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Development of a new clean methodology with ultrasound-assisted extraction for analysis of sodium in pet food

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Abstract

Sodium is essential to all living beings, including humans and animals; in higher heterotrophic organisms, it is responsible for regulating the osmotic pressure of tissues and maintaining the sodium-potassium pump. However, even though the presence of sodium is vital, in excess it can cause problems such as increased blood pressure and kidney stone formation. The official methodology for the analysis of sodium in pet food employs corrosive acids and high temperatures during sample preparation, making the process time-consuming and prone to error. In this work, a new methodology is proposed for the extraction of sodium from pet food, using ultrasonic irradiation to enhance the transfer of the analyte to solution, with subsequent determination by flame photometry. This new method, which is coherent with the principles of green chemistry, provided a linear range of 1-20 mg L$^{-1}$ ($R = 0.998$), and limits of detection (LOD) and quantification (LOQ) of 0.26 and 0.90 mg L$^{-1}$, respectively. Recoveries were in the range 98.4-104%. The technique was successfully applied to different brands of commercial pet food and compared favorably with the official methodology (at a 95% confidence level). In comparative tests of the two extraction methods, the proposed methodology showed good repeatability, selectivity, precision, and accuracy.

Keywords: Sodium, ultrasonic extraction, green analytical methodology.
1. Introduction

Sodium is one of the Earth’s most abundant elements. It is essential to all living organisms, including humans and animals, where it contributes to maintaining osmotic balance\cite{1,2} and participates in the sodium-potassium pump that controls the electrical potential of cells\cite{3}. Salt (sodium chloride) has played a crucial role in the development of human society, because it is a key component used to preserve foods such as meat, fish, and pet food. The sodium in the diets of humans and animals is mainly present in the form of sodium chloride.

In recent years, dietary sodium has become one of the more controversial nutrient elements in foods. Some commercial pet foods contain high levels of sodium, which some veterinarians have suggested might be unsafe\cite{4}. Signs of sodium deficiency include increased restlessness, elevated heart rate, reduced urine specific gravity, and dry mucous membranes\cite{5}. However, the intake of sodium in excess can cause several problems, such as increased blood pressure and the onset of kidney stones\cite{4}.

Cases of acute salt toxicosis have been reported in dogs ingesting extremely high amounts of sodium\cite{6}.

In the case of pets, excessive intake of sodium is related to the feed provided to the animals, where sodium is not only present in the form of NaCl, but also as preservatives such as sodium benzoate and sodium nitrite, as well as dyes.

In humans, excess salt increases oxidative stress, which is one of the hallmarks of renal failure\cite{7}.

In the case of dogs and cats, uncertainty remains concerning the effects of high dietary sodium intake on renal disease\cite{4}. In Brazil, the agency responsible for the control of this type of product is the Ministry of Agriculture, Livestock, and Food Supply (MAPA)\cite{8}, but no limits have been established concerning the addition of salt to animal feed.

Due to its importance in biochemical processes, control of the levels of Na in pet food is needed, which in turn requires efficient methods of sample preparation that enable fast and accurate quantification of the element. The literature reports few studies concerning mapping of the elements present in animal feed\cite{9,10}, and the sample treatments that have been used (such as in the official
methodology) are time-consuming and environmentally unfriendly, employing high temperatures and strong corrosive acids, which are contrary to the principles of green chemistry\textsuperscript{11}.

The implementation of green chemistry techniques has become increasingly popular since the 1990s and is based on 12 principles that aim to minimize environmental impacts, reduce or eliminate possible harm to the operator, and provide faster and more efficient analyses\textsuperscript{11,12}.

Ultrasonic irradiation is an environmentally friendly alternative to solid-liquid extraction that can be used for the pretreatment of solid samples, enabling faster performance of steps such as dissolution, extraction, and leaching, amongst others\textsuperscript{13}. Ultrasonication increases extraction efficiency due to a combination of acoustic cavitation and mechanical effects. Microbubbles formed during cycles of compression and decompression increase in size and eventually collapse, which enhances the removal into solution of sodium and other minerals present in the solid sample. The use of ultrasound therefore helps to increase the amount of analyte extracted from the sample\textsuperscript{14-19}.

In this paper, we evaluate the efficiency of a new methodology for the extraction of sodium from pet food, using deionized water as the solvent and ultrasonication to enhance the extraction. The determination of sodium was performed by flame photometry, which is simple, fast, inexpensive, and selective for the analyte in question. The proposed methodology was applied in the analysis of different pet food samples.

2. Experimental

2.1. Materials, reagents, and solutions

Sodium chloride (analytical grade, 100\% purity) was purchased from Mallinckrodt. Prior to use, the reagent was dried in an oven for 1 h at 250 °C. Concentrated nitric acid (60-65\%) was purchased from Synth, and the dilutions utilized in the reference method (20\% and 1 mol L\textsuperscript{-1} nitric acid) were prepared using deionized water (18 MΩ cm) obtained from a Milli-Q system (Millipore). Polyethylene bottles and beakers were used throughout the experiments.
A stock standard solution of 100 mg L$^{-1}$ Na$^+$ was prepared using deionized water. Working standard solutions (1-20 mg L$^{-1}$) were prepared by appropriate dilution of the stock solution. For the official method, a stock standard solution of 100 ppm Na$^+$ was prepared in 1 mol L$^{-1}$ nitric acid.

Seven commercial pet foods for dogs and cats were acquired from local shops in Araraquara (São Paulo, Brazil).

2.2. Equipment

Initial sample homogenization employed a domestic blender together with a porcelain mortar and pestle. A forced air recirculation and renewal oven (Model TE-394/1, Tecnal) was used for drying the samples. An ultrasonic bath (Model T14, Thornton) operated at a frequency of 40 kHz and power of 60 W was used during the proposed extraction process. A muffle furnace (Model MA 385, Marconi) was used for sample preparation according to the official AOAC method$^{10}$. The determination of sodium present in the samples was performed with a flame photometer (Model B262, Micronal).

2.3. Sample preparation and extraction of sodium using the proposed method

The proposed pretreatment of the animal feed samples was performed using a household blender followed by reduction to a fine powder with the aid of a porcelain mortar and pestle. An appropriate mass of the powder was then oven-dried for 6 h at 100 °C and transferred to a polyethylene beaker. It was necessary to dry the samples, because the sodium contents were calculated on a dry mass basis. As recommended by the AOAC$^{10}$, heating for 6 hours was used to ensure that all the water present in the samples of pet food was eliminated. It is not recommended to perform the ultrasound-assisted extraction without first drying the sample in an oven. After the drying step, deionized water was added at a rate of approximately 100 mL for each 1 g of sample, and the mixture was placed under sonication for an optimized time of 20 min. The samples were then filtered through common paper filter, followed by filtration with Whatman qualitative filter paper No.1, and the resulting filtrates were...
collected in polyethylene bottles. Each sample was prepared in triplicate, and sodium determination was performed using flame photometry.

2.4 Optimization of experimental conditions

The optimization of the conditions of the ultrasound bath was based on studies by Nascentes et al.\textsuperscript{16}, using 1.5 L of water in the bath and six polyethylene vials containing the samples with extraction solvent (water, T = 25 °C), corresponding to two samples in triplicate. The vials were placed in the central region of the bath. The sample mass was kept constant at 1.00 g, and the volume of water inside the polyethylene vials ranged from 25 to 150 mL.

The sodium extraction time was optimized by ultrasonicking seven aliquots of the same sample for times ranging from 0 to 60 min, after which the sodium contents were determined by flame photometry.

2.5 Study of matrix interferences

The possible interference of matrix components was investigated by means of the addition and recovery of standards, using fortification levels from 50 to 250%, with subsequent determination of sodium by flame photometry.

2.6 Analytical curves

A series of sodium solutions were prepared at suitable dilutions (1.00, 2.50, 5.00, 7.50, 10.0, 15.0, and 20.0 mg L\textsuperscript{-1}). A calibration graph was then prepared by plotting relative emission intensity against sodium concentration in the range 1.00 to 20.0 mg L\textsuperscript{-1}.

2.8 Reference method
In the case of the AOAC method\textsuperscript{10}, the glassware was first cleaned with 20% HNO\textsubscript{3} solution and then rinsed with deionized water. The feed samples were dried in an oven for 6 h at 100 °C, followed by heating in a muffle furnace at 525 °C for 7 h until a grayish-white color was obtained. The calcined samples were then dissolved in an appropriate volume of 1 mol L\textsuperscript{-1} HNO\textsubscript{3}. These solutions were stored in polyethylene bottles prior to analysis using the recommended reference method.

3. Results and Discussion

3.1. Optimization of the experimental conditions

3.1.1. Ultrasonic extraction time

Analysis of the sodium concentrations in the solutions obtained after different periods of sonication revealed the formation of a plateau after 15 min (Figure 1). A sonication time of 20 min was therefore adopted, which was sufficient to ensure quantitative extraction of sodium, without extending the extraction process for an unnecessarily long time.

3.1.2. Effect of the water volume in the extraction vials

Variation of the relative amounts of sample and extraction solvent did not significantly influence the determination, as shown in Figure 2.
3.2. Construction of analytical curve and determination of LOD and LOQ

An analytical curve of the relative emission intensity (RE) as a function of sodium concentration ([Na⁺]) was constructed using the solutions prepared with deionized water. The equation for the linear regression was \( RE = 0.576 + 5.4872[Na^+] \) and the correlation coefficient value was 0.998. The limits of detection (LOD) and quantification (LOQ), determined as recommended by IUPAC\(^{20}\), were 0.26 and 0.90 mg L\(^{-1}\), respectively.

3.3. Study of matrix interferences

Possible matrix interferences were investigated using the recovery of analyte after the addition of standards at levels ranging from 50% to 250%. The recoveries obtained were between 98.4% and 104% (Table 1). These data indicated that the composition of the commercial product did not significantly interfere in the analysis of sodium, so sample clean-up steps were not necessary.

3.4. Determination of sodium in pet food

The proposed method was applied using seven commercial pet food samples and the results were compared with those obtained with the comparative method. Statistical evaluation using the t-test (95% confidence interval, 2 degrees of freedom) showed that the two sets of data were consistent with each other (Table 2). The calculated t values were lower than the tabulated values, indicating that there was no significant difference between the two methods in terms of precision and accuracy.

The concentrations of sodium found in the samples were from 2775 to 10822 mg kg\(^{-1}\). Samples A, C, F, and G were different cat foods, while samples B, D, and E were dog foods. Sample G was a special food for pets with kidney problems and therefore contained less sodium.
4. Conclusions

This study demonstrated the viability of ultrasound-assisted extraction of sodium present in pet food. The developed method has the advantage of being more environmentally friendly than the traditional technique; it uses reagents that do not pose any risk to the operator or the environment, and compared to the official method is faster, cheaper, and equally efficient. The main advantages of ultrasound-assisted extraction therefore include a shorter extraction time and the absence of any requirement for solvents or corrosive acids. In addition, ultrasound-assisted extraction can be carried out at a lower temperature. The developed method was successfully applied to the extraction and determination of sodium present in animal feed. A final observation is that there were high concentrations of sodium in the animal feeds tested, which could be a potential cause of contemporary health problems observed in pet animals.

Acknowledgements

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References


Figure captions

**Figure 1.** Effects of ultrasonication time on the extraction of sodium.

**Figure 2.** Effect of the volume of water inside the polyethylene vials on the extraction of sodium (emission intensity, %). The sample mass was kept constant at 1.00 g and the volume ranged from 25 to 150 mL.
Table 1 - Recovery data for sodium spiked into feed (brand C).

<table>
<thead>
<tr>
<th>Added (mg L⁻¹)</th>
<th>Found (mg L⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>2.50</td>
<td>100</td>
</tr>
<tr>
<td>5.00</td>
<td>5.19</td>
<td>104</td>
</tr>
<tr>
<td>7.51</td>
<td>7.69</td>
<td>102</td>
</tr>
<tr>
<td>10.0</td>
<td>10.2</td>
<td>102</td>
</tr>
<tr>
<td>12.5</td>
<td>12.3</td>
<td>98.4</td>
</tr>
</tbody>
</table>

Table 2 – Determination of sodium in seven commercial brands of pet food.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Proposed methoda</th>
<th>Official methoda</th>
<th>Calculated tb</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9410 ± 251</td>
<td>9092 ± 149</td>
<td>1.978</td>
</tr>
<tr>
<td>B</td>
<td>6302 ± 143</td>
<td>6728 ± 180</td>
<td>2.865</td>
</tr>
<tr>
<td>C</td>
<td>10822 ± 79</td>
<td>10425 ± 173</td>
<td>3.056</td>
</tr>
<tr>
<td>D</td>
<td>3840 ± 111</td>
<td>4058 ± 22</td>
<td>4.004</td>
</tr>
<tr>
<td>E</td>
<td>4184 ± 94</td>
<td>4309 ± 17</td>
<td>2.388</td>
</tr>
<tr>
<td>F</td>
<td>6820 ± 231</td>
<td>7014 ± 20</td>
<td>1.645</td>
</tr>
<tr>
<td>G</td>
<td>2775 ± 106</td>
<td>2893 ± 78</td>
<td>2444</td>
</tr>
</tbody>
</table>

aResults expressed in mg(Na⁺) kg⁻¹ of feed.

bTabulated value of t (Student’s t-test) equal to 4.303 for two degrees of freedom and a confidence interval of 95%.
150x106mm (96 x 96 DPI)
Analytical Methods

Proportion of sample mass (g): volume of extractor solvent (mL)

Emission intensity (%) vs. Proportion of sample mass (g): volume of extractor solvent (mL)

150x106mm (96 x 96 DPI)