the absorption of vitamin A and D, inactivate thiamin and cause phosphate depletion (Cooke *et al*., 1978). Antacids containing calcium may result in having too high a level of calcium which has been associated with kidney stones and constipation.

Corresponding Author: Maher M. Al-Dabbas, Department of Nutrition and Food Technology, Faculty of Agriculture,
University of Jordan, Amman 11942, Jordan

Other adverse effects of antacids include alkalosis, arterial hypertension, heart failure, vomiting and renal disease (Gabriely *et al*., 2008).

In addition to widely used antacids, home remedies also are quite common. In Jordan, heartburn may be treated by drinking a baking soda solution, milk, licorice or locust bean (carob) extracts or by eating almonds, lentils, chickpeas, lupine, apples and lettuce stems. While the effectiveness and advisability of any of these "treatments" is beyond the scope of this study, the basis for the effectiveness of any of these remedies is likely due to their buffering capacity.

Because of the widespread use of legumes as a home remedy for acid indigestion, the purpose of this study was to measure *in vitro* the buffering capacity potential of legumes and other foods commonly used in Jordan as heartburn remedies to determine the ability of these products to de-acidify, neutralize acid, or increase pH levels of an acid and a base solution.

MATERIALS AND METHODS

Sample preparation: The seeds of five legumes: Broad beans (*Vicia faba*), chickpeas (*Cicer arietinum*), kidney bean (*Phaseolus vulgaris*), sweet lupine (*Lupinus termis*) and lentils (*Lens esculenta*), along with almond (*Prunus amygdalus*), peanuts (*Arachis hypogaea*), lettuce stem (*Lactuca sativa* var. *longifolia*), carob (*Ceratonia siliqua*), licorice root (*Glycyrrhiza glabra* L.) and raw cow's milk were selected for testing of their acid buffering abilities. All produce products were obtained from local markets in Amman, dried at 102°C to constant weight, finely ground to achieve 0.25 mm particle size in a Krups coffee grinder and stored in airtight polyethylene bags in a refrigerator (4°C) until testing. Fresh raw cow's milk was obtained directly from the dairy plant of Jordan University.

Chemical analysis: Moisture, total ash, crude fiber and protein levels of the food samples were determined by AOAC methods (AOAC, 1990). Calcium, K and Na content of food samples were determined via emission spectroscopy (Atomic absorption spectrophotometer, Thermo S1, MA, USA) after wet ashing (AOAC, 1995). Phosphorus content of food samples was determined via spectroscopy (AOAC, 1995) and the absorbance of samples, blank and standards were measured at 650 nm (UVD-2950 spectrophotometer, Labomed CA, USA). Aspartic and glutamic amino acids were detected using hydrolysis and the accelerated procedure of Spaceman, on samples exposed to 6 N HCl at 110±1°C for 24 h under vacuum

conditions using a Beckman 6300 AA analyzer (CA, USA) (Spackman *et al*., 1958).

Titrations: A calculated amount from each produce sample equivalent to five grams dried weight was finely ground, suspended separately in 100 mL of distilled water and stirred continuously with a magnetic stir bar to obtain 5% food suspension homogenate. Forty two grams of fresh cow's milk (equivalent to five grams on dried weight) were taken directly for titration. A commercial antacid tablet (680 mg $CaCO_3$ and 80 mg $MgCO_3$) was dissolved in 100 mL distilled water and used as comparison standard. Forward titration was performed on the 5% homogenate for each sample by gradual addition of 0.5 mL of standard 0.1 N HCl until the pH decreased to 1.5 (the normal pH of stomach). The samples were then back titrated until the pH increased to 10.0 (the initial pH of commercial antacid) by gradual addition of 0.5 mL of standard 0.1 N NaOH using a HANNA 211 microprocessor pH meter (Hanna instruments, RI, USA) adjusted to room temperature. Initial pH level and all further measurements taken during titration were recorded following a 1 min equilibration period after addition of acid or base. The total volume of acid or base added to each sample from the initial pH to 1.5 or from pH 1.5-10.0 was recorded separately and then multiplied by the normality to calculate the total titrable acidity or alkalinity. Alkalinity of ash (%NaOH) was determined by direct titration of the water dissolved dry ash with standard of 0.1 N HCl using phenolphthalein indicator (AOAC, 1990). Titrable acidity (% citric acid) for each of tested samples was determined by direct titration with standard 0.1 N NaOH using phenolphthalein indicators (AOAC, 1990).

Acid-buffering capacity assay: The acid-buffering capacity of the tested samples was calculated by dividing titrable acidity (from its respective initial pH to 1.5) of each sample by its total changes in pH units.

Statistical analysis: Data in triplicate were analyzed using statistical analysis system, SAS program (SAS Institute Inc., Cary, NC, USA). Significant Differences between means (LSD) were determined. Differences at $p < 0.05$ were considered to be significant. Correlation coefficients (R) were determined by MS Excel software (2007).

RESULTS AND DISCUSSION

Chemical analysis and titration curves: Table 1, reports levels of moisture, ash, crude fiber, crude

protein, aspartic and glutamic amino acids, Na, K, Ca, P contents and alkalinity of ash (%) of the studied samples on a dry weight basis.

Crude protein contents varied significantly (p<0.05) ranging from 42.5% in lupine to 4.8% in carob powder. Similarly, lupine had the highest content of aspartic (5.3%) and glutamic (8.3%) amino acids, while carob powder had the lowest content of aspartic (0.5%) and glutamic (0.38%) amino acids.

The crude fiber contents of the tested samples varied from 29.3% in lettuce stem to 0.9% in cow's milk and ash content from 8.2% in licorice to 3.0% in almond.

Alkalinity of ash varied significantly (p<0.05) ranging from 15.8% in licorice to 0.0 in lettuce stem, while titrable acidity (%) in Table 2 ranged from 0.72 in carob powder to 0.03 in lettuce stem.

Phosphorus content ranged from 1.36% in cow's milk to 0.29% in licorice, Ca content from1.6% in licorice to 0.094% peanut, K content from 3.9% in lettuce stem to 0.087% in licorice and Na content from 0.34% in carob powder to 0.017% in chickpea.

Forward and back titration curves of each sample 5% homogenate acidification and neutralization are shown in Fig. 1-3. Gaps (hysteresis) indicating a shift in the pH and in buffering capacity between forward and back titrations curves were observed in the pH range of

samples initial pH to about pH 2 for antacid, cow's milk, licorice, peanut, almond, lupine, beans, lentils, cowpea and chickpea samples. This hysteresis might be due to the interaction of food components such as proteins, fibers and minerals with the added acid and base, resulting in a higher amount of acid mopped up before attaining pH 2 than the amount of base consumed for neutralization. The variation in each sample's protein and in particular the sequence, number, spatial structure and the environment of protein makes some ionizabel groups become accessible for titration within a protein after a change in pH or denaturation (Singh *et al*., 1997). The protonation of carboxyl groups of acidic amino acids (aspartic and glutamic acids, pka 4.6) present in these samples protein may also contributes to samples hysteresis.

It also may be the case that interactions of minerals and, in particular Na, K, Ca and P with acid-base groups of proteins increased the samples' hysteresis by changing the acid-base equilibrium of the protein and by altering the protein spatial structure that affected proton exchanges during titration. It has been reported by Wohlt *et al*. (1987), that the mineral content of feeds could influence there buffering capacity. Harmsen *et al*. (1971) reported that the pH titration curve of bovine serum albumin is affected by the presence of calcium.

Table 1: Chemical composition of the tested samples on dry matter basis[1]

Food items	Moisture	Protein	Crude fiber	Ash	Na (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	P (mg/100 g)	Aspartic acid (mg/100 g)	Glutamic acid (mg/100 g)	Alkalinity of ash
Bean, broad	9.5±0.6[d]	26.8±0.7[c]	6.4±0.3[e]	4.0±0.1[d]	48.3±7.1[e]	895.5±6.5[f]	151.3±5.3[h]	410.0±10.0[d]	2920.6±45.2[e]	4047.2±43.3[g]	6.0±0.1[d]
Chickpea	9.5±0.6[d,e]	21.7±0.6[d]	3.8±0.4[g]	3.4±0.1[f,g]	17.4±5.1[f]	990.9±7.3[e]	214.0±6.1[g]	391.4±11.9[d]	2606.9±47.3[g]	3566.7±44.7[h]	5.3±0.1[f]
Kidney bean	9.3±0.5[d,e]	25.4±0.8[c]	5.0±0.7[f]	4.9±0.0[c]	91.6±4.3[d]	1501.9±3.2[b]	257.3±2.0[f]	488.5±41.8[b]	2783.9±41.1[f]	4384.0±47.1[f]	7.9±0.0[b]
Lentils	10.8±0.6[d]	28.8±0.9[b]	3.6±0.8[g]	3.4±0.1[f,g]	28.7±5.5[f]	991.4±4.8[e]	150.4±5.3[h]	423.7±0.3[d]	3479.8±43.7[b]	4686.1±48.0[e]	4.7±0.1[g]
Lupine	8.0±0.8[f,e]	42.5±0.6[a]	9.8±0.5[d]	3.7±0.1[e]	55.7±8.2[e]	1077.6±7.4[c]	245.5±9.7[f]	481.0±25.7[c]	5297.3±46.8[a]	8275.6±45.3[a]	5.5±0.1[e]
Peanut	3.7±0.7[g]	25.6±0.4[c]	3.5±0.2[h,g]	2.6±0.1[i]	31.6±6.3[f]	687.1±5.2[g]	93.9±7.1[i]	432.6±9.6[d]	3322.9±44.2[c]	5568.0±42.1[d]	1.3±0.1[l]
Almond	3.5±0.8[g]	22.3±1.2[d]	2.8±0.3[h]	3.0±0.1[h]	26.4±6.1[f]	702.1±6.1[g]	334.0±8.0[e]	511.0±0.6[b]	3049.7±47.6[d]	7091.1±43.6[b]	0.9±0.1[j]
Carob powder	14.3±1.0[c]	4.8±1.0[f]	12.8±0.6[c]	3.3±0.1[g]	336.2±9.6[a]	1044.3±9.2[d]	477.4±8.5[d]	396.0±0.7[d]	521.6±48.0[k]	375.7±46.0[k]	7.7±0.1[c]
Licorice	7.5±0.5[f]	6.8±0.5[e]	25.1±0.3[b]	8.2±0.1[a]	197.9±4.6[c]	86.5±2.6[h]	1596.3±5.3[a]	292.9±0.9[e]	918.9±43.5[j]	1654.0±43.4[j]	15.8±0.1[a]
Milk, cow	87.9±1.7[b]	28.9±1.4[b]	0.0±0.9[i]	5.8±0.2[b]	88.0±12.2[d]	760.3±13.9[g]	1009.1±9.6[b]	1356.2±9.2[a]	2016.5±52.2[l]	5867.7±49.0[C]	3.3±0.1[h]
Lettuce	94.2±2.0[a]	22.4±1.8[d]	29.3±0.8[a]	3.5±0.2[f]	310.3±11.6[b]	3879.3±9.6[a]	569.9±10.3[c]	534.5±9.0[b]	2413.8±51.6[h]	2931.0±56.6[i]	0.0±0.1[k]

[1] Results are means of three replicate determinations ± SD, Values in the same columns followed by different letters are significantly different (p<0.05) ND, not detected

Table 2: pH, acid added, base added, acid-buffering capacity and acidity (as citric acid) of 5% homogenate suspensions[1, 2]

Food items	pH (initial)	Acid added (mls)	Base added (mls)	Acid-buffering capacity	Acidity as citric acid (%)
Bean, broad	6.58±0.10[b]	49.0±0.4[g]	48.5±0.9[f,g]	0.96±0.04[f,g,h]	0.35±0.08[b,c,d]
Chickpea	6.00±0.72[d,e,f]	38.5±0.2[j]	48.0±0.8[i]	0.84±0.05[h]	0.22±0.06[d,e,f]
Kidney bean	6.21±0.66[d,e]	41.2±0.9[i]	50.1±0.3[h]	0.87±0.08[g,h]	0.14±0.04[e,f,g]
Lentils	5.87±0.90[g,f]	45.1±0.7[h]	52.0±0.6[g]	1.03±0.09[e,f,g]	0.38±0.07[b,c]
Lupine	6.23±0.20[c,d]	55.3±0.3[f]	68.2±0.4[d]	1.16±0.1[f]	0.27±0.06[b,c,e]
Peanut	5.60±0.81[g]	59.2±0.4[e]	54.3±0.2[f]	1.43±0.07[d]	0.07±0.07[f,g]
Almond	5.90±0.61[e, g,f]	60.3±0.5[e]	50.0±0.8[h]	1.37±0.06[e]	0.28±0.1[c,d,e]
Carob powder	5.05±1.13[h]	70.0±0.7[d]	78.2±1.0[c]	1.97±0.10[b]	0.72±0.09[a]
Licorice	5.75±0.50[g,f]	80.3±1.0[c]	88.0±0.6[a]	1.88±0.8[b]	0.46±0.07[b]
Milk, cow	6.49±0.12[b,c]	98.1±1.6[b]	62.3±1.7[e]	1.96±0.17[b]	0.09±0.25[f,g]
Lettuce stem	5.79±1.82[g,f]	71.2±1.4[d]	84.2±1.5[b]	1.65±0.19[c]	0.03±0.22[g]
Antacid tablet	9.82±0.14[a]	185.4±0.1[a]	30.3±0.01[j]	2.23±0.67[a]	0.00±0.00[g]

[1]: Results are means of three replicate determinations ± SD, [2]: Values in the same columns followed by different letters are significantly different (p<0.05)
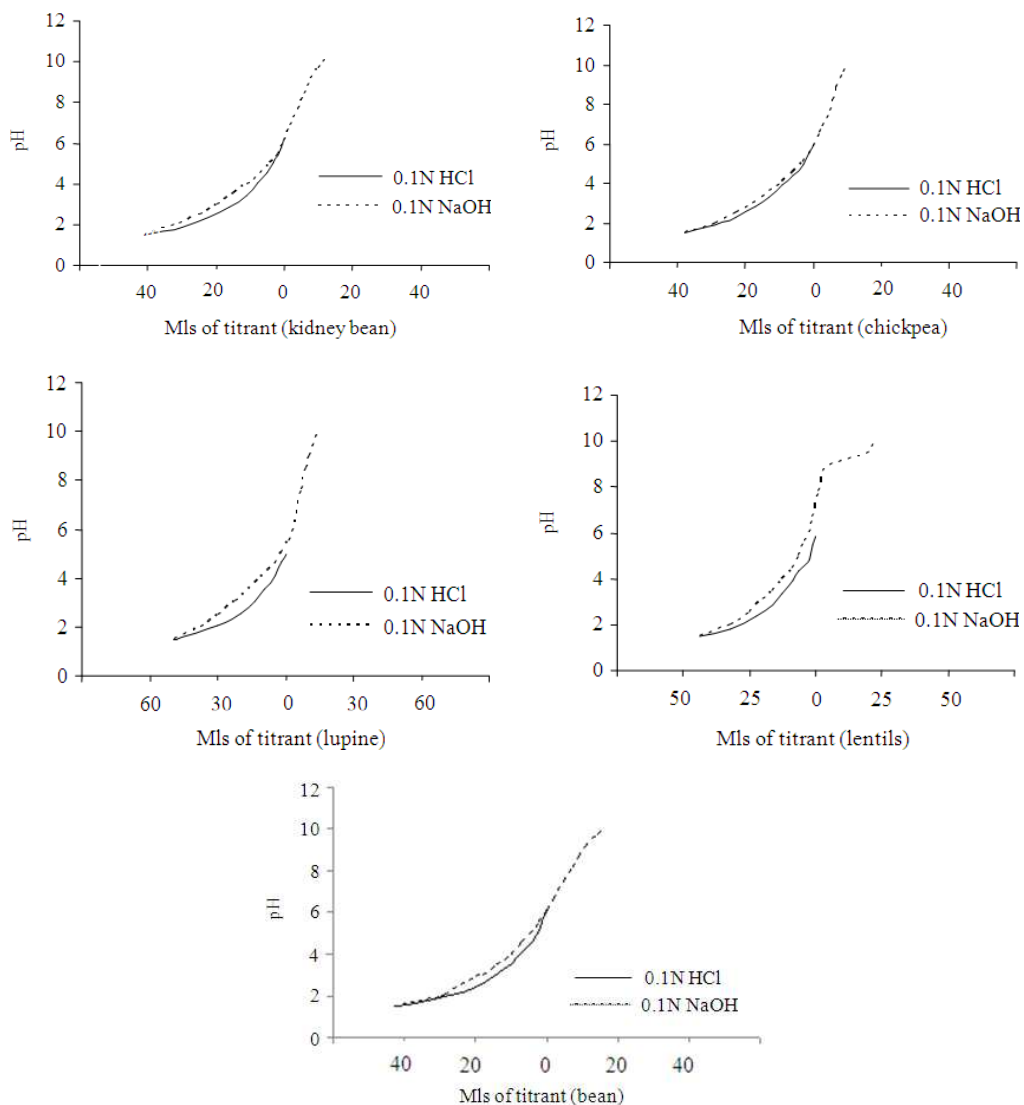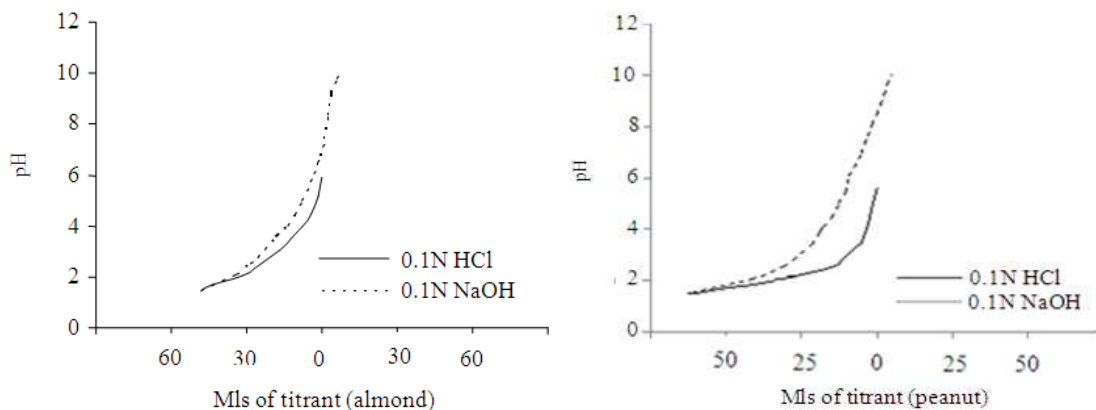
Fig. 1: Forward (———— 0.1 N HCl) and backward (------ 0.1 N NaOH) titration curves of 5% homogenized suspension of legumes
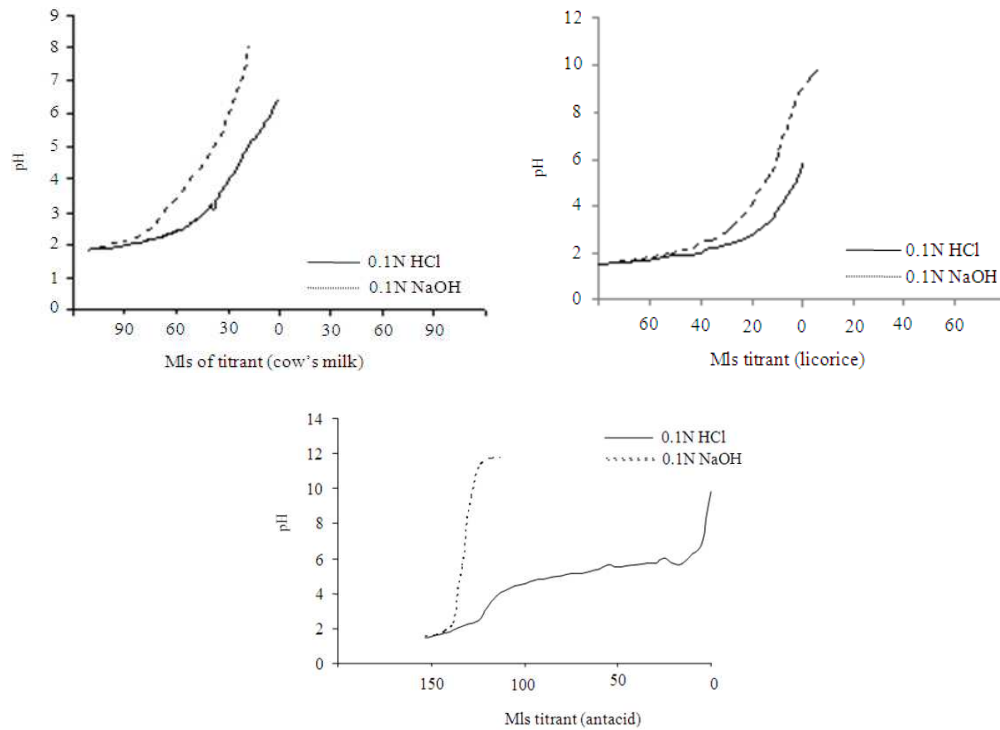
Fig. 2: Forward (―― 0.1N HCl) and backward (------0.1N NaOH) titration curves of 5% homogenized suspension of almond, peanut, cow's milk, licorice and antacid tablet
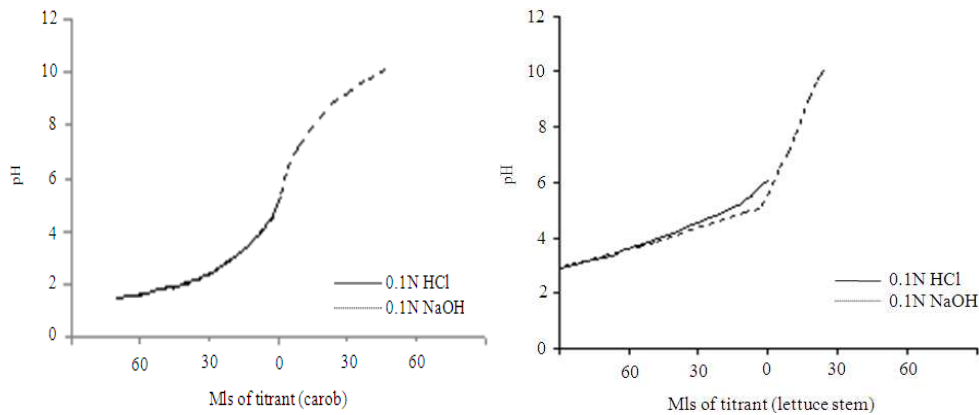

Fig. 3: Forward (―― 0.1N HCl) and backward (------0.1N NaOH) titration curves of 5% homogenized suspension of carob and lettuce stem

Carob and lettuce stem curves were almost completely coinciding, indicating that titration curves not affected by the tested food composition and the amount of acid added was neutralized by back titration with base.

Buffering capacity: Antacid showed the highest ($p<0.05$) acid-buffering capacity due to its neutralization reaction as a result of its alkaline character (basic salts). The antacid raises the pH upon acid addition by forming alkaline bicarbonate (HCO_3-, pka 6.36) or carbonic acid (H_2CO_3, pka 10.25), which are vital components of the pH buffering system, thus higher amount of acid is required to alter this buffering system and neutralizing the formed alkaline compounds.

The buffering capacities of the food samples were evaluated using the acid-Buffering Capacity (BC) method. Based on these values we categorized the samples into three buffering level groups (Table 2). The first group represented the highest BC values (between 1.65 and 1.97) representing carob, cow's milk, licorices and lettuce stem.

The high BC of cow's milk is likely related to its high protein content (28.9%), glutamic (5.8%) and aspartic (2%) acids, phosphate, organic acids and ash (Table 1). Previous studies have reported that in normal cow's milk protein and organic acids content and solubilization of colloidal calcium phosphate are responsible for its acid reducing properties (Salaun *et al.*, 2005; Lucey *et al.*, 1996; Krichmeier, 1980; Lucey *et al.*, 1993b).

The high BC of carob may be attributable to its high acidity (0.72%), relatively high alkalinity of ash (7.7%), high fiber content (12.8%) and to some extent to its crude protein content (Tables 1 and 2). The BC of the fiber found in carob might be also act as a buffering agent due to the high cations exchange capacity on its surface, which serves as a bank for exchanging K, Ca, Na, Mg for hydrogen when acid is added (McBurney *et al.*, 1986).

The high BC for licorice is likely due to its high ash content (8.2%), alkalinity of ash (15.8%) and to its high content of crude fiber (25.1%). Moreover, because licorice has a relatively high acidity (0.46%) resulting largely from its natural content of weak organic acids mainly glycyrrhizic and glycyrrhetinic acids. The protein and aspartic and glutamic amino acids contents also are known to contribute to the BC of licorice (Cooney and Fitzsimons, 1996).

Finally, the BC of lettuce stem might be due mainly to its high content of protein (22.4%), crude fiber (29.3%), aspartic (2.4%) and glutamic (2.9%) amino acids.

The second group, which had BC levels in the range 1.37-1.64, included almond and peanut. Although, the protein content of the components of this group is relatively higher than those of the first group they showed lower BCs. This finding might be due to their higher content of fat (about 49%). The fat may act as barrier reducing the interaction between protein and the acid added leading to low BC, i.e., that small amount of acid produce drastic drops in pH. Kargul (2007), reported that the increase of fat level in yoghurt decreases its BC.

The third group of foods, which were the legumes included lupine, broad bean, chickpea, kidney bean and lentils had BCs in the range 0.84-1.36. The BCs of this group was probably due to their content of protein (increases there initial pH), aspartic and glutamic amino
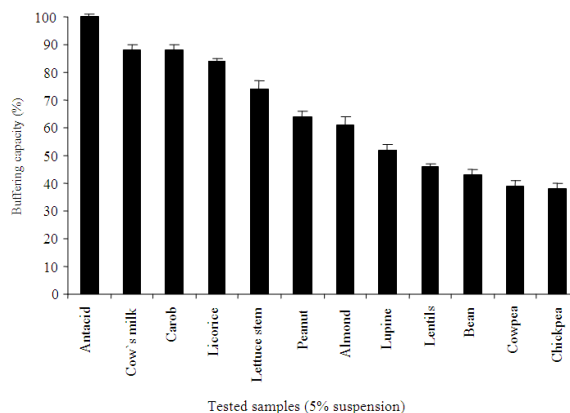

Fig. 4: Buffering capacity of 5% samples homogenate calculated as one commercial antacid tablet equivalent (%) by acid-Buffering Capacity (BC). Bars having different letters are significantly different (p<0.05)

acids and phosphorus. The BCs of these legumes showed strong correlations, R = 0.95, 0.94, 0.89 with the protein, aspartic, glutamic amino acids, but weak correlation with phosphate (R = 0.38). The alkalinity of the above samples ash was relatively high (Table 1), which may contribute in raising the needed volumes of acid for neutralization.

Although, the protein content in the tested legumes was high, they showed the lowest BCs. This might be attributed to the distribution of protein in the starchy legumes and its hydrophobicity. The high starch content also may reduce the availability of protein to interact with the acid added. Furthermore, the hydrophobicity of legume proteins indicates that the surface of protein contains low amount of charged amino acids that interact with the acid added resulting in low BCs. The relative BC of tested samples to the control antacid tablet is shown in Fig. 4.

CONCLUSION

The results of the present study demonstrate that differences in BC within and among studied samples were largely due to differences in chemical composition. The acid-buffering capacity was in the following order:

Antacid>carob = cow's milk>licorice>lettuce stem>peanut>almond>lupine>lentils>bean>kidney bean>chickpea

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