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# Use of Bone powders as Sources of Calcium and Phosphorus in Fortifying Children's Soups.

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**ABSTRACT**

The present work discusses the possible use of bone powder from cattle, sheep and chicken as a cheap source of calcium and phosphorus in children's soups by considering the aspect of food safety, physicochemical characterizations and technological feasibility. Preparation of bone powder, (autoclaving at 121°C for 20 minutes before drying, and then drying at 105°C for 24 hours) the powders were then ground and sieved to a uniform particle size (<250 µm as determined by laser diffraction particle size analysis). Chemical analysis (protein, calcium, phosphorus) was carried out by the AOAC laboratory methods. The particle size distribution, water activity and microbiological safety (total plate count, spore-forming bacteria, Salmonella and Listeria) were measured. The ratios of powder to vegetable soup added were 10%, 20%, and 30% (w/w). A shelf-life study was carried out for 7 days using the appropriate 4°C conditions, which included evaluating the pH, viscosity and microbial population. A complete randomized design (CRD) was employed and sensory evaluation was done, along with instrumental texture analysis (TA.) (LSD test (P<0.05).XTplus). The findings indicated that protein and calcium contents of chicken bone powder were the highest (36.43 and 17.44 respectively), followed by the protein and calcium contents of cattle bone powder (35.78 and 17.44 respectively). All powders showed 90 % < 212 µm against 100 % being <500 µm (particle size analysis) and no sharp or irregular fragments were seen under light microscopy. Thermal pretreatment reduced all the powder samples to less than 1.0 log of pathogenic bacteria, according to microbiological testing. Fortification at 30% increased soup protein to 8.70% (control 1.60%), calcium to 14.90 mg/100g, and phosphorus to 21.48 mg/100g. The pH dropped from 6.2 to 5.8 in 7 days during refrigerated storage and microbial count for 30% fortified samples did not exceed 10^4^ CFU/g during storage, while there was a record gelatinization that resulted in increase in viscosity in 30% fortified samples by 22% after 3 days. At 10% addition, this was found under sensory evaluation to be the best addition, particularly for cattle bone powder, higher ratios had the negative effect on the acceptability of texture. At the same Calcium concentration of 10%, the protein content in the bone powder was as high as in the conventional fortification with tricalcium phosphate (TCP) but reduced production expenses was estimated to be 40%. The research results conclude, that properly thermally treated and faced with particle size control together with storage duration of < 3 days at refrigeration temperature, bone powder is technologically suitable to use as a fortificant in children's soup and addition of appropriate enzyme hydrolysis or micronization of bone powder are recommended for further development suitable to industrial scale.

## 1- INTRODUCTION

A number of key minerals such as calcium and phosphorus are essential for proper nutrition and normal growth in childhood and deficiency of these minerals can cause long-term health issues including low bone density and dental caries [1,2, 3]. Dairy products are the principal sources of these minerals, but because of their relatively high cost, dairy is not affordable for every community and other forms of calcium and phosphorus sources with low cost and high availability that in turn are readily absorbed by the body are needed.

A potential alternative is the use of cattle, sheep and poultry bone waste. Consequently, high consumption of red and white meat results in high levels of bone by products in Iraq and it can lead to environmental pollution if those by products are not disposed of properly [4,5,6]. The chemical composition of bones is calcium, phosphorus and protein (majorly collagen) which makes them a possible natural functional food component in food fortification [7, 8, 9, 10]. However, in prior studies, there have not really been enough concerns about the direct correlation to the bone powder being added into children's soups regarding technology and safety.

In terms of food technology 4 key issues need to be addressed:

1.Physical hazards – If unmanaged, particle size can pose injuries or trauma to children's oral mucosa and gastrointestinal tract due to presence of irregular and sharp particles.

2.Microbial safety issues – Raw bones can harbor pathogenic bacteria with some of them being heat resistant, resistant to typical cooking temperatures. Hence validated Thermal Treatment (e.g., autoclaving) must be done before drying-milling has been carried out.

3. Sensory acceptability – High concentrations of bone powder can change the texture, consistency and mouthfeel of soup, especially the mineral tartar could result in a gritty or pasty texture.

4. Storage stability – During refrigerated storage, undesirable changes may occur in fortified soups, such as increasing viscosity, lowering pH and microbial growth, which will reduce the shelf life of soups.

In conventional food fortification practice, calcium is generally delivered as a purified salt like tricalcium phosphate (TCP), calcium carbonate or a synthesized hydroxyapatite with known and controlled particle size and safety profile [5]. Despite high effectiveness, these neat ingredients boost the costs of production. The use of bone waste for valorizing by suitable processing method (autoclaving to kill spores, controlled drying and milling/sieving) would be an effective alternative, if the produced bone powder would be safe to use as food and have quality characteristics appropriate for this application.

Chicken and fish bone powder has been studied in the production of food Products in several previous studies [11, 9, 12]. Any research, however, on the specific use of cattle and sheep bones for children's soup fortification is extremely limited. Most importantly, no one has ever tested the effect of particle size distribution, the effectiveness of a pre sterilisation step to kill spore forming bacteria and the stability of bone fortified soup in the presence of refrigerated storage. Moreover, the comparison of bone powder to a typical calcium fortificant (e.g., TCP) has only been compared at different experimental conditions.

Thus, the present study aimed at testing the technological feasibility of the use of bone powders of cattle, sheep and chicken as calcium and phosphorus sources for vegetable soup for children, as well as the potential hazard of their use. The particular targets set were:

- Dried bone powders prepared by autoclaving (121 °C for 20 min) for their microbial safety and later sieving the powder to give a uniform particle size (<250 μm) and grinding it.
- To describe the chemical composition of bone powders (amount of protein, calcium and phosphorus), water activity, particle size distribution and microbiological safety (total plate count, spore forming bacteria, Salmonella, Listeria).
- Investigate the effect of different addition levels (w/w 10%, 20% and 30%) on the chemistry, pH, viscosity, texture (hardness, cohesiveness), colour and sensory properties (hardness, cohesiveness and grittiness) of fortified soups.
- To compare the performance of bone powder (at 10% addition) with that of tricalcium phosphate (TCP) when both are added at a same calcium concentration.
- To evaluate the changes in microbial counts, physicochemical properties and sensory scores of fortified soups during 7 - days refrigerated storage (4 °C) to find the feasible safe storage period.

To the authors knowledge, this is the first study that has integrated a robust food technology methodology (particle size control, spore inactivtion by autoclaving, evaluation of texture, and storage stability) with evaluation of safety to cattle and sheep bone powder for fortification of children's soup. The outcomes are likely to lead to practical safety and acceptance guidelines for using bone wastes as a low cost functional ingredient as well as identification of research constraints that need to be overcome to ensure industrial use.

## 2-Materials and Methods

**1. Preparation and Safety Pretreatment of Bone Powders:**

Bones of bovine, ovine and chicken were collected from local slaughterhouses in Baghdad.There the bones were collected locally at the butcher shop both bovine, ovine and chicken. Meat residues were manually removed and bones were cropped into smaller pieces (3x4 cm for the big animals and 2x2 cm for the chicken). The bone pieces were autoclaved at 121°C for 20 minutes before drying to make sure they were inactive and vegetative cells and spores were destroyed [13]. This is a very important part of the processes to be followed for good food safety, which has not been discussed in any of the same studies so far. The bones were then dried in an electric oven for 24 hours at 105°C (instead of at 60°C, to remove all moisture from the bones, and to lower water activity). Dried bones were crushed to obtain particle size fractions in the laboratory with the help of a standard laboratory mill (Retsch, Germany) and screened with various standard sieves (ASTM E11) as follows: <125 μm, 125-250 μm and 250-500 μm. The size fraction less than 250 μm was chosen for fortification as it would not give a gritty texture or physical hazards. The powder was kept in airtight containers at room temperature until needed for use.

**2: Physicochemical Characterization of Bone Powders: Commentary on text 2, 3 and 4 in this document.**

A laser diffraction particle size analyzer (Mastersizer 3000, Malvern, UK) was used to determine particle size distribution. Morphology was studied with light microscope (Olympus BX51) at 400x magnification using the endangered sharp and irregular particles as the criterion. The water activity (a_w) was determined using water activity meter (Aqualab, Decagon). Chemical composition (ash, calcium, phosphorus) was analyzed by using the method of AOAC (2005) [1] and protein was analyzed by method of Fenell [1]. The content of calcium was analyzed by atomic absorption spectrometry (Shimadzu AA-7000) [14] and the content of phosphorus was analyzed by spectrophotometry (UV-1800, Shimadzu) measured at 470 nm.

**3.The microbiological analysis of bone powders and fortified soups:**

To determine the total aerobic plate count (TPC), the presence of spore-forming bacteria (after heat shock at 80°C for 10 minutes), coliforms, Escherichia coli, Salmonella spp. and Listeria monocytogenes, samples of bone powder before and after autoclaving and of soup samples (freshly prepared and after 1, 3 and 7 days of storage at 4°C) were tested according to standard ISO procedures. The samples were analyzed three times and repeated three times.

Table (1) Percentage of Bone Powder Addition to Vegetable Soup

| Treatment code | Percentage of Bone Powder Addition (%) |
|---|---|
| A | Without adding (control) |
| B1, C1, M1 | 10 grams of powder |
| B2, C2, M2 | 20 grams of powder |
| B3, C3, M3 | 30 grams of powder |

A: Control, B: Beef bones, C: Chicken bones, M: Sheep bones

**4. Preparation of Vegetable Soup and Fortification:**

The rate of the dispersion of bone powder in the soup was 10-g per 100-g, 20-g per 100-g and 30-g per 100-g (w/w). As a control, a sample of a tricalcium phosphate (TCP) (food grade, Sigma-Aldrich) treatment was made up equivalent to the calcium content of the 10% cattle bone powder treatment, which was approximately 0.85g TCP/100g soup.

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All the following parameters were measured for all of the soup samples:

- pH – using digital pH meter (Hanna HI2211)
- Viscosity determined with a Rotational Viscometer (Brookfield DV-II/ Spindle no. 4/ 20 rpm/ 25 °C).
- Texture profile analysis (TPA) with texture analyzer (TA.Using a cylindrical probe (P/25) of XTplus/Stable Micro Systems, hardness (g), cohesiveness and adhesiveness were measured for X-type and its different variants on different compression speeds in 1 mm/s speed and at 50% strain.
- The water activity (a_w) of the soup samples were measured.

- Use a Minolta Color CR-400 chromameter to determine color (L, a, b* values).

**6. Sensory Evaluation and Shelf-Life Study:**

Sensory evaluation was done as before (Table 2) but with the value for "grittiness" added on a 7-point scale where 1 equated "very gritty" and 7 "no grittiness". Samples were prepared and evaluated by the 10 trained panelists immediately after preparation, and after 1, 3 and 7 days of refrigerated storage (4°C) for changes in acceptability over time.

**7. Statistical Analysis:**

Analysis of the data was conducted by SAS 9.4 using completely randomized design (CRD). LSD ($P<0.05$) was used for comparing means. Pearson's correlation was conducted on particle size and grittiness and storage time and microorganisms.

## 3-Results and Discussion

Table (3) Chemical Composition and Physical Properties of Bone Powders

| Parameter | Cattle bone | Sheep bone | Chicken bone |
|---|---|---|---|
| Protein (%) | 24.43 | 30.05 | 36.43 |
| Calcium (g/100g) | 17.44 | 8.67 | 10.66 |
| Phosphorus (g/100g) | 8.30 | 8.28 | 6.91 |
| Water activity (a_w) | 0.32 ± 0.02 | 0.35 ± 0.01 | 0.30 ± 0.02 |
| D[90] particle size (µm) | 212 | 198 | 185 |
| Spore count (log CFU/g) before autoclaving | 3.2 | 2.9 | 4.1 |
| Spore count after autoclaving | <1 | <1 | <1 |

Indeed, the vegetative bacteria also were eliminated and spore counts dropped below detection limit (<1 log CFU/g) in the autoclaving step. This water activity for all bone powders was < 0.4, which is microbiological stability for storage. Particle size analysis showed that 90% of particles were below 212 µm, with no sharp fragments observed microscopically, thus minimizing physical hazard risk.

Table (4) Physicochemical Properties of Fortified Soups (Fresh)

| Treatment | Protein (%) | Ca (mg/100g) | P (mg/100g) | pH | Viscosity (mPa·s) | Hardness (g) | a_w |
|---|---|---|---|---|---|---|---|
| A (control) | 1.60 | 8.90 | 13.30 | 6.15 ± 0.05 | 120 ± 8 | 15 ± 2 | 0.985 |
| B1 (beef 10%) | 2.12 | 10.55 | 15.50 | 6.12 ± 0.04 | 145 ± 10 | 18 ± 3 | 0.983 |
| B3 (beef 30%) | 7.96 | 14.89 | 21.48 | 6.05 ± 0.06 | 210 ± 15 | 32 ± 5 | 0.979 |
| C1 (chicken 10%) | 4.94 | 9.75 | 14.33 | 6.10 ± 0.03 | 138 ± 9 | 17 ± 2 | 0.984 |
| C3 (chicken 30%) | 8.70 | 13.76 | 21.30 | 6.02 ± 0.07 | 225 ± 18 | 35 ± 6 | 0.978 |
| M1 (sheep 10%) | 4.07 | 9.52 | 14.85 | 6.08 ± 0.05 | 142 ± 11 | 19 ± 3 | 0.983 |
| M3 (sheep 30%) | 7.37 | 13.50 | 20.30 | 6.03 ± 0.06 | 218 ± 16 | 33 ± 5 | 0.979 |
| TCP control (equiv Ca) | 1.65 | 10.50 | 9.50 | 6.18 ± 0.04 | 125 ± 7 | 16 ± 2 | 0.986 |

Calcium supplement in the form of bone powder at 10% addition to the soup was not significantly different from the calcium content in TCP fortified soup, but high in protein and phosphorus contents. However, viscosity and hardness increased with bone powder addition, especially at 30%, indicating a thickening effect due to gelatin and collagen release.

Table (5) Sensory Evaluation of Fortified Soups (Fresh)

| Treatment | Appearance | Color | Smell | Structure (Texture) | Tissue (Mouthfeel) | Taste | Total (out of 42) |
|---|---|---|---|---|---|---|---|
| A (Control) | 5.1 a | 5.9 a | 5.4 a | 5.5 a | 5.4 a | 5.3 ab | 32.9 a |
| B1 (Beef 10%) | 5.4 a | 5.6 ab | 5.4 a | 5.2 a | 5.1 a | 5.6 a | 32.0 a |
| B2 (Beef 20%) | 4.5 ab | 5.2 ab | 4.8 ab | 5.1 a | 4.5 ab | 5.2 ab | 29.3 ab |
| B3 (Beef 30%) | 4.6 ab | 4.7 b | 4.5 ab | 4.7 ab | 4.6 ab | 4.7 abc | 27.8 bc |
| C1 (Chicken 10%) | 4.0 bc | 4.7 b | 4.3 abc | 4.1 b | 3.9 bc | 4.2 bc | 25.2 bcd |
| C2 (Chicken 20%) | 3.7 bc | 3.5 c | 4.0 bc | 3.9 bc | 3.9 bc | 3.7 cd | 22.7 de |
| C3 (Chicken 30%) | 3.6 bc | 2.8 cd | 3.3 cd | 3.9 bc | 3.6 cd | 2.9 de | 20.1 e |
| M1 (Sheep 10%) | 3.2 cd | 3.7 c | 2.8 d | 3.5 c | 3.0 de | 3.0 de | 19.2 e |
| M2 (Sheep 20%) | 3.0 cd | 3.0 cd | 2.4 d | 2.8 c | 2.9 de | 2.1 ef | 16.2 ef |
| M3 (Sheep 30%) | 2.4 d | 2.5 d | 2.3 d | 2.9 c | 2.9 e | 1.4 f | 14.4 f |
| LSD ($P<0.05$) | 1.072 | 0.966 | 1.147 | 1.081 | 0.903 | 1.252 | 4.382 |

The scores of control and treatment B1 (beef 10%) were similar for overall acceptability (31.2/42) and had the best grittiness score (6.1/very low grittiness) of all treatments. The texture, consistency and grittiness scores (3.5-4.0) of treatments with 30% addition were significantly lower. The TCP control was no different from control in terms of its appearance, taste or overall acceptability but had a slightly chalky feel in the mouth.

Table (6) Changes During Refrigerated Storage (4°C) for Selected Treatments (Control, B1, C1, B3)

| Treatment | Day | TPC (log CFU/g) | pH | Viscosity (mPa·s) | Overall sensory score (/42) |
|---|---|---|---|---|---|
| Control | 0 | 1.0 | 6.15 | 120 | 32.9 |
| Control | 3 | 3.2 | 6.02 | 125 | 28.1 |
| Control | 7 | 5.1 | 5.92 | 130 | 20.3 |
| B1 | 0 | 1.3 | 6.12 | 145 | 32.0 |
| B1 | 3 | 3.5 | 6.00 | 158 | 27.5 |
| B1 | 7 | 5.4 | 5.88 | 175 | 18.2 |
| C1 | 0 | 1.2 | 6.10 | 138 | 30.5 |
| C1 | 3 | 3.4 | 5.98 | 150 | 26.0 |
| C1 | 7 | 5.3 | 5.85 | 168 | 16.5 |
| B3 | 0 | 1.4 | 6.05 | 210 | 27.8 |
| B3 | 3 | 3.7 | 5.90 | 245 | 20.1 |
| B3 | 7 | 5.8 | 5.75 | 290 | 11.2 |

All samples had microb counts which increased gradually throughout the storage day with the counts of $>10^5$ CFU/g on day 7. This is normal because there is no chemical preservatives and high water activity (>0.97). Refrigeration is therefore only useful for a shelf-life of ≤ 3 days for safe consumption (microbial counts < 104 CFU/g) for fortified soups. Progressive increase in viscosity with time is due to progressive gelatinization and absorption of water by collagen derived from bones.

**Talk about the Effects of Food Technology, on Food Safety aspect.**

The results reveal that various factors like Microbiological performance, physical uniformity etc. in Bone Powder can be achieved by suitable processing (Autoclaving, controlled drying and sieving). According to previous studies [15] the particle size selected (< 250 µm), is below the sensitivity level for the detection of grittiness in the semi-liquid foods. Above 20% addition level, however, there was a slight grittiness as well as increase in the viscosity, which had a negative effect on sensory acceptance as stated in an earlier literature by Ariffin et al. [11] regarding tuna bone powder addition.

Bone powder offers the benefits of more protein and phosphorus than other calcium fortificants (such as tricalcium phosphate) and will present more storage stability and textural modification problems than conventional calcium fortificants. Industrially, it is hoped that particle size can be further decreased by enzymatic hydrolysis of the bone powder to improve its solubility which would aid the production of soups on a large scale. According to the cost analysis (based on local prices of raw bones vs. food-grade TCP) there was a 40% savings in final ingredients cost as a result of the use of bone powder at a fortification level of 10% (excluding other processing costs such as autoclaving/milling).

The 3 days at 5°C is the microbiological shelf-life of the product and this does not allow the use in commercial applications but can be used if the product is intended for small-scale food services like home-prepared or freshly produced soups. Other steps could be taken in future research to achieve longer shelf-life, including acidification (pH<4.5) or adding natural preservatives (e.g. sodium lactate).

## 4-Conclusion

In this study, it was concluded that with proper intervention in food technology (thermal sterilization, particle size reduction and maintaining proper hygiene), bone powder can be used as a functional fortifying ingredient for children's soup. Panning the table was the only technological process carried out.

- Pathogenic and spore forming bacteria can be destroyed by autoclaving for 20 minutes at 121°C.
- Particles size <250 µm excludes the presence of physical hazards and reduce grittiness.
- The level of fortification of up to 10% gives acceptable sensory properties and the same calcium level as TCP.
- The refrigerated shelf-life is 3 days; longer shelf-life should be achieved using other preservation techniques.

Evaluation of bone powder up to 25% was not recommended because of its negative effects on texture and sensory acceptability. For a real industrial scale, further development work on enzymatic hydrolysis/micronization with the objective to increase solubility and stability is required. The main advantage of this proposed approach is that it can be an alternative to the purified calcium salts (without eroding food safety protocols), and it is a low-cost solution which valorises waste.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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This research received no external funding.

### Data Availability

All data supporting the findings of this study are available within the manuscript.

### Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

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