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

Maria Izabel Milani[a], Eduardo Luiz Rossini[a], Leonardo Pezza[a], Helena Redigolo Pezza[a],*

[a] Instituto de Química, Universidade Estadual Paulista "Julio de Mesquita Filho", UNESP,

R. Prof. Francisco Degni 55, P.O. Box 355, 14800-900, Araraquara, SP, Brazil

* Corresponding author

Fax: +55-16-33327932

E-mail address: hrpezza@iq.unesp.br (H.R. Pezza)

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.

63   **1.   Introduction**

64   Sodium is one of the Earth's most abundant elements. It is essential to all living organisms,

65   including humans and animals, where it contributes to maintaining osmotic balance[1,2] and participates

66   in the sodium-potassium pump that controls the electrical potential of cells[3]. Salt (sodium chloride) has

67   played a crucial role in the development of human society, because it is a key component used to

68   preserve foods such as meat, fish, and pet food. The sodium in the diets of humans and animals is

69   mainly present in the form of sodium chloride.

70   In recent years, dietary sodium has become one of the more controversial nutrient elements in

71   foods. Some commercial pet foods contain high levels of sodium, which some veterinarians have

72   suggested might be unsafe[4]. Signs of sodium deficiency include increased restlessness, elevated heart

73   rate, reduced urine specific gravity, and dry mucous membranes[5]. However, the intake of sodium in

74   excess can cause several problems, such as increased blood pressure and the onset of kidney stones[4].

75   Cases of acute salt toxicosis have been reported in dogs ingesting extremely high amounts of sodium[6].

76   In the case of pets, excessive intake of sodium is related to the feed provided to the animals, where

77   sodium is not only present in the form of NaCl, but also as preservatives such as sodium benzoate and

78   sodium nitrite, as well as dyes.

79   In humans, excess salt increases oxidative stress, which is one of the hallmarks of renal failure[7].

80   In the case of dogs and cats, uncertainty remains concerning the effects of high dietary sodium intake

81   on renal disease[4]. In Brazil, the agency responsible for the control of this type of product is the

82   Ministry of Agriculture, Livestock, and Food Supply (MAPA)[8], but no limits have been established

83   concerning the addition of salt to animal feed.

84   Due to its importance in biochemical processes, control of the levels of Na in pet food is

85   needed, which in turn requires efficient methods of sample preparation that enable fast and accurate

86   quantification of the element. The literature reports few studies concerning mapping of the elements

87   present in animal feed[9,10], and the sample treatments that have been used (such as in the official

88 methodology) are time-consuming and environmentally unfriendly, employing high temperatures and

89 strong corrosive acids, which are contrary to the principles of green chemistry[11].

90 The implementation of green chemistry techniques has become increasingly popular since the

91 1990s and is based on 12 principles that aim to minimize environmental impacts, reduce or eliminate

92 possible harm to the operator, and provide faster and more efficient analyses[11,12].

93 Ultrasonic irradiation is an environmentally friendly alternative to solid-liquid extraction that

94 can be used for the pretreatment of solid samples, enabling faster performance of steps such as

95 dissolution, extraction, and leaching, amongst others[13]. Ultrasonication increases extraction efficiency

96 due to a combination of acoustic cavitation and mechanical effects. Microbubbles formed during

97 cycles of compression and decompression increase in size and eventually collapse, which enhances the

98 removal into solution of sodium and other minerals present in the solid sample. The use of ultrasound

99 therefore helps to increase the amount of analyte extracted from the sample[14-19].

100 In this paper, we evaluate the efficiency of a new methodology for the extraction of sodium

101 from pet food, using deionized water as the solvent and ultrasonication to enhance the extraction. The

102 determination of sodium was performed by flame photometry, which is simple, fast, inexpensive, and

103 selective for the analyte in question. The proposed methodology was applied in the analysis of

104 different pet food samples.

105

106 **2. Experimental**

107 *2.1. Materials, reagents, and solutions*

108 Sodium chloride (analytical grade, 100% purity) was purchased from Mallinckrodt. Prior to

109 use, the reagent was dried in an oven for 1 h at 250 °C. Concentrated nitric acid (60-65%) was

110 purchased from Synth, and the dilutions utilized in the reference method (20% and 1 mol L^{-1} nitric

111 acid) were prepared using deionized water (18 MΩ cm) obtained from a Milli-Q system (Millipore).

112 Polyethylene bottles and beakers were used throughout the experiments.

113   A stock standard solution of 100 mg L^{-1} Na^+ was prepared using deionized water. Working

114   standard solutions (1-20 mg L^{-1}) were prepared by appropriate dilution of the stock solution. For the

115   official method, a stock standard solution of 100 ppm Na^+ was prepared in 1 mol L^{-1} nitric acid.

116   Seven commercial pet foods for dogs and cats were acquired from local shops in Araraquara

117   (São Paulo, Brazil).

118

2.2. Equipment

120   Initial sample homogenization employed a domestic blender together with a porcelain mortar

121   and pestle. A forced air recirculation and renewal oven (Model TE-394/1, Tecnal) was used for drying

122   the samples. An ultrasonic bath (Model T14, Thornton) operated at a frequency of 40 kHz and power

123   of 60 W was used during the proposed extraction process. A muffle furnace (Model MA 385, Marconi)

124   was used for sample preparation according to the official AOAC method[10]. The determination of

125   sodium present in the samples was performed with a flame photometer (Model B262, Micronal).

126

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

128   The proposed pretreatment of the animal feed samples was performed using a household

129   blender followed by reduction to a fine powder with the aid of a porcelain mortar and pestle. An

130   appropriate mass of the powder was then oven-dried for 6 h at 100 °C and transferred to a polyethylene

131   beaker. It was necessary to dry the samples, because the sodium contents were calculated on a dry

132   mass basis. As recommended by the AOAC[10], heating for 6 hours was used to ensure that all the water

133   present in the samples of pet food was eliminated. It is not recommended to perform the ultrasound-

134   assisted extraction without first drying the sample in an oven. After the drying step, deionized water

135   was added at a rate of approximately 100 mL for each 1 g of sample, and the mixture was placed under

136   sonication for an optimized time of 20 min. The samples were then filtered through common paper

137   filter, followed by filtration with Whatman qualitative filter paper No.1, and the resulting filtrates were

138   collected in polyethylene bottles. Each sample was prepared in triplicate, and sodium determination

139   was performed using flame photometry.

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142   *2.4 Optimization of experimental conditions*

143   The optimization of the conditions of the ultrasound bath was based on studies by Nascentes et

144   al.[16], using 1.5 L of water in the bath and six polyethylene vials containing the samples with extraction

145   solvent (water, T = 25 ^{o}C), corresponding to two samples in triplicate. The vials were placed in the

146   central region of the bath. The sample mass was kept constant at 1.00 g, and the volume of water

147   inside the polyethylene vials ranged from 25 to 150 mL.

148   The sodium extraction time was optimized by ultrasonicating seven aliquots of the same

149   sample for times ranging from 0 to 60 min, after which the sodium contents were determined by flame

150   photometry.

151

152   *2.5 Study of matrix interferences*

153   The possible interference of matrix components was investigated by means of the addition and

154   recovery of standards, using fortification levels from 50 to 250%, with subsequent determination of

155   sodium by flame photometry.

156

157   *2.6. Analytical curves*

158   A series of sodium solutions were prepared at suitable dilutions (1.00, 2.50, 5.00, 7.50, 10.0,

159   15.0, and 20.0 mg L^{-1}). A calibration graph was then prepared by plotting relative emission intensity

160   against sodium concentration in the range 1.00 to 20.0 mg L^{-1}.

161

162   *2.8. Reference method*

163   In the case of the AOAC method[10], the glassware was first cleaned with 20% HNO_3 solution

164   and then rinsed with deionized water. The feed samples were dried in an oven for 6 h at 100 °C,

165   followed by heating in a muffle furnace at 525 °C for 7 h until a grayish-white color was obtained. The

166   calcined samples were then dissolved in an appropriate volume of 1 mol L^{-1} HNO_3. These solutions

167   were stored in polyethylene bottles prior to analysis using the recommended reference method.

168

3. Results and Discussion

170   *3.1. Optimization of the experimental conditions*

171   *3.1.1. Ultrasonic extraction time*

172   Analysis of the sodium concentrations in the solutions obtained after different periods of

173   sonication revealed the formation of a plateau after 15 min (Figure 1). A sonication time of 20 min was

174   therefore adopted, which was sufficient to ensure quantitative extraction of sodium, without extending

175   the extraction process for an unnecessarily long time.

176

177   [Insert Figure 1]

178

179   *3.1.2. Effect of the water volume in the extraction vials*

180   Variation of the relative amounts of sample and extraction solvent did not significantly influence

181   the determination, as shown in Figure 2.

182

183   [Insert Figure 2]

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185

186 *3.2. Construction of analytical curve and determination of LOD and LOQ*

187 An analytical curve of the relative emission intensity (RE) as a function of sodium

188 concentration ($[Na^+]$) was constructed using the solutions prepared with deionized water. The equation

189 for the linear regression was RE = 0.576 + 5.4872$[Na^+]$ and the correlation coefficient value was

190 0.998. The limits of detection (LOD) and quantification (LOQ), determined as recommended by

191 IUPAC[20], were 0.26 and 0.90 mg L^{-1}, respectively.

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193 *3.3. Study of matrix interferences*

194 Possible matrix interferences were investigated using the recovery of analyte after the addition

195 of standards at levels ranging from 50% to 250%. The recoveries obtained were between 98.4% and

196 104% (Table 1). These data indicated that the composition of the commercial product did not

197 significantly interfere in the analysis of sodium, so sample clean-up steps were not necessary.

198

199 *3.4. Determination of sodium in pet food*

200 The proposed method was applied using seven commercial pet food samples and the results

201 were compared with those obtained with the comparative method. Statistical evaluation using the t-test

202 (95% confidence interval, 2 degrees of freedom) showed that the two sets of data were consistent with

203 each other (Table 2). The calculated t values were lower than the tabulated values, indicating that there

204 was no significant difference between the two methods in terms of precision and accuracy.

205 The concentrations of sodium found in the samples were from 2775 to 10822 mg kg^{-1}. Samples

206 A, C, F, and G were different cat foods, while samples B, D, and E were dog foods. Sample G was a

207 special food for pets with kidney problems and therefore contained less sodium.

208

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.

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260   **Figure captions**

261

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

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264   **Figure 2.** Effect of the volume of water inside the polyethylene vials on the extraction of sodium

265   (emission intensity, %). The sample mass was kept constant at 1.00 g and the volume ranged from 25

266   to 150 mL.

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271   **Table 1 -** Recovery data for sodium spiked into feed (brand C).

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Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
2.50	2.50	100
5.00	5.19	104
7.51	7.69	102
10.0	10.2	102
12.5	12.3	98.4

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

| Samples	Proposed method[a]	Official method[a]	Calculated t[b]
A	9410 ± 251	9092 ± 149	1.978
B	6302 ± 143	6728 ± 180	2.865
C	10822 ± 79	10425 ± 173	3.056
D	3840 ± 111	4058 ± 22	4.004
E	4184 ± 94	4309 ± 17	2.388
F	6820 ± 231	7014 ± 20	1.645
G	2775 ± 106	2893 ± 78	2444

[a]Results expressed in mg(Na$^+$) kg^{-1} of feed.

[b]Tabulated value of t (Student's t-test) equal to 4.303 for two degrees of freedom and a confidence interval of 95%.

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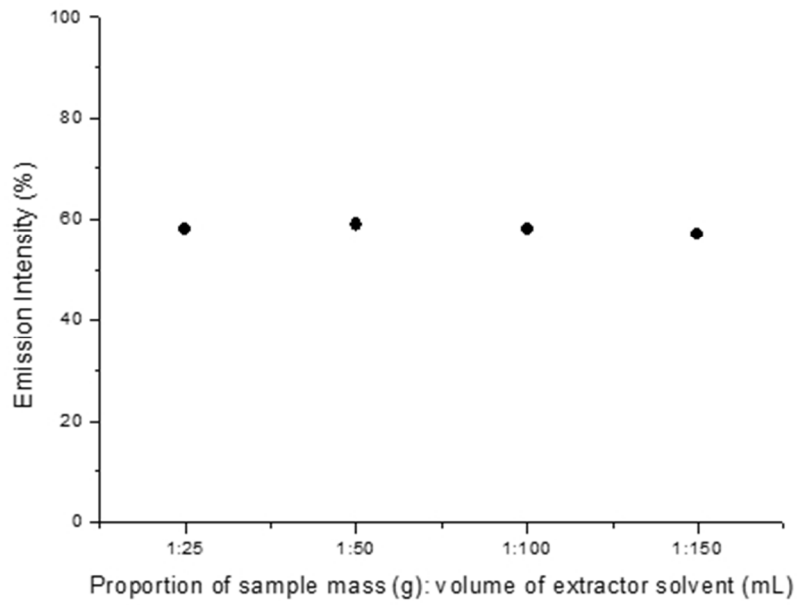43
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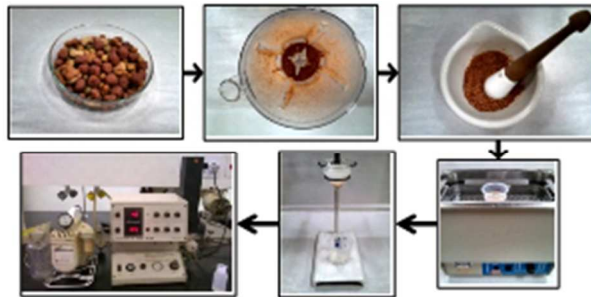
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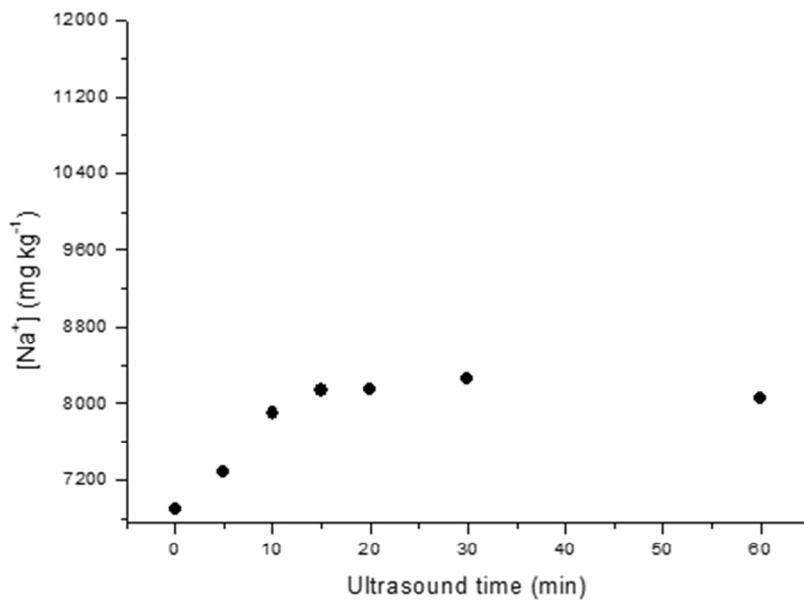
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