

Determination of phosphorus additives in instant noodle samples by Phosphorus-31 nuclear magnetic resonance (^{31}P -NMR)

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Abstract:

Instant noodles are widely consumed in Vietnam and are especially popular among young people. In the production of instant noodles, phosphate additives are commonly added to enhance the crispiness and chewiness of the product. In this study, three phosphate species, including sodium pyrophosphate (SPP), sodium tripolyphosphate (STPP), and sodium hexametaphosphate (SHMP), in instant noodle samples collected from the Vietnamese market were rapidly analysed using the ^{31}P -NMR method. The phosphate species in instant noodle samples were simply extracted using deionised water. For ^{31}P -NMR measurement, a D_2O /deionised water mixture (9:1) was added. Chlorpyrifos ethyl was used as an internal standard and the pH was adjusted to 9-10. The ^{31}P -NMR spectra were then obtained in 1D mode using a pulse program that involved a 90° hard pulse. The method detection limits for SPP, STPP, and SHMP determination were 0.60, 0.30, and 0.74 mg/g, respectively. The repeatability of SPP in instant noodles was found to be smaller than 5%. The linearity was tested with R^2 (R-squared) values greater than 0.99 for all three analytes. The analysis of instant noodle samples revealed the presence of only SPP, a common additive known for its significant impact on enhancing food quality. The SPP content was estimated to be around 25-54% of the total phosphate, determined by UV-Vis based on the heteropoly blue phosphomolybdate complex.

Keywords: instant noodle, phosphomolybdate, sodium pyrophosphate, total phosphate, ^{31}P -NMR.

Classification numbers: 2.2, 2.3

1. Introduction

Phosphates are crucial substances for life, playing vital roles in metabolising and absorbing nutrients from food [1]. Phosphorus (P) additives are incorporated into convenience and processed foods for various purposes, such as leavening, colour and moisture retention, anti-caking properties, and flavour enhancement [2]. In food technology, P additives (inorganic, organic, and natural P-containing) exist in different forms (single, di-, tri-phosphate, and polyphosphate), identified by E numbers [3, 4]. Previous research has suggested that SPP, STPP, SHMP, sodium acid pyrophosphate (SAPP), and their phosphate blends at various concentrations (0.3, 0.5, and 0.7%) significantly impact the quality and characteristics of food products, such as pH, water-holding capacity, and colour of ground meat patties [5]. Acceptable levels of phosphorus content vary depending on the type of food, ranging from 251 mg of phosphorus per person per day in infants to 1,625 mg per person per day for adults. High exposure levels range from 331 mg of phosphorus per person per day in infants to 2,728 mg per person per day for adults [1]. Various types of phosphorus additives are observed across different food categories, with lecithin (E 322), pyrophosphate (E 450), and triphosphate (E 451)

being the most common [3]. Therefore, it is essential to analyse phosphate species in food to ensure food safety.

In terms of conventional analytical methods for determining total phosphate in food samples, spectrophotometric techniques such as the molybdenum blue, vanadomolybdate complex, and malachite green methods are commonly employed [6]. These methods offer sufficient sensitivity and accuracy. However, for determining phosphorus species, chromatography is the preferred method in laboratories due to its selectivity and low detection limit [7]. Some studies have utilised high-performance liquid chromatography (HPLC) for the quantitative analysis of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) in litchi fruit pericarp tissue [8], as well as for analysing soluble reactive phosphate (SRP) in marine and freshwater samples [9].

Comparatively, quantitative nuclear magnetic resonance (NMR) spectroscopy offers several advantages over time-consuming chromatography when applied to food analysis. It presents a simple operation, allows for simultaneous procedures, and enables quick analysis without the need for extensive sample preparation, making it an inexpensive method [10]. Specifically, quantitative ^{31}P -NMR serves as a

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flexible analytical tool for phosphate determination across various food samples, including meat [11-14], milk [15], and beverages [16-18]. While sample preparation for ^{31}P -NMR analysis may require some time, the method offers a high method detection limit and good specificity comparable to other analytical methods. However, this high detection limit may potentially limit its effectiveness in detecting trace levels of polyphosphates in complex food matrices like certain types of food products.

In studies focusing on phosphorus additives, considerable attention is directed towards compounds such as $\text{Na}_5\text{P}_3\text{O}_{10}$, $\text{Na}_3\text{P}_3\text{O}_9$, $\text{K}_4\text{P}_2\text{O}_7$, $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$, and $\text{K}_4\text{P}_2\text{O}_7$, among others. Researchers highlight the utility of the proposed method for monitoring the hydrolysis of poly- and pyrophosphates, alongside the analysis of samples resulting from such hydrolysis or alkaline extraction. To ensure the completeness of the method, the use of internal reference standards such as hexamethylphosphoramide (HMPA) [14], methylphosphonic acid (MPA) [19], or triphenylphosphate with solvents like D_2O and modified $\text{CDCl}_3/\text{MeOH}/\text{D}_2\text{O}$ -EDTA- Cs^+ (1.0 M) (400:80:5 v/v) [13] is essential. The effectiveness of these techniques relies on the types and concentrations of phosphorus compounds present in food samples, ensuring good selectivity and high accuracy, particularly when applied to complex matrix samples.

This study aims to introduce a method for determining SPP, STPP, and SHMP in instant noodle samples using ^{31}P -NMR. The experimental conditions are optimised to enhance accuracy and reliability. An innovative approach is employed by utilising Chlorpyrifos ethyl, a readily available and cost-effective internal standard, for quantification. The validation and efficacy of the ^{31}P -NMR method in accurately assessing specific phosphate additives in instant noodle samples offer a novel and accessible approach for quality control in food analysis.

2. Materials and methods

2.1. Chemicals and reagents

The following primary standards were purchased from Sigma-Aldrich, with mass fraction purities indicated in parentheses: Sodium triphosphate pentabasic ($\text{Na}_5\text{P}_3\text{O}_{10}$, $\geq 98.0\%$), SPP tetrabasic ($\text{Na}_4\text{P}_2\text{O}_7$, $\geq 95.0\%$), and SHMP ($(\text{NaPO}_3)_6$, 96%). Working standard solutions were prepared by diluting the original stock standards, which were stored in Falcon tubes and kept in the freezer. These solutions were used within a month of preparation.

A stock standard solution of 1000 ppm phosphorus (P) was prepared from KH_2PO_4 stock standard solution obtained from Merck. A 0.08 M ammonium molybdate solution was prepared by dissolving 4.996 grams of $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ in 50 ml of deionised water, followed by complete dissolution using an ultrasonic machine. Additionally, a mixture of ascorbic acid (0.375 M) and sulphuric acid (2.5 M) was prepared using

commercially available reagents. An internal standard solution of Chlorpyrifos ethyl was prepared from a phosphate-based plant protection drug containing 44.5% Chlorpyrifos ethyl. This was achieved by taking 1.0 ml of the pesticide and diluting it to 10 ml in a 10 ml volumetric flask using deionised water.

D_2O solution (100 atom %D), utilised in this research, was sourced as a pure chemical and was exclusively used in the laboratory of ARMAR Chemicals (Switzerland).

All reagents used were of analytical grade, and deionised water (DI) was utilised throughout. Solutions were prepared in volumetric flasks, transferred to glass jars, and stored at 25°C, shielded from sunlight. These solutions were used within 24 hours of reconstitution to maintain their integrity and accuracy.

2.2. Equipment and instruments

The UV-Vis spectrophotometer (UV-1650PC, Shimadzu, Japan) equipped with a deuterium lamp and tungsten filament, along with a plastic cuvette (1 cm thickness), was utilised for the spectrophotometric analysis of total phosphate.

For the analysis of phosphorus species, the NMR spectrometer Bruker Avance III equipped with a 5 mm PABBO BB/19F-1H/D probe with Z gradient, featuring auto-matching and auto-tuning capabilities, operating at a frequency of 550 Hz in the mode of liquid sample, was used. Nitrogen (N_2) was used as the cooling liquid gas.

2.3. Samples and sample preparation

Sampling: Three instant noodle samples from different manufacturers were collected randomly from grocery stores and supermarkets in Vietnam, with expiry dates at least three months away. The samples were kept in the manufacturer's packaging and stored in a refrigerator until sample processing. All samples were used to determine total phosphate concentration by the UV-Vis method.

Sample preparation for phosphomolybdate method: Instant noodle samples were treated as described in AOAC 995.11 [20]. A mixture of 1.5 g of sample with 0.5 g of ZnO catalyst was heated in an oven at 550°C for 4 hours, cooled, then dissolved with 5 ml of HCl (3:1) and 5 ml of DI water, then boiled for 15 minutes. After filtering, the solution was neutralised with 0.1 M NaOH solution until a white precipitate ($\text{Zn}(\text{OH})_2$) appeared. Then a few drops of dilute acid (0.1 M HCl) were added to the precipitate to dissolve it. The sample was filtered and transferred to a 50 ml volumetric flask and then graduated up to the mark. Accurately pipette 5.0 ml of treated solution into a 50 ml volumetric flask containing 15 ml of DI. To this flask, 7 ml molybdate-ascorbic acid solutions (1.5 ml of 0.08 M ammonium molybdate, 3.5 ml of 2.5 M sulphuric acid, and 2 ml of 0.375 M ascorbic acid) were added. The blank samples were also prepared by a home-made instant noodle manufacturing process involving the combination of wheat flour and acetylated starch to create adhesion and enhance the chewiness of the noodle strands.

The absorbance of the produced complex was measured at the maximum wavelength (820 nm) and the calibration curve method was studied and applied in this study. The instrument detection limit and quantification limit were 0.09 and 0.29 ppm, respectively. The method detection limit was 0.47 mg/g of total phosphorus species.

Sample preparation for ^{31}P -NMR method: Samples were prepared according to the E. Szlyk, et al. (2011) [13]'s process with slight modification. To the exact weight of ground sample (1.5 g for instant noodles), 10 ml of DI was added, then it was shaken for 60 minutes. Next, the solution was centrifuged at 4000 rpm for 1 hour. The clear solution was transferred into a 50 ml volumetric flask and filled to the mark. For quantitative determination of phosphorus content by ^{31}P -NMR, the standard and D_2O were mixed in a ratio of 9:1. An internal standard was added at 0.089%, and the pH was adjusted to about 9-10 with 0.1 M NaOH solution. Then 1 ml of these solutions was transferred into NMR tubes, and NMR spectra were recorded. To quantify the phosphate species by ^{31}P -NMR, the relationship between the area of the obtained signal and the concentration of the compounds was analysed.

The ^{31}P -NMR spectra were obtained in 1D mode. The pulse programme involved a 90° hard pulse. The relaxation delay was set at 2 seconds, five times the relaxation time of the ^{31}P nucleus, to ensure total spin relaxation, followed by an acquisition time of 0.402 seconds. The receiver gain was calculated by the command "rga" in Topspin 3.2 (Bruker Biospin). Other parameters were fixed: spectral width 81521.7 Hz (-400 to 400 ppm),

acquired size 32768. A line broadening function of 1.0 Hz was applied prior to Fourier transform. It resulted in a spectral size of 65536 points.

3. Results and discussion

3.1. Optimisation of experimental conditions

Optimisation of experimental conditions was studied by recording ^{31}P -NMR spectra of standards and the internal standard (Chlorpyrifos ethyl) in a mixture (Fig. 1). The signal was calibrated with an instrumental blank, which is a mixture of H_2O and D_2O in a 90:10 % v/v ratio.

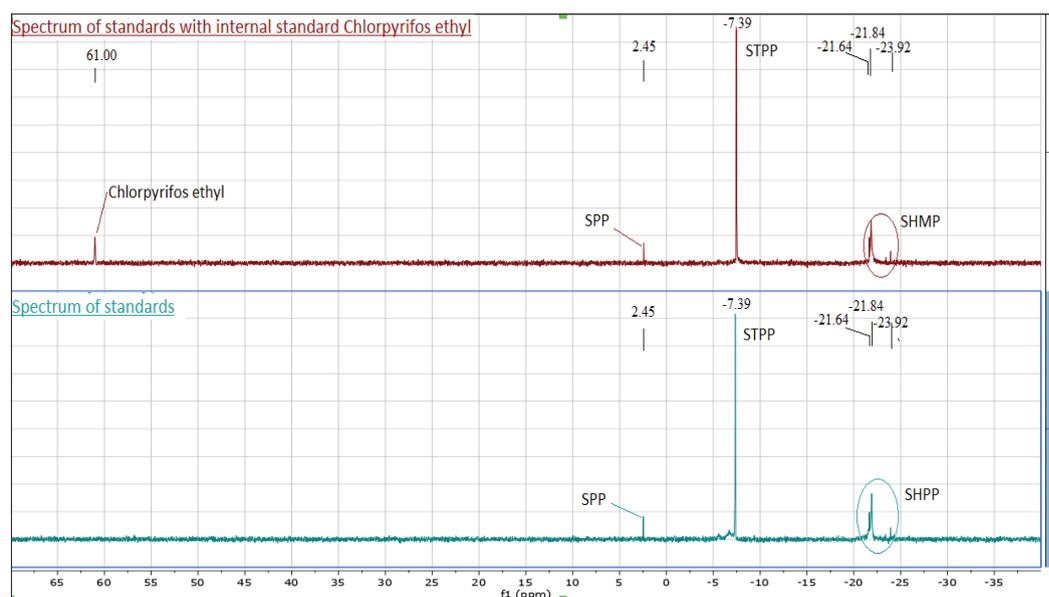


Fig. 1. ^{31}P -NMR spectra of standards and internal standard (chlorpyrifos ethyl) in a mixture of H_2O and D_2O (90:10 % v/v).

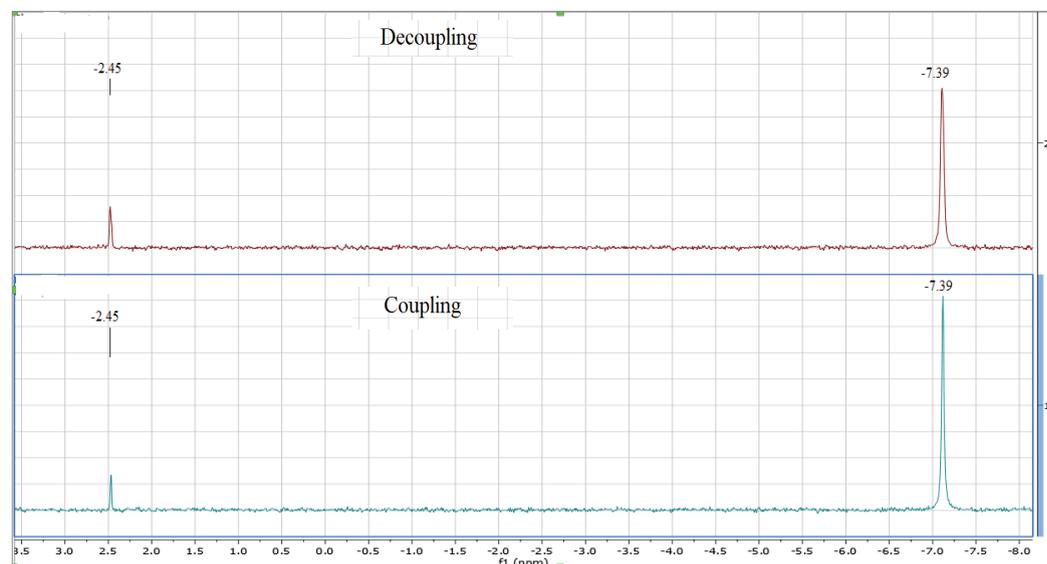


Fig. 2. Effect of scan mode on the ^{31}P -NMR signal.

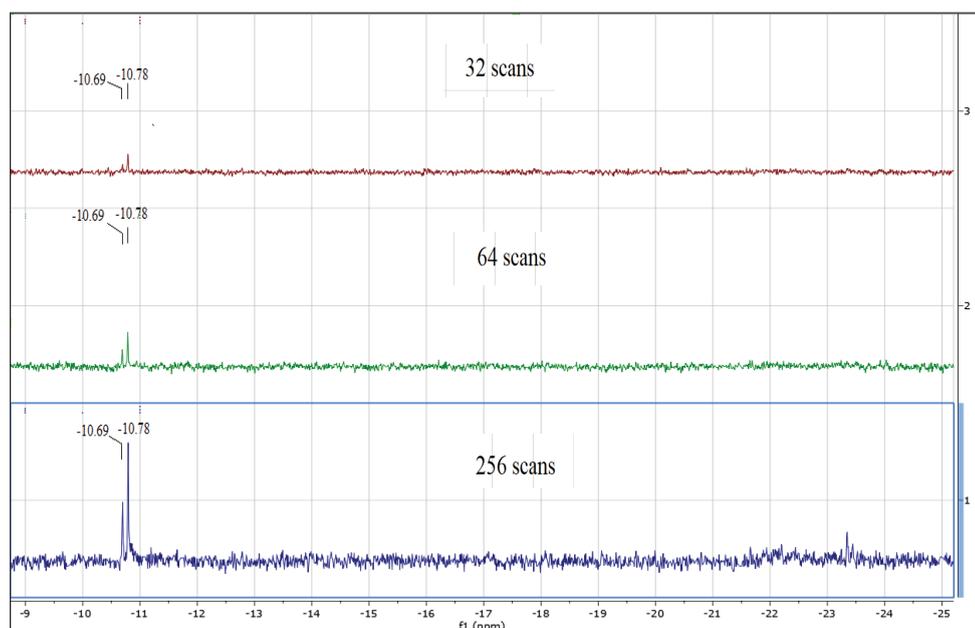


Fig. 3. Effect of the number of scans on the ^{31}P -NMR signal.

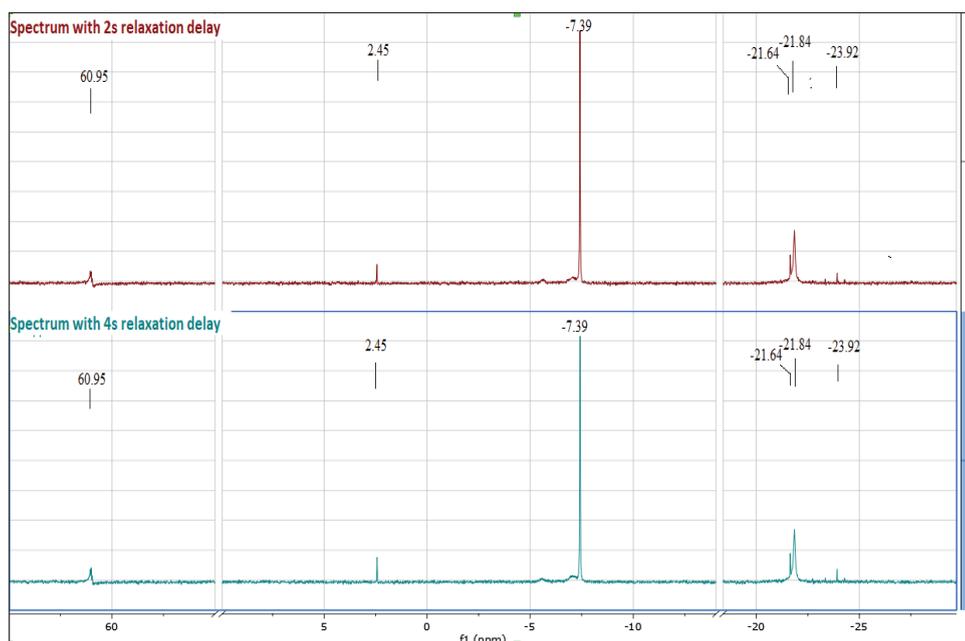


Fig. 4. ^{31}P -NMR spectra of standards and internal standard at different relaxation times.

Effect of measurement mode: Two measurement modes, coupling and decoupling, were investigated. The coupling mode scans the effects and interactions of atoms in the analyte molecule, while the decoupling mode ignores these interactions, resulting in a clearer measurement spectrum. However, the signal intensity in coupling mode is observed to be higher than in decoupling mode, despite a small difference in the area integral of the two modes (Fig. 2). Consequently, utilising decoupling mode may result in more challenging quantification. Therefore, experiments on measuring signals were conducted with pyrophosphate $\text{Na}_4\text{P}_2\text{O}_7$ 300 ppm with

64 scans and a relaxation delay time of 2 seconds at 26°C (Fig. 3).

Effect of the number of scans: As the number of scans increases, the spectral signal becomes clearer. However, excessive scans result in longer scanning times, which is not necessary.

In practice, 1024 scans were selected in this case as it provided a sufficiently clear signal for analysis.

Effect of relaxation time: Relaxation time theoretically affects signal clarity similar to the number of scans. However, experiments with different relaxation times showed very similar results, with insignificant differences (Fig. 4).

As the relaxation time increases, the measurement duration also becomes longer. Therefore, a relaxation time of 2 seconds was deemed reasonable and selected for future experiments.

Effect of pH: Variations in pH lead to changes in the measuring signal. At higher pH levels, the inorganic spectra shift towards the positive (left side) and the signal intensity increases, whereas the organic compound signals remain almost unchanged (Fig. 5).

The pH 10 sample controlled by the NaOH and HCl were considered as suitable experiment conditions.

Investigation of selection of internal standards: The ^{31}P -NMR spectrum of instant noodles shows a clear peak at 2.5 ppm and a smaller peak at approximately 5.5-6 ppm (Fig. 6). The peak at 2.5 ppm is identified as SPP, but the peak at 5.5-6 ppm does not match any of the three reference standards studied and thus remains unidentified [17-19]. Therefore, it is evident that the current method, with optimised conditions and Chlorpyrifos ethyl as the internal standard, can determine the presence of SPP in instant noodle samples.

It was found that the additive in instant noodles contains only SPP and no STPP or SHMP. Therefore, the experiments in this study focused on analysing SPP in instant noodles.

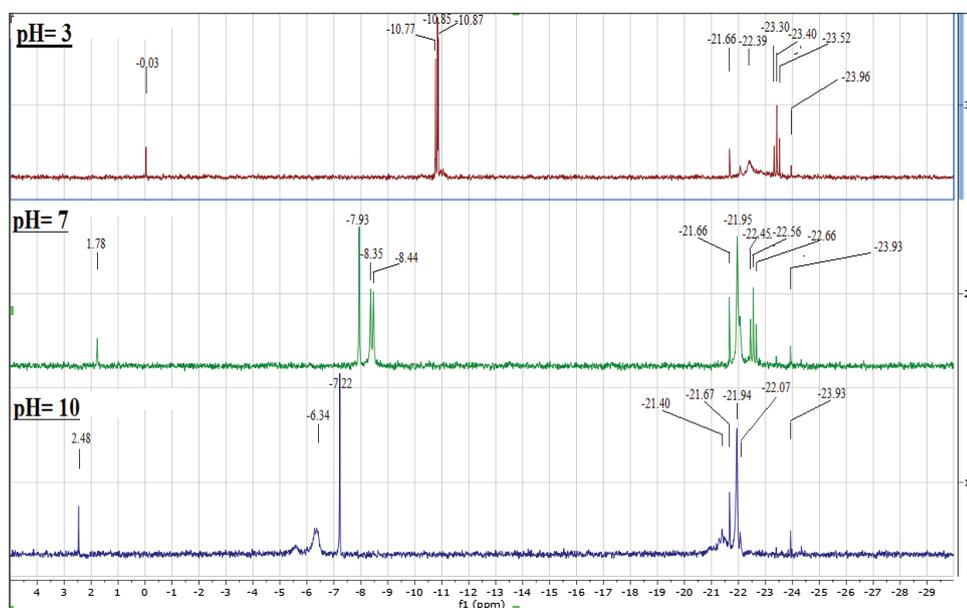


Fig. 5. Effect of pH on ^{31}P -NMR spectra.

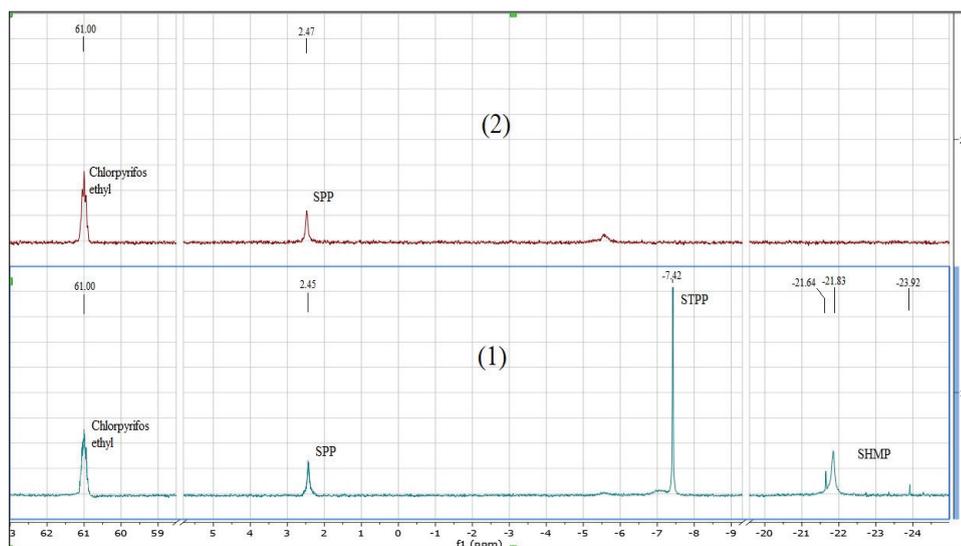


Fig. 6. ^{31}P -NMR spectra of (1) instant noodle with chlorpyrifos ethyl as the internal standard and (2) instant noodles added to standards sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate with chlorpyrifos ethyl as the internal standard.

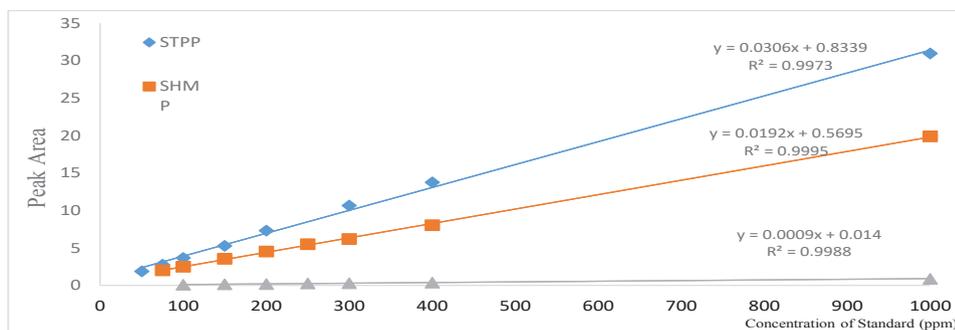


Fig. 7. Calibration curves of sodium pyrophosphate, sodium hexametaphosphate, and sodium tripolyphosphate in ^{31}P -NMR.

3.2. Validations of the ^{31}P -NMR method

Calibration curves and linearity: The peak areas obtained from the analysis of 12 standard samples for the ^{31}P -NMR method were used to establish calibration curves and assess linearity. For the evaluation of linearity, correlation coefficients and working ranges were considered. The calibration curve for SPP utilised a concentration range of 100-1000 ppm. The STPP calibration curve was prepared within the range of 50-1000 ppm, while the SHMP calibration curve was established within the range of 75-1000 ppm. The obtained correlation coefficients for all three calibration curves were greater than 0.99, indicating excellent linearity of the regression models. Additionally, the relative deviation values of concentrations calculated from the calibration curves were all below 15%. This suggests that the calibration curves are reliable and accurately predict the concentrations of SPP, STPP, and SHMP within the specified concentration ranges (Fig. 7).

Detection and quantitative limits: For the method detection limit (MDL) and method quantitative limit (MQL), instant noodle samples of the same product (IN2) were processed and analysed. Six samples of IN2 noodles (containing only SPP) and spiked noodle samples (with low concentrations of STPP and SHMP added) were processed according to the same procedure and analysed under the same conditions to determine the MDL and MQL of SPP. The instrument detection limits (IDL)

indicate that the method is not highly sensitive and is not suitable for determining trace or hypertrace concentrations directly. However, for food samples, the detection limit and quantitative limit of the method are reasonable and acceptable (Table 1).

Table 1. Instrument detection limit, method detection limit, and method quantitative limit of sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate determination by ³¹P-NMR method.

Parameters	SPP	STPP	SHMP
IDL (mg/g)	0.6	0.3	0.74
MDL (mg/g) (For instant noodles samples)	0.31	0.25	0.32
MQL (mg/g) (For instant noodles samples)	1.00	0.83	1.10

Trueness: To determine the trueness of the method, the recovery of the method was assessed using spiked samples of instant noodles. The results in Table 2 show recoveries for SPP, STPP, and SHMP in instant noodle samples between 90 and 110%, indicating good trueness of the analytical procedure.

Table 2. Recovery of sodium pyrophosphate determined in instant noodle samples.

	Conc. added (ppm)	Conc. in sample (ppm)	Conc. found (ppm)	Spiked conc. found (ppm)	Recovery (%)
SPP	100	63.89	157.71	93.82	93.82
STPP	100	N.D.	104.36	104.36	104.36
SHMP	100	N.D.	95.05	95.05	95.05

Accuracy: Repeatability was evaluated by analysing the same sample 6 times, and reproducibility was validated by analysing the previously used sample 6 times the following day (Table 3). Relative standard deviations (RSD_r) for repeated analysis of phosphorus in instant noodle sample matrices within the same day were all lower than 3%, and RSD_R (%) values were smaller than 3%, indicating consistent results between the two sets of experiments.

Table 3. Repeatability and reproducibility.

Entry	Day 1	Day 2
	SPP (ppm)	SPP (ppm)
1	64.29	65.00
2	63.89	63.99
3	63.23	64.15
4	64.00	64.19
5	63.51	63.97
6	64.44	64.69
Mean	63.89	64.33
SD	0.459	0.418
RSD_r (%)	0.719	0.651

Precision: Relative standard deviations (RSD_r) for repeated analysis of phosphorus in instant noodle sample matrices within the same day were all lower than 3%, confirming the repeatability of the analytical procedure. Comparison between two sets of experiments on day 1 and day 2 was carried out to validate reproducibility across different days. The t_{cal} and s_{pooled} values for SPP concentration in instant noodles were 1.736 and 0.439, respectively. The t_{cal} value (1.736) was smaller than $t_{0.95,10}$ (2.228), and the RSD_R (%) value (0.745) was smaller than 3%, indicating consistent results between the two sets of experiments.

3.3. ³¹P-NMR quantitative determination of phosphate compounds in instant noodle sample

³¹P-NMR spectra of three analytical subjects (SPP, STPP, and SHMP) were detected separately from each other and from the internal standard spectrum (Chlorpyrifos ethyl) (Fig. 1). Quantitative determination of phosphate types in some instant noodle samples was performed using ³¹P-NMR. The results are shown in Table 4.

Table 4. Concentration of phosphate in samples.

Sample	Conc. of SPP in analytical solution (ppm)	Conc. of SPP in instant noodles sample (mg/g)	Total phosphate (determined by UV-Vis) (mg/g)
IN1	120±1.3	1.2±0.045	4.3±0.082
IN2	128±1.63	2.6±0.054	4.7±0.12
IN3	93±0.41	0.93±0.02	3.6±0.08

The concentration of SPP in three instant noodle samples from three different brands ranged from approximately 0.93 to 2.6 mg/g. The percentage of SPP to total phosphate in the sample, as analysed by the UV-Vis method, ranged from approximately 28 to 54%, depending on the product of each producer. The percentage of SPP additives in instant noodles was relatively low, and other additives (STPP and SHMP) were not detected. An unknown spectrum appearing at approximately 5.5 ppm was recorded. This suggests that the phosphate in instant noodles may also include various other forms of phosphorus compounds, such as water-insoluble phosphorus (since the reporting solvent used was water) and other unreported phosphorus compounds. The amount of SPP additives used in instant noodle products varied among different companies. IN2 noodles were found to have the highest percentage of SPP additives and the largest amount of total phosphate among the three types of noodles. Meanwhile, the percentage of SPP in IN1 and IN3 was similar.

4. Conclusions

The ³¹P-NMR method successfully detected the presence of SPP as the main phosphate additive in three instant noodle samples from different brands. The percentage of SPP to total phosphate in these samples, as analysed by the

UV-Vis method, ranged from approximately 28 to 54%, varying depending on the specific product of each producer. Interestingly, other additives such as STPP and SHMP were not detected in any of the samples. This suggests that the phosphate content in instant noodles may also consist of various other forms of phosphorus compounds, including water-insoluble phosphorus (as the reporting solvent used was water) and potentially other unreported phosphorus compounds. Moreover, it was observed that the amount of SPP additives used in instant noodle products varied among different companies. The proposed ^{31}P -NMR procedure proved to be highly useful for the quantitative analysis of phosphate types in food due to its excellent selectivity and straightforward implementation. This method offers valuable insights into the composition of phosphate additives in food products, aiding in quality control and regulatory compliance efforts within the food industry.

CRedit author statement

Trung Quang Pham: Conceptualisation, Methodology, Data collection, Writing - Original draft preparation; Lan Anh Thi Nguyen: Validation, Formal analysis; Nhung Thi Dao: Sampling and Sample preparation; Thao Thi Ta: Methodology, Investigation, Supervision, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] European Food Safety Authority, M. Younes, G. Aquilina, et al. (2019), "Re-evaluation of phosphoric acid-phosphates-di-, tri- and polyphosphates (E 338-341, E 343, E 450-452) as food additives and the safety of proposed extension of use", *EFSA Journal*, **17**(6), DOI: 10.2903/j.efsa.2019.5674.

[2] R.A. Molins (1991), *Phosphates in Food*, CRC Press, 272pp.

[3] M. Tuominen, H.J. Karp, S.T. Itkonen (2022), "Phosphorus-containing food additives in the food supply - An audit of products on supermarket shelves", *Journal of Renal Nutrition*, **32**(1), pp.30-38, DOI: 10.1053/j.jrn.2021.07.010.

[4] A.S.R. Anjaneyulu, N. Sharma, N. Kondaiah (1989), "Evaluation of salt, polyphosphates and their blends at different levels on physicochemical properties of buffalo meat and patties", *Meat Science*, **25**(4), pp.293-306, DOI: 10.1016/0309-1740(89)90047-8.

[5] W. Li, J.A. Bowers, J.A. Craig, et al. (1993), "Sodium tripolyphosphate stability and effect in ground turkey meat", *Journal of Food Science*, **58**(3), pp.501-504, DOI: 10.1111/j.1365-2621.1993.tb04310.x.

[6] E.A. Tosi, A.F. Cazzoli, L.M. Tapiz (1998), "Phosphorus in oil. Production of molybdenum blue derivative at ambient temperature using noncarcinogenic reagents", *Journal of The American Oil Chemists' Society*, **75**(1), pp.41-44, DOI: 10.1007/s11746-998-0007-x.

[7] G. Helmerich, P. Koehler (2003), "Comparison of methods for the quantitative determination of phospholipids in lecithins and flour improvers", *Journal of Agricultural Food Chemistry*, **51**(23), pp.6645-6651, DOI: 10.1021/jf0345088.

[8] H. Liu, Y. Jiang, Y. Luo, et al. (2006), "A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography", *Food Technology Biotechnology*, **44**(4), pp.531-534.

[9] J.L. Haberer, J.A. Brandes (2003), "A high sensitivity, low volume HPLC method to determine soluble reactive phosphate in freshwater and saltwater", *Marine Chemistry*, **82**(3-4), pp.185-196, DOI: 10.1016/S0304-4203(03)00069-0.

[10] V. Ramakrishnan, D.L. Luthria (2017), "Recent applications of NMR in food and dietary studies", *J. Sci. Food Agric.*, **97**(1), pp.33-42, DOI: 10.1002/jsfa.7917

[11] A. Jastrzębska, E. Szlyk (2009), "Application of ^{31}P NMR for added polyphosphate determination in pork meat", *Chemical Papers*, **63**(4), pp.414-419, DOI: 10.2478/s11696-009-0042-6.

[12] H.C. Bertram, H.J. Ersen (2004), "Applications of NMR in meat science", *Annual Reports on NMR Spectroscopy*, **53**, pp.157-202, DOI: 10.1016/S0066-4103(04)53003-X.

[13] E. Szlyk, P. Hrynczyszyn (2011), "Phosphate additives determination in meat products by ^{31}P -phosphorus nuclear magnetic resonance using new internal reference standard: Hexamethylphosphoramide", *Talanta*, **84**(1), pp.199-203, DOI: 10.1016/j.talanta.2010.12.046.

[14] A. Chiba, M. Hamaguchi, M. Kosaka, et al. (1991), "Quality evaluation of fish meat by ^{31}P -phosphorus nuclear magnetic resonance", *Journal of Food Science*, **56**(3), pp.660-664, DOI: 10.1111/j.1365-2621.1991.tb05351.x.

[15] J. Belloque, M.A.D.L. Fuente, A.M. Ramos (2000), "Qualitative and quantitative analysis of phosphorylated compounds in milk by means of ^{31}P -NMR", *Journal of Dairy Research*, **67**(4), pp.529-539, DOI: 10.1017/s0022029900004477.

[16] S.M. Ackermann, K. Dolsophon, Y.B. Monakhova, et al. (2017), "Automated multicomponent analysis of soft drinks using 1D ^1H and 2D ^1H - ^1H J-resolved NMR spectroscopy", *Food Analytical Methods*, **10**, pp.827-836, DOI: 10.1007/s12161-016-0643-y.

[17] M. Kõllo, M. Kudrjašova, M. Kulp, et al. (2013), "Methylphosphonic acid as a ^{31}P -NMR standard for the quantitative determination of phosphorus in carbonated beverages", *Analytical Methods*, **5**(16), pp.4005-4009, DOI: 10.1039/C3AY40743H.

[18] L. Medhurst, F. Shahnaz, N. Ramnarine, et al. (2016), "Measurement of phosphates in soft drinks: A general chemistry experiment using NMR, in NMR spectroscopy in the undergraduate curriculum: First year and organic chemistry courses", *ACS Symposium Series*, **2**, pp.31-37, DOI: 10.1021/bk-2016-1221.ch003.

[19] P. Hrynczyszyn, A. Jastrzębska, E. Szlyk (2010), "Determination of phosphate compounds in meat products by ^{31}P -phosphorus nuclear magnetic resonance spectroscopy with methylenediphosphonic acid after alkaline extraction", *Analytica Chimica Acta*, **673**(1), pp.73-78, DOI: 10.1016/j.aca.2010.05.018.

[20] AOAC International (1995), "AOAC official method 995.11 phosphorus (total) in foods: Colorimetric methods", <https://img.21food.cn/img/biaozhun/20100108/177/11285243.pdf>, accessed 5 June 2023.