Determination of Sensory Thresholds of Selected Calcium Salts and Formulation of Calcium-fortified Pocket-type Flat Bread

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ABSTRACT: Pita bread loaves were prepared from flours fortified with calcium carbonate, calcium sulfate, and tricalcium dicitrate at 8 ascending levels to provide ranges of 800 to 2500, 700 to 1500, and 400 to 2000 mg of added Ca/100 g flour, respectively. The detection thresholds of calcium salts in pita bread were determined by the 3-alternative forced choice (3-AFC) test and construction of dose-response curves. Detection thresholds determined by calculating geometric mean of individual best estimate thresholds, using criterion of 50%-above-chance and probit analysis of 3-AFC data, were in the middle region of calcium concentrations. Analysis of dose-response curves yielded values for thresholds outside the range of surveyed calcium concentrations. The detection threshold of $CaSO_4$ (2724 mg/100 g) in pita bread was significantly higher ($P < 0.01$) than those of calcium carbonate (1984 mg/100 g) and tricalcium dicitrate (2132 mg/100 g). Calcium-fortified pita bread was similar (*$P < 0.01$*) to its regular counterpart when formulated to contain 1254.6, 1772.5, or 1155 mg/100 g of $CaCO_3$, $CaSO_4$, or tricalcium dicitrate, respectively. At the indicated levels of fortification, calcium-fortified pita bread is expected to provide between 61% and 126.5% of the recommended daily intake for calcium for Middle Eastern populations.

Keywords: calcium, sensory, thresholds, pita bread, fortification

Introduction

Adequate calcium intake during childhood and adolescence is crucial for attaining peak bone mass and attenuating bone loss and predisposition to osteoporosis in later years. In addition to being 1 of the determinants of peak bone mass, adequate calcium intake has protective effects against hypertension (Jorde and Bonna 2000) and colorectal cancer (Parodi 2001; Hambly and others 2002) and may ameliorate toxicity of environmental lead (Ballew and Bowman 2001). The perceived positive health effects of calcium prompted the Inst. of Medicine of the Natl. Academy of Sciences to revise upward the recommended intakes for calcium for many population groups and to set a safe upper limit of 2500 mg/d (Inst. of Medicine 1997). Milk and milk products provide more than 50% of total calcium intake of people in Western Europe and North America (New 2001; Cashman 2002). In the Middle East and Persian Gulf regions, dairy products do not contribute significantly to calcium intakes of populations in view of their reported low consumption (Baba 1998; Musaiger and Abuirmeileh 1998; Musaiger and Miladi 2000). Scant information exists on calcium intakes of populations in the aforementioned regions. Calcium intakes of school children in Lebanon, with a mean age of 13.3 y, have been reported to range between 487 and 787 mg/d (El-Hajj Fuleihan and others 2001). Such intakes are lower than those recommended, for this population group, in the European Union (800 to 1000 mg/d; EU 1993) and the United States (1300 mg/d; Inst. of Medicine 1997).

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Fortification of staple foods is universally recognized as an effective means for meeting daily requirements for a range of nutrients (Richardson 1997). Pocket-type flat bread, known as pita bread in Europe and North America, is the main dietary staple of people in the Middle East, Nile Valley, and Persian Gulf States. Consequently, fortification of bread with calcium has the potential of significantly increasing calcium intakes of populations in the aforementioned regions. For successful fortification, the calcium fortificant must not impart off-flavors to foods, especially since many calcium salts possess intrinsically characteristic flavors (Richardson 1997; Flynn and Cashman 1999). To safeguard against possible changes in sensory profile of the fortification vehicle, fortificants must be added at levels below their detection thresholds. The sensory thresholds of a number of calcium salts, in water, have been reported (Tordoff 1996). However, sensory thresholds of calcium salts in bread systems are largely unknown. Sensory thresholds of chemical stimuli are determined by the ascending forced-choice procedures or through construction of dose-response curves (Lawless and Heymann 1998).

The objectives of the present work were to (1) determine sensory thresholds of 3 calcium salts (calcium carbonate, calcium citrate, and calcium sulfate) that are commonly used in food fortification and (2) establish the maximum level of fortification at which the calcium-fortified pocket-type flat bread is judged similar to its regular counterpart.

Materials and Methods

A commercial baker's flour (protein-N×5.7-, 12.2%; ash, 0.75%) was obtained from Dora Mills (Beirut, Lebanon). Active dry yeast (Fermipan, Gist Brocades Co., Delft, Holland), table sugar, and salt were procured from the local market. Calcium carbonate, tricalcium dicitrate (calcium citrate), and calcium sulfate, of analytical grade, were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). NBS wheat flour (Standard Reference Material 1567) was obtained from Natl. Bureau of Standards (Washington, D.C.).

Determination of calcium

Calcium contents were determined as described by Martin and others (2002). Calcium citrate, wheat flour, and bread samples were dry-ashed at 600 °C for 24 h and the resulting ash dissolved in 1 mol/L HCl. Calcium carbonate and calcium sulfate were dissolved in 1 mol/L HCl. Aliquots of digested wheat flour and bread and calcium salt samples were appropriately diluted with 0.5 mol/L HCl containing 0.9% $LaCl_3$ and calcium contents determined by atomic absorption spectrophotometry (GBC 902 Double Beam Atomic Absorption Spectrophotometer, Scientific Equipment PTY Ltd, Victoria, Australia). Determinations were carried out in triplicate. The accuracy of calcium determination was assessed through analysis of reference wheat flour with average replicate analysis being 186.17 $\pm$ 3.0 μg/g (C.V. 1.63%) compared with the reference's content of 190 $\pm$ 10 μg/g.

Preparation of bread samples

Wheat flour was fortified with calcium carbonate and calcium sulfate at 8 ascending levels, differing by half log cycle, to provide ranges of 800 to 2500 and 700 to 1500 mg of added Ca/100 g flour, respectively. Calcium citrate was added at 8 ascending concentrations, differing by 1 log cycle, to provide flours containing between 400 and 2000 mg of added Ca/100 g flour. Flours were prepared by diluting flours fortified at the highest levels of calcium with control flour. Flours were sifted to ensure uniform distribution in the baked product and stored in polyethylene bags at 5 °C until used. Levels of fortification were chosen, on the basis of preliminary screening experiments, to provide a reasonable bracketing of threshold. Pita bread loaves were prepared as described previously (Toufeili and others 1999). Doughs contained flour (100 parts), sugar (3 parts), salt (1.5 parts), yeast (1 part), and distilled water to optimum dough consistency (Farinograph 850 Brabender Units line). The ingredients were mixed for 12 min in a dough mixer (E.B. 12, Crypto Ltd., London, U.K.) until a smooth continuous dough was obtained. The dough was fermented at 40 °C for 15 min, divided into balls of 30 g each, and the dough balls proofed at 40 °C for 30 min. The balls were flattened into sheets 1.7 mm thick, proofed at 40 °C for 15 min, and baked at 500 °C for 1 min. The loaves were allowed to cool to room temperature, placed in polyethylene bags to prevent moisture loss, and stored at –20 °C until used.

Determination of detection thresholds

Determination by the 3-alternative forced-choice (3-AFC) method. Twenty-five subjects (8 males, 17 females; age, 20 to 55 y) were recruited from staff, student, and faculty of the Faculty of Agricultural and Food Sciences at the American Univ. of Beirut. The subjects had no self-reported problems in their sense of taste or smell and were not compensated for serving on the panel. Breads were removed from frozen storage and left for 30 min to equilibrate to room temperature. Samples for sensory testing were prepared by cutting rectangular pieces (2 cm × 5 cm) from the loaf layers. The rectangular pieces were placed in polyethylene bags (2 pieces/bag), coded with 3-digit numbers, and kept at 5 °C until served. Thresholds were determined according to American Society for Testing and Materials procedure E 679-79 (ASTM 1997), an ascending forced-choice method of limits, as modified by Lawless and others (2000). Three samples, 2 regular breads and 1 calcium-fortified bread, at each fortification level were presented at room temperature to panelists. Calcium-fortified breads were presented in ascending order with samples being arranged in a balanced random order within each triad to eliminate positional bias. Panelists were asked to chew and swallow samples and to indicate which sample, from the 3, contained calcium and to give a certainty judgment (guessing or not guessing). Each panelist evaluated an average of 3 triads in a day at the rate of 1 triad per session and with 30-min interval between sessions. Sensory assessments required 3 d of testing and were repeated the following week for each calcium salt. One day before testing, a 30-min training session was conducted to familiarize panelists with taste of calcium-fortified bread and logistics of the 3-AFC test. Sensory assessments were carried out in partitioned booths equipped with daylight. Assessors were asked to rinse their mouths with distilled water after each sample to minimize adaptation and carryover effects. The individual best estimate threshold was taken as the geometric mean of the last concentration with an incorrect response and the 1st concentration with a correct response indicated "without guessing." For subjects indicating a "guessing" response for the correct choice, the individual best estimate threshold was taken as the geometric mean of the guessed correct choice concentration and the next concentration step to account for the possibility of chance correct responding (Lawless and others 2000). Group thresholds were determined by computing the geometric means of the individual best estimate thresholds. The logarithms of individual detection thresholds were arranged as a randomized complete block design with calcium salts as treatments, panelists as blocks and with 2 observations per block, and subjected to analysis of variance using the fixed effects model. Means were separated by the least significant difference test when F values were significant (Gacula and Singh 1984).

Another estimate of detection thresholds, of calcium salts, was the concentration at which correct responses were 50% above chance, which corresponds to 66.7% correct in the 3-AFC test (Antinone and others 1994; Lawless and Heymann 1998).

Detection thresholds were also estimated by probit analysis of the 3-AFC data. The percents correct in the 3-AFC test were corrected for chance performance using Abbot's formula: adjusted percent correct = (percent correct – chance)/(1 – chance) (Lawless and Heymann 1998). The adjusted percents correct were converted to probits, and detection threshold values, corresponding to adjusted 50% correct, were interpolated from the fitted probit function with the 95% confidence intervals being constructed as the values predicted at 30% and 70% adjusted percents correct (Finney 1971; Brown and others 1978; Lawless and Heymann 1998).

Determination from dose-response curves. Nine panelists (6 females, 3 males; age 20 to 55 y), selected from the 3-AFC panel on the basis of availability and motivation, participated in this part of the study. Dose-response curves of the calcium salts were constructed as outlined by Marin and others (1991). Each panelist evaluated 16 samples presented in 4 rows of 4 samples. Each row consisted of a sample identified as control, followed by a coded (3-digit number) control and 2 coded samples containing adjacent concentration levels from a series of 8 increasing concentration of added calcium. The rows were presented in ascending order of added calcium concentration. Subjects rated the magnitude of difference between coded samples and the control on a 9-point category scale anchored as follows: 0 = no difference; 1 = just detectable difference; 2 = slight difference; 3 = slight to moderate difference; 4 = moderate difference; 5 = moderate to large difference; 6 = large difference; 7 = large to extreme difference; and 8 = extreme difference. Panelists evaluated 2 rows of samples in the morning and 2 rows in the afternoon at the rate of 1 row in a session with a 30-min interval between sessions. Panelists were trained for 2 1-h sessions, 1 d before testing, on the use of scale and logistics of the test. Sensory assessments were replicated 4 times and were conducted as described in the previous section.

The blank rating of each panelist was subtracted from the 2 test sample ratings, in each row, when the correlation coefficients between blank ratings and deviations of sample ratings from the row mean were ≥0.5 to reduce bias when blank and sample ratings contain equal amounts of random error (Marin and others 1991; Chapman and others 2002). The mean ratings (9 panelists over 4 replications) of samples containing 8 ascending levels of calcium were fitted to the following equation:

$$\text{Rating} = m + \frac{(8 - m)e^{s(x-c)}}{1 + e^{s(x-c)}} \qquad (1)$$

where m is the lower asymptote, the upper asymptote being the response scale maximum at 8, x is log concentration of stimulus, s a slope parameter, and c the concentration at the point where the slope is maximum. Threshold is defined as the point where response shows maximum change with increasing concentration of stimulus. The concentration of the stimulus at this point, C_{max}, corresponds to the apex of the 2nd derivative of the logistic function and is determined by taking the lower value of stimulus concentration where the 3rd derivative of the logistic function is zero (Marin and others 1991).

The parameters m, s, and c were estimated, using the Levenberg-Marquardt estimation method, by fitting the mean panelist ratings to Eq. 1 using the nonlinear regression module of SPSS software (SPSS Inc. 2002). The logistic functions [$f(x)$], corresponding to Eq. 1, of calcium salts and their 2nd derivatives [$f''(x)$] were plotted and C_{max} determined from $f''(x)$, using Maple software (WMI 2000).

Similarity testing

One hundred eight subjects (43 males, 65 females; age 13 to 68 y), recruited from the American Univ. of Beirut campus, participated in this part of the study. Breads were prepared from flours fortified with calcium carbonate, calcium citrate, or calcium sulfate at successive calcium levels lower than corresponding determined threshold values by 100 mg Ca/100 g flour and tested for similarity to regular bread. The triangle test was used and subjects were instructed to identify the sample that was most different from the other 2. Half of the triads contained 2 calcium-fortified breads and 1 regular bread while the other half of triads contained 2 regular and 1 calcium-fortified bread samples. Samples were presented in a balanced random order with sample size and testing logistics (sample coding, testing area, mouth rinsing) being as described in previous sections.

Testing was done at an α-level of 0.2 (probability of concluding that a perceptible difference exists when 1 does not), a β-level of 0.01 (probability of concluding that no perceptible difference exists when 1 does) and a P_d of 25% (true proportion of population able to detect a difference between samples). The significance of the test was ascertained by reference to tables of correct responses required to establish significance in the triangle test (Meilgaard and others 1999). The confidence limits on the proportion of the population that can distinguish between samples were determined by calculating $1.5 \times c/n - 0.5 - z_\alpha(1.5)\sqrt{[(c/n)(1 - c/n)/n]}$ and $1.5 \times c/n - 0.5 - z_\beta(1.5)\sqrt{[(c/n)(1 - c/n)/n]}$, where c is the number of correct responses in triangle test, n total number of assessors, and Z_α and Z_β are critical values of the standard normal distribution (Meilgaard and others 1999).

Results and Discussion

Calcium contents of $CaCO_3$, $CaSO_4$, and calcium citrate were 40.6 ± 1.46 g, 21.6 ± 0.42 g, and 21.3 ± 0.36 g/100 g, respectively. Wheat flour used in preparation of bread samples contained 23 ± 1.67 mg Ca/100 g. At the indicated levels of flour fortification, $CaCO_3$, $CaSO_4$, and calcium citrate contents of breads ranged between 1384 ± 47.5 and 4726.2 ± 140.57 mg/100 g, 2390.8 ± 72.56 and 4614.3 ± 61.58 mg/100 g, and 1186.4 ± 66.5 and 6703.2 ± 147.69 mg/100 g, respectively.

Table 1—Individual and group detection thresholds for $CaCO_3$, $CaSO_4$, and $Ca_3C_{12}H_{10}O_{14}$ (tricalcium dicitrate) in pita bread[a,b]

	Individual threshold (mg/100 g bread)		
Panelist nr	$CaCO_3$	$CaSO_4$	$Ca_3C_{12}H_{10}O_{14}$
1	1570	2641	1460
2	2506	3541	2156
3	1704	2307	1160
4	1643	2378	1709
5	2064	3646	2967
6	1520	2307	2685
7	1657	2652	1761
8	1749	2996	1926
9	1580	2857	1317
10	2927	2845	2497
11	1568	2441	1709
12	2330	2584	2840
13	1803	2357	2967
14	2645	2307	2042
15	2800	2494	3910
16	1616	3385	3697
17	1616	2652	2800
18	2645	3305	1661
19	2196	2718	1926
20	2577	2441	1590
21	1910	2930	2431
22	2359	2860	1590
23	1660	2643	2180
24	1657	2441	1950
25	2506	2987	3025
Group mean threshold	1984b	2724c	2132b
Standard log deviation	0.096	0.058	0.138

<table footnote>[a]Average of 2 replicate determinations. As per the ASTM Method E 679-79 (1997).
[b]Values with different superscripts are different ($P < 0.01$) by the least significant difference test.

Detection thresholds of subjects, determined by calculating geometric mean of individual best estimate thresholds, for the calcium salts in pita bread ranged over 1.58 to 3.37 orders of magnitude with differences being smallest for $CaSO_4$ and largest for tricalcium dicitrate (calcium citrate) (Table 1). Differences spanning 4 orders of magnitude in detection thresholds of aqueous solutions of organic and inorganic calcium salts have been reported (Tordoff 1996). Analysis of variance indicated significant differences ($F_{2,75} = 11.1$, $P < 0.01$) between detection thresholds of the calcium salts. The detection threshold of $CaSO_4$ (2724 mg/100 g bread) was higher ($P < 0.01$) than those of $CaCO_3$ (1984 mg/100 g bread) and calcium citrate (2132 mg/100 g bread) with no significant differences being detected between thresholds of the 2 latter salts. Using the criterion of 50%-above-chance the detection thresholds of $CaCO_3$, $CaSO_4$, and calcium citrate were between 1616 and 1705 mg/100 g, 2516 and 2655 mg/100 g, and 2017 and 2356 mg/100 g, respectively (Figure 1). Probit analysis predicted detection thresholds of $CaCO_3$, $CaSO_4$, and calcium citrate at 1765 mg/100 g (95% confidence interval [CI], 1533 to 2308 mg/100 g), 2595 mg/100 g (95% CI, 2352 to 3117 mg/100 g), and 2348 mg/100 g (95% CI, 1869 to 3304 mg/100 g) mg/100 g, respectively. The 3 criteria used for determining thresholds yielded values in the middle region of surveyed concentrations. The criterion of 50%-above-chance yielded intervals for threshold values in view of the scatter of data points relating percents correct to concentration (Figure 1). Calculation of geometric means of individual best estimate thresholds and probit analysis yielded point estimates of thresholds that differed by 5.0% to 12.4%. Estimates of thresholds are known to be shaped by the methods used in their determination (Lawless and Heymann 1998; Meilgaard and others 1999). However, in contrast to determination from geometric mean, which takes into consideration all data points, probit analysis uses data on percent correct and ignores number of panelists and replications, thereby leading to unrealistically large confidence intervals (Brown and others 1978). These factors render estimates obtained by calculating geometric means more reliable than those obtained by the other 2 procedures.

The mean ratings of stimuli for $CaCO_3$-fortified breads were adequately described by Eq. 1, with coefficient of determination (R^2) of 0.934, yielding estimates for *m*, *s*, and *c* of –0.34, 2.38, and 7.03, respectively. The concentration of $CaCO_3$ at the point of maximum curvature on the dose response curve, which corresponds to the threshold, determined from the apex of the 2nd derivative of the logistic curve (Figure 2) was 1173 mg/100 g bread. The coefficients of determination of logistic functions fitted to mean ratings of $CaSO_4$-fortified and calcium citrate–fortified breads were 0.987 and 0.979, and the corresponding parameter estimates were ($m = -10.05$, $s = 3.02$, $c = 6.41$) and ($m = -1.93$, $s = 1.95$, $c = 6.47$), respectively. The threshold values of $CaSO_4$ and calcium citrate, in pita bread, as determined form the 2nd derivatives of logistic curves (data not shown) were 1529 and 1098 mg/100 g, respectively. In all cases, the determined thresholds were lower than the surveyed ranges of calcium concentrations. The predicted low values for thresholds could be due, at least in part, to the presence of 2 sensitive individuals on the panel who were able to correctly identify the calcium-containing samples ≥93.75% of the time, in 3-AFC tests, and would, therefore, possibly have detection thresholds lower than the surveyed range of calcium concentrations. Notwithstanding the presence of sensitive individuals on the panel, and the training acquired by panelists in the 3-AFC tests, perusal of the data indicated that panelists tended to exaggerate the magnitude of difference from the control at the lowest concentrations of calcium in response to questions on differences (expectation error; Meilgaard and others 1999) especially as percents correct responses for $CaCO_3$, $CaSO_4$, and calcium citrate, in 3-AFC tests, at the 2 lowest levels of stimulus ranged between 52% to 54%, 52% to 58%, and 44% to 62%, respectively (Figure 1). These factors contributed to the creation/establishment of a point of maximum change in response with change in stimulus concentration, defined as threshold value, below the surveyed range of calcium concentrations.

In pocket-type flat bread-consuming countries, bread serves as an intermediary between food and the person consuming it. In addition to its use as a pocket to hold food, as practiced in Europe and North America, pocket breads are split into their constituent layers and topped with food and rolled into a sandwich or cut into conveniently sized pieces and shaped into a scoop for carrying food into the mouth. The regular consumption of bread with meals would render consumers intolerant to any foreign taste/flavor in the product. This is particularly important in calcium-fortified analogues of the bread, especially as the product-evoked sensations described as astringent, bitter, chalky, sour, metallic, irritating, and throat-catching at suprathreshold concentrations of the calcium fortificants. Triangle tests indicated that calcium-fortified breads were similar ($P < 0.01$) to regular bread when formulated from flours fortified at levels lower by 400 and 300 mg of added Ca/100 g flour, than corresponding threshold values obtained by calculating geometric means of individual best estimate thresholds, with $CaCO_3$ and $CaSO_4$, or calcium citrate, respectively. More specifically, pita bread containing $CaCO_3$ (1254.6 ± 8.03 mg/100 g), $CaSO_4$ (1772.5 ± 18.26 mg/100 g), and calcium citrate (1155.0 ± 27.58 mg/100 g) are similar to regular bread with the 99% CI, of the true proportion of population that can detect differences between samples, ranging between 0% and 20% for the $CaCO_3$-fortified product and 0% to 19% for the product fortified either with $CaSO_4$ or calcium citrate.

The per capita consumption of bread in the Middle East has been estimated at 323 g/d (WHO 1998). This relatively high intake coupled with the reported high bioavailability of calcium from bread fortified with $CaCO_3$, $CaSO_4$, or calcium citrate, being similar to that of dairy calcium (Ranhotra and others 1997, 2000; Juma and others 1999; Martin and others 2002), render calcium-fortified analogues of pita bread–rich sources of dietary calcium. At the indicated level of consumption, calcium-fortified pita bread that is similar sensorily to its regular counterpart, would provide 1644, 1236, and 793.1 mg Ca/d when baked from flour that has been fortified with $CaCO_3$, $CaSO_4$, and calcium citrate, respectively. At recom-

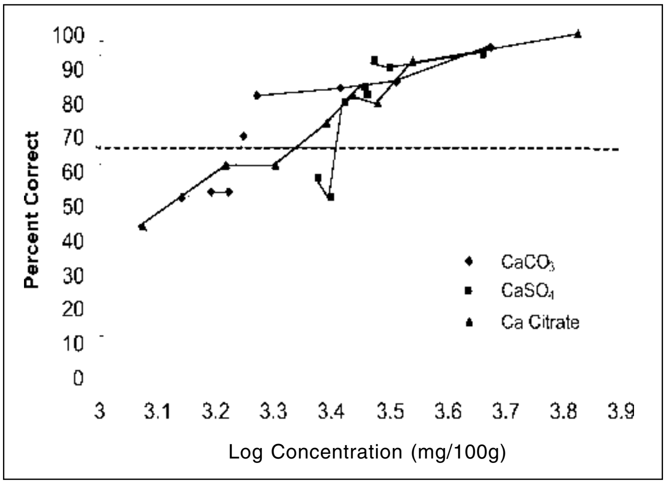

Figure 1—Percentage of correct responses (25 panelists, 2 replicate determinations) in 3-alternative forced choice (3-AFC) tests of calcium-fortified pita bread. The dashed line represents 50% above chance performance in the 3-AFC test.

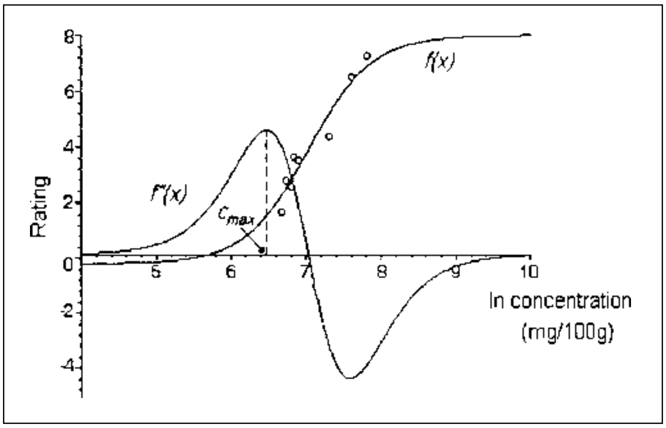

Figure 2—*f(x)*: Logistic curve fitted to the joint panelists' ratings (9 panelists, 4 replicate determinations) for $CaCO_3$ in pita bread in procedure for constructing dose-response curves; *f''(x)*: 2nd derivative of logistic curve; C_{max} = concentration associated with point of maximum curvature that corresponds to threshold.

mended daily intakes (RDI) of 1300 mg Ca/d and at the indicated level of consumption, bread fortified with $CaCO_3$, $CaSO_4$, and calcium citrate would provide 126.5%, 95%, and 61%, respectively, of the RDI for calcium for Middle Eastern populations.

Conclusions

The detection thresholds, determined by calculating geometric mean of individual best estimate thresholds obtained by 3-AFC tests, of $CaCO_3$, $CaSO_4$, and tricalcium dicitarte in pita bread were 1984, 2724, and 2132 mg/100 g. Pita bread was judged similar to its regular counterpart when formulated to contain levels lower by 36.8%, 35%, and 45.8% than determined thresholds for $CaCO_3$ and $CaSO_4$, or tricalcium dicitrate, respectively. The calcium-fortified pita bread analogues provide 61% to 126.5% of the recommended daily intakes for calcium for Middle Eastern populations and, in view of their increasing popularity, are potentially good sources of calcium for vegetarians and other population groups that do not consume dairy products, in Europe and North America.

Acknowledgments

The financial support provided by the Univ. Research Board of the American Univ. of Beirut is gratefully acknowledged.

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