Chromium speciation in foodstuffs: a review

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Highlights
- A comprehensive review of chromium speciation in foodstuffs is presented in this article
- Cr(VI) concentrations from off-line extraction and analytical techniques should be questioned
- Isotope dilution techniques can monitor species interconversions during extraction
- New speciation techniques should also focus on the measurement of Cr(III)

Abstract
Numerous critical reviews have evaluated exposure to toxic and carcinogenic hexavalent chromium (Cr(VI)) from a number of pathways; including workplace air, cement and packaging materials. The contribution of foodstuffs to dietary Cr(VI) has been increasingly under investigation, however no summary of this work has been carried out. The objective of this article is to review the last twenty years of chromium speciation research in foodstuffs. Alkaline extraction, used for chromium speciation in other solids, is the most widely-reported procedure. Previous measurement of Cr(VI) in foodstuffs is questionable due to the reducing power of organic matter and antioxidants, leading to the development of speciated isotope dilution mass spectrometry (SIDMS) techniques to monitor interconversions. Evaluation of the genotoxicity of trivalent chromium (Cr(III)), which acts through a different pathway to that of Cr(VI), requires reconsideration towards measurement of Cr(III), which is present at higher concentrations in foodstuffs following reduction of the more-bioavailable Cr(VI).

1.0 Introduction

1.1 Background
Chromium (Cr) is a transition metal that exists in the environment as Cr(III) (trivalent) and Cr(VI) (hexavalent) forms. These naturally occurring oxidation states differ significantly in their mobility, bioavailability and toxicity (Oliveira, 2012). Trivalent Cr is present as cationic species and is considered to be essential for insulin regulation and glucose metabolism (Office of Dietary Supplements: National Institute of Health, 2013), whereas Cr(VI), which is largely anthropogenic in origin and exists as anionic species, is toxic and a known carcinogen through inhalation (McCarroll, Keshava, Chen, Akerman, Kligerman, & Rinde, 2010). The EH40/2005 Workplace Exposure Limit (WEP) assigned for Cr(VI) is 0.05 milligrams per cubic metre of air averaged over an 8-hour period (Health and Safety Executive, 2013). However, there is evidence of genotoxicity of Cr(III) compounds.
(Fang, Zhao, Zhen, Chen, Shi, & Huang, 2014); their use in nutritional supplements has been the subject of a number of research articles (Bailey, 2014).

The main applications of Cr are in the metallurgical and tanning industries (Stasicka, 2000). Owing to its hardness and resistance to corrosion, Cr is used to anodise aluminium (Kendig, Jeanjaquet, Addison, & Waldrop, 2001), as an additive in primer paint for aerospace and automobile refinishing (Basketter, Slodovnik, Merimes, Trattner, & Ingber, 2000), and in the production of steel and alloys (Dhal, Das, Thatoi, & Pandey, 2013). Trivalent Cr salts are used to tan leather by cross-linking collagen fibres (Brown, 1997). In the United States, the mineral crocoite (PbCrO$_4$) was used as a yellow pigment for school buses because it does not degrade in light and has a strong colour, but this has declined due to environmental concerns over its disposal (Gettens, 1966). Lead chromate was historically used as a food adulterant to bulk flour and enhance the yellow colour in turmeric (Ellis, Brewster, Dunn, Allwood, Golovanov, & Goodacre, 2012). Chromium-containing pesticides, such as chromated copper arsenate, were utilised in the timber industry (Hingston, Collins, Murphy, & Lester, 2001), although their use in the UK has been highly restricted since 2004 (Mercer & Frostick, 2012). This widespread use has led to concern over Cr(VI) pollution of the environment (A. M. Zayed & Terry, 2003). Chromium is one of four priority soil-metal contaminants for the U.S. Department of Defense, owing to the use of Cr(VI) compounds for aircraft fuselages (Salatas, Lowney, Pastorok, Nelson, & Ruby, 2004).

1.2 Chromium in soil-water systems

When discussing the total concentration and speciation of Cr in foodstuffs, it is important to consider the sources of Cr and the chemical forms present in these sources prior to plant uptake. Whilst mitigating the issues associated with rapid urbanisation and water scarcity, it is well documented that the irrigation of crops with untreated domestic and industrial effluents results in accumulation of heavy metals in soils (Stasinos & Zabetakis, 2013) which increases the potential for transfer into crops. Therefore, an understanding of the chemical behaviour of Cr in soil-water systems is essential for predicting species stability and the likelihood of transfer into foodstuffs.

Chromium in water originates from natural sources such as the weathering of rocks and precipitation, with elevated concentrations attributed to wastewater from industrial sources (Stasicka, 2000). Chromium has a strong affinity with ultrabasic and basic rocks (peridotite, serpentinite, gabbro, dolerite and basalt), because some pyroxenes (e.g. kosmochlor) have undergone isomorphic substitution of Cr(III) for Al (Oze, Fendorf, Bird, & Coleman, 2004). Chromium in soils typically originates from fallout and washout of Cr-containing particles (Stasicka, 2000) through geochemical processes such as weathering, diagenetic reactions and volcanic eruptions (Prado, Hilal, Chocobar-Ponce, Pagano, Rosa, & Prado, 2016). Agricultural use of sewage sludge as a fertiliser can also introduce Cr into soils (Loubna, Hafidi, Silvestre, Kallerhoff, Merlina, & Pinelli, 2015). Background concentrations of Cr in European soils are typically between 5 and 68 mg kg$^{-1}$ (Utermann, Düwel, & Nagel, 2006). Disposal of industrial waste can lead to percent levels of Cr in soil, with the highest concentrations reported in India, East Africa, South America and China (Shahid, Shamshad, Rafiq, Khalid, Bibi, Niazi, et al., 2017).

Due to its presence as cationic species such as Cr$^{3+}$, Cr(OH)$^{2+}$ and Cr(OH)$_2$$^+$, Cr(III) tends to form strong complexes with alumino-silicate clays, soil organic matter and Fe/Al hydrous oxides in soil,
which restricts the mobility and bioavailability of Cr(III). The affinity of soil for Cr(III) increases with
pH due to the increased negative charge on soil particles (Ritchie & Sposito, 2007). By contrast, the
negative charge of Cr(VI) repels the predominantly-negatively charged clay minerals and humus in
soil, accounting for the increased mobility and bioavailability of Cr(VI) as pH rises (Jardine, Stewart,
Barnett, Banta, Brooks, Fendorf, et al., 2013). In the form of CrO$_4^{2-}$ or HCrO$_4^-$, Cr(VI) is adsorbed by
Fe/Al hydrous oxides (James & Bartlett, 1983). The oxidation of Cr(III) and reduction of Cr(VI) in soils
is dependent on pH, oxygen concentration and the presence of appropriate redox coupling agents.
Hexavalent Cr as CrO$_4^{2-}$ and HCrO$_4^-$ can be reduced by Fe(II) and sulphide (James & Bartlett, 1983),
with Fe(II) dominating at pH > 5.5 and sulphide dominating at lower pH (Fendorf, Wielinga, & Hansel,
2000). Oxidation of Cr(III) to Cr(VI) can occur in the presence of oxidised manganese as Mn(IV)O$_2$
(Bartlett & James, 1979).

The toxicity of Cr to plants has been extensively covered in a number of reviews (Shanker, Cervantes,
Loza-Tavera, & Avudainayagam, 2005). Chromium has no essential role in plant metabolism (Mertz,
1969) and is poorly translocated within plants (Singh, Mahajan, Kaur, Batish, & Kohli, 2013). This is
likely due to Cr(III) binding to cell walls (A. Zayed, Lytle, Qian, & Terry, 1998), leading to increased
concentrations of Cr in the roots (Kabata-Pendias, 2010). Both Cr(III) and Cr(VI) can be absorbed,
although preferential uptake of Cr(VI) through sulphate carriers has been observed (Banerjee,
Nayak, Chakrabortty, & Lahiri, 2008). Ultimately Cr(VI) is reduced to Cr(III) in roots by Fe(III)
reductase enzymes (A. Zayed, Lytle, Qian, & Terry, 1998). Deleterious effects, including restricted
root growth, biomass reduction and distortion of leaf appearance have been recorded for plants
grown in media with excess Cr(III) and Cr(VI) (Dube, Tewari, Chatterjee, & Chatterjee, 2003).

The toxicity and greater mobility of Cr(VI) has led to a number of research articles concerned with
quantitatively evaluating the Cr(VI) content in a variety of foodstuffs, including milk (Lameiras, Elisa
Soares, Lourdes Bastos, & Ferreira, 1998), mushrooms (Figueiredo, Soares, Baptista, Castro, &
Bastos, 2007), tea (Chen, Zhu, He, & Lu, 2014), bread (Soares, Vieira, & Bastos, 2010) and beer
(Vieira, Soares, Kozior, Krejpcio, Ferreira, & Bastos, 2014), utilising different sample preparation
techniques and analytical methodologies. Meat, dairy products, bread and tea are the most studied
as they constitute the main sources of Cr in the human diet (Lendinez, Lorenzo, Cabrera, & López,
2001). The key objective is to assess the oral genotoxicity of Cr(VI) from foodstuffs to reduce the risk
to human health from this pathway (Proctor, Otani, Finley, Paustenbach, Bland, Speizer, et al., 2002).
It is likely that any ingested Cr(VI) will be reduced to Cr(III) in the acidic conditions of the stomach
(Milacic & Stupar, 1994), and any Cr(VI) transferred from soil to plant will also be reduced. However,
there are other sources of Cr(VI) introduced during the manufacture and processing of foodstuffs
(e.g. stainless steel vats) that justify the need to undertake Cr speciation. It is therefore vital to
understand the limitations and interferences of each speciation technique. To the author’s
knowledge, this article represents the first literature report to compile and critically review the
speciation of Cr in foodstuffs over the last twenty years.

2.0 Analytical methods for chromium speciation in foodstuffs

The approach to Cr speciation in foodstuffs can be broken down into two categories: off-line
determination of Cr species following sample pre-treatment and on-line speciation of the pre-
treated sample using hyphenated techniques. The pre-treatment step is designed to solubilise all
forms of Cr(VI) in the sample without inducing oxidation of Cr(III) or reduction of Cr(VI) (Unceta,
Séby, Malherbe, & Donard, 2010). This is particularly important because, depending on the sample matrix, the concentration of Cr(III) can be between 10 and 1000 times greater than the concentration of Cr(VI) (Ndung’u, Djane, Malcus, & Mathiasson, 1999). The use of solid-state speciation techniques for foodstuffs has not been reported in the literature and is therefore outside the scope of this review. Table 1 is a summary of the reported literature for the speciation of Cr in foodstuffs, with analytical merits indicated; the table is arranged according to foodstuff. A number of additional articles have summarised the speciation of Cr in other solid matrices (Unceta, Séby, Malherbe, & Donard, 2010). Table 2 is a summary of the reported Cr(VI) concentrations for the literature in Table 1.
The extraction of Cr(VI) is most commonly undertaken in an alkaline medium due to the stability of Cr(VI) in high-pH matrices (Zhao, Sobecky, Zhao, Crawford, & Li, 2016). The U.S. Environmental Protection Agency (EPA) developed a number of extraction methods that are in common use; for example (1) Method 3060A Alkaline Digestion for Hexavalent Chromium (United States Environmental Protection Agency, 1996) and (2) Method 7196A Hexavalent Chromium by Colorimetry (United States Environmental Protection Agency, 1992). The latter is based on the reaction of Cr(VI) with diphenylcarbazide (DPC) to form a Cr-diphenylcarbazone complex which can be detected using UV-Vis Spectrophotometry at 540 nm. The main disadvantages of this technique are comparatively high detection limits (typically 0.5 mg L$^{-1}$) and susceptibility to interfering species including molybdenum, vanadium, mercury and iron. The release of humic compounds during the extraction can also bias determination of Cr(VI), as these also absorb at 540 nm (Pettine & Capri, 2005).

To account for this, and the possibility of Cr(III) oxidation under alkaline conditions, Method 3060A was developed; this involves extracting the sample using 0.28M Na$_2$CO$_3$/0.5M NaOH at 90-95°C for 60 minutes. In cases of high Cr(III) concentrations the addition of Mg$^{2+}$ can suppress oxidation of Cr(III) to Cr(VI). The resulting solution can then be analysed using a suitable off-line or on-line analytical technique (United States Environmental Protection Agency, 1996). The majority of Cr(III) in the sample precipitates out in the form of hydroxides, oxides and carbonates (Rahman, Kingston, Towns, Vitale, & Clay, 2005) which can be filtered out of the solution prior to analysis. The species present in the insoluble solid can then be determined and a Cr mass balance employed to confirm the efficiency of the entire procedure (Wolle, Rahman, Skip Kingston, & Pamuku, 2014).

Ammonium nitrate (NH$_4$NO$_3$) (Soares, Vieira, & Bastos, 2010) and ammonium hydroxide (NH$_4$OH) (Vacchina, de la Calle, & Séby, 2015) have also been used for selective extraction of Cr(VI). Aside from alkaline extraction, solid phase extraction (SPE) columns can be used to selectively retain Cr(III) or Cr(VI); this method also enables pre-concentration of the retained species for improved detection limits (Ahmad, Murthy, & Chandra, 1990), (Narin, Kars, & Soylak, 2008). Solid phase extraction can utilize alumina columns (Mahmoud, Yakout, Ahmed, & Osman, 2008) or nanotubes (Chen, Zhu, He, & Lu, 2014), occasionally with a reduction step to convert Cr(VI) to Cr(III) for retention on the SPE medium.

Cloud point extraction (CPE) has recently been used by Tiwari et al. for speciation of Cr in turmeric to investigate food adulteration (Tiwari, Deb, & Sen, 2017). Cloud point extraction has been used for speciation of Cr in waters (Kiran, Kumar, Prasad, Suvardhan, Lekkala, & Janardhanam, 2008), (Zhu, Hu, Jiang, & Li, 2005) and is considered to be a “greener” method of pre-concentration and speciation owing to the use of fewer harmful chemicals (Samaddar & Sen, 2014). Tiwari et al. used diffuse reflectance-Fourier transform infrared spectroscopy (DRS-FTIR) to analyse Cr(VI) complexed with 1,5-diphenylcarbazide, offering a lower cost alternative to other techniques at the expense of higher detection limits.
Off-line analytical techniques can be described as instrumentation with no separation technique at the point of analysis, requiring the separation of chemical species through selective extraction prior to determination of Cr concentration. Off-line techniques require minimal sample preparation and post-analysis processing. Up until c. 2013, the speciation of Cr in foodstuffs was undertaken using off-line techniques, with the most common detection method being electrothermal atomic absorption spectrometry (ETAAS) (Figueiredo, Soares, Baptista, Castro, & Bastos, 2007; Lameiras, Elisa Soares, Lourdes Bastos, & Ferreira, 1998; Panichev, Mandiwana, Kataeva, & Siebert, 2005; Soares, Vieira, & Bastos, 2010), followed by inductively coupled plasma mass spectrometry (ICP-MS) (Ambushe, McCrindle, & McCrindle, 2009) and flame atomic absorption spectroscopy (FAAS) (Uluozlu, Tuzen, & Soyak, 2009). ETAAS is a mono-elemental technique capable of detection limits lower than 1 µg L\(^{-1}\) using sample volumes less than 100 µl (Daftsis & Zachariadis, 2007), making it suitable for the sensitive determination of Cr. The larger linear dynamic range of FAAS is applicable to samples with high concentrations of Cr(VI) (Marqués, Salvador, Morales-Rubio, & Guardia, 2000).

Aside from spectrometric techniques, electroanalytical speciation of Cr in foodstuffs was reported by Abbasi et al. (Abbasi & Bahiraei, 2012) using catalytic adsorptive stripping voltammetry. The speciation of Cr(VI) in vinegar was achieved with a detection limit of 0.002 µg L\(^{-1}\). Electroanalytical speciation has also been used for speciation of Cr in soil samples (Grabarczyk, Korolczuk, & Tyszczuk, 2006). The main issue with this form of speciation is the underlying assumption that all of the Cr measured in the extract is in the form of Cr(VI), and that no species interconversions have taken place during and/or after the extraction procedure.

Prior to the development and implementation of speciated isotope dilution mass spectrometry (SIDMS), a number of research articles reported Cr(VI) concentrations in bread (Soares, Vieira, & Bastos, 2010) and tea (Mandiwana, Panichev, & Panicheva, 2011) and estimated daily intakes of Cr(VI) based on these results (Novotnik, Zuliani, Scancar, & Milacic, 2013). The organic content of bread and presence of antioxidants in tea is likely to induce reduction of Cr(VI) to Cr(III) (Vacchina, de la Calle, & Séby, 2015), which would be highlighted by on-line separation techniques.

### 2.3 On-Line Analytical Techniques

The uncertainty surrounding off-line analytical techniques has led to a number of on-line methods incorporating pre-instrument separation and subsequent determination of Cr species. The ease of coupling chromatographic methods, such as high performance liquid chromatography (HPLC), to ICP-MS has led to an increase in the number of published studies using this approach to undertake Cr speciation in foodstuffs. These methods also allow for isotopic labelling of Cr(III) and Cr(VI) for SIDMS applications, where species interconversions can be monitored throughout the digestion and analysis procedures. EPA Method 6800 (Samaddar & Sen, 2014) utilises the same alkaline extraction as Method 3060A, but during the preparation step known quantities of isotopically-labelled Cr(III) and Cr(VI) are added and equilibrated with the sample. By monitoring the isotope of each species, redox conversions can be corrected for through the application of deconvolution calculations (Meija, Yang, Caruso, & Mester, 2006).

The enriched Cr isotopes \(^{50}\)Cr(III) and \(^{53}\)Cr(VI) are most commonly used as isotopic spikes due to their ease of preparation, with a few laboratories offering bespoke products designed for SIDMS applications (Applied Isotope Technologies). Single-spike isotope dilution mass spectrometry (IDMS)
has also been explored, where samples are spiked with only one isotope corresponding to the species of interest. Guidotti et al. demonstrated reliable extraction and quantification of Cr(VI) in contaminated soils using $^{53}\text{Cr(VI)}$ and ethylenediaminetetraacetic acid (EDTA) extraction, with minimal reduction of Cr(VI) even in soils with high Fe and organic matter content (Guidotti, Abad, Rodríguez-González, Alonso, & Beone, 2015).

Anion-exchange columns are most frequently used to separate Cr species (Sabty-Daily, Luk, & Froines, 2002). If the sample pre-treatment step does not include a chelating agent, such as EDTA, Cr(VI) will be the only species attracted to the stationary phase of the column. The presence of polyatomic interferences associated with the extraction matrix, such as $^{40}\text{Ar}^{12}\text{C}^{+}$ and $^{35}\text{Cl}^{16}\text{O}^{+}$, can be controlled using the collision or reaction cell of the ICP-MS; this is usually achieved by pressurising with NH$_3$ (Chang & Jiang, 2001) or He (Hagendorfer & Goessler, 2008).

### 2.4 Reference Materials- Validation of Speciation Methodology

One of the main limitations associated with elemental speciation is the verification of the accuracy of the speciation methodology and sample preparation procedures. Non-aqueous matrix-specific certified reference materials (CRMs) are difficult to produce due to potential species instability and interconversions during the extraction procedure (Oliveira, 2012). Commonly the accuracy of these methodologies is determined through spike recoveries, but these fail to take into account the possibility for species interactions with matrix components (James, Petura, Vitale, & Mussoline, 1995). Although currently no CRM exists for speciation of Cr in foodstuffs, the National Institute of Standards and Technology (NIST) have produced the only CRM for Cr speciation in contaminated soils, Standard Reference Material® SRM 2701 (Nagourney, Wilson, Buckley, Kingston, Yang, & Long, 2008). Data from EPA Method 6800 were used to produce a certified value for Cr(VI) in this material, with the conclusion that this CRM should be included as an external quality control sample to confirm the accuracy of Cr(VI) analyses in Cr-containing waste. The development of CRMs for Cr(VI) in a range of foodstuffs, particularly bread, dairy products and tea that may be adulterated by manufacturing and processing methods, is an important consideration for the validation of future work.

### 2.5 Disparity of results between off-line and on-line analytical techniques

As mentioned previously, the results obtained from off-line and on-line analytical techniques have led to conflicting conclusions regarding the content of Cr(VI) in foodstuffs. One particular example is the presence of Cr(VI) in bread. Prior to the implementation of SIDMS, Soares et al. investigated bread samples using alkaline extraction and ETAAS, reporting Cr(VI) as representing 12% of the total Cr concentration ($5.65 \pm 5.44$ µg kg$^{-1}$ for white bread and $6.82 \pm 4.88$ µg kg$^{-1}$ for wholegrain bread) (Soares, Vieira, & Bastos, 2010). Novotnik et al. repeated the experiment using SIDMS, showing that Cr(VI) is not stable in bread extracts and is partially reduced to Cr(III) by organic matter within the sample, with the extent of reduction greater in wholegrain bread compared to white (Novotnik, Zuliani, Scancar, & Milacic, 2013). Cr(VI) values could not be reported for alkaline extracts of bread in this work. This highlights the issue of reporting species concentrations without a speciation step; selective extraction alone cannot account for poor extraction efficiency or interconversions, and any assessments of dietary intake could be significantly skewed as a result. More recently, Mathebula et al. reported that between 33 and 73% ($58.17 \pm 5.12$ µg kg$^{-1}$ to $156.1 \pm 6.66$ µg kg$^{-1}$) of total Cr in...
bread samples from South Africa was Cr(VI) using selective extraction and high resolution continuum source atomic absorption spectrometry, although it was concluded that average consumption would not exceed maximum acceptable concentrations of Cr(VI) of 0.003 mg kg\(^{-1}\) bw\(^{-1}\) day\(^{-1}\) according to the US EPA (Mathebula, Mandiwana, & Panichev, 2017). Therefore, the lack of an on-line speciation procedure and the proven issues with Cr(VI) reduction in bread extracts casts doubt on results and conclusions for Cr(VI) concentrations in bread determined using off-line analytical techniques.

Another example is the Cr(VI) content of tea, namely tea leaves and tea infusions. Mandiwana et al. used alkaline extraction and ETAAS to carry out Cr speciation in black, green and herbal teas (Mandiwana, Panichev, & Panicheva, 2011). Cr(VI) concentrations were reported between 0.03 and 3.15 µg g\(^{-1}\) for black tea and 0.03 and 0.14 µg g\(^{-1}\) for green tea; Cr(VI) in herbal tea was below the detection limit. Based on the preparation of 200 ml of black tea using 2.0 g tea material, it was determined that 17.5 µg L\(^{-1}\) of Cr(VI) could be consumed per unit cup. Novotnik et al. repeated this experiment using on-line speciation techniques with isotopic tracers (\(^{53}\)Cr(III) and \(^{50}\)Cr(VI)), proving that Cr(VI) is reduced to Cr(III) by antioxidants in tea below the detection limit of 0.06 µg L\(^{-1}\). In highly alkaline extracts Cr(III) can be partially extracted as Cr(OH)\(_4\)^-, and if no on-line speciation procedure is used then this will be interpreted as Cr(VI) during the measurement step. Chen et al. used titanium dioxide nanotubes (TDNTs) to carry out solid phase extraction on tea infusions, reporting Cr(VI) concentrations of 0.12 ± 0.011 µg g\(^{-1}\). Cr(VI) was determined as the difference between total Cr and Cr(III) retained on the TDNTs. However, Cr(III) in tea infusions can form neutral and negatively-charged complexes with organic ligands which will not be retained on the TDNTs, leading to erroneous results as no on-line speciation technique or isotopic tracing was performed to identify the formation of these complexes (Novotnik, Zuliani, Ščančar, & Milačič, 2015). These issues of analytical artefacts can only be prevented through the use of on-line analytical techniques; widespread adoption of these speciation methods will therefore lead to more reliable assessment of the Cr(VI) content in foodstuffs.

2.6 Measurement of Cr(III)

The majority of the analytical methods reviewed in this article prioritise measurement of Cr(VI) over Cr(III) due to the known genotoxicity of Cr(VI). However, the use of Cr(III) compounds in nutritional supplements has generated a number of research articles assessing the biological importance and potential toxicity of Cr(III) (Lukaski, 2007). The use of Cr(III)-containing supplements has increased over the last 15 years (Prado, Hilal, Chocobar-Ponce, Pagano, Rosa, & Prado, 2016), with emphasis being placed on the putative ability of the supplements to control diabetes and aid in weight loss. There are a number of Cr(III) compounds used in nutritional supplements, with Cr picolinate being the most common due to its relatively high bioavailability (A. M. Zayed & Terry, 2003). Sales of Cr(III)-containing supplements are approximately $100 million per year (U.S. Department of Health and Human Services), accounting for 6% of all supplement sales (Chen, Zhu, He, & Lu, 2014). Despite this large market it has been shown that, while Cr(III) may play a role in the management of type II diabetes (Bailey, 2014), there is no significant improvement or acceleration of weight loss that can be attributed solely to supplementation of Cr(III) (Dhal, Das, Thatoi, & Pandey, 2013). Recently, Wu et al. found that adipocytes treated with Cr(III) displayed localised regions or “hotspots” of Cr(V) and Cr(VI), likely formed during cell signalling where oxidising agents such as hydrogen peroxide are created with the potential to oxidise Cr(III) (Wu, Levina, Harris, Cai, Lai, Vogt, et al., 2016).
Concerning the genotoxicity of Cr(III), a recent article by Fang et al. (Fang, Zhao, Zhen, Chen, Shi, & Huang, 2014) reported that both Cr(III) and Cr(VI) induce genetic mutations and DNA damage in yeast cells, with the effect of Cr(III) being greater than that of Cr(VI). The mechanism of DNA damage is also different; Cr(III) interferes with base pair stacking whilst Cr(VI) intercalates within DNA. As the research focus shifts to Cr(III) genotoxicity, and the potential for oxidation to Cr(VI) following ingestion, the need to measure Cr(III) as well as Cr(VI) is likely to become more commonplace. The likelihood of Cr(VI) reducing to Cr(III) during transfer into staple crops could increase the concentration of Cr(III) within the foodstuff (Lytle, Lytle, Yang, Qian, Hansen, Zayed, et al., 1998), potentially leading to an increased risk of exposure to Cr(VI) following Cr(III) re-oxidation in cells. Therefore, more research is needed to explore this possible route for Cr(VI) exposure, with future speciation methods incorporating measurement of both Cr(III) and Cr(VI).

3.0 Conclusion

This article presented a review of the analytical techniques used to undertake Cr speciation in foodstuffs over the last twenty years. Selective extraction using an alkaline medium, commonly a mixture of NaOH/Na₂CO₃ or NaOH/NH₄NO₃, is the most widely used technique for liberating Cr(VI) from the foodstuff prior to determination using off-line (FAAS, ETAAS, ICP-MS) or on-line (HPLC-ICP-MS) analytical methods. For all techniques the main goal is to maximise the amount of Cr(VI) extracted from the foodstuff whilst minimising species interconversions. The introduction of SIDMS has highlighted problems with studies that utilised selective extraction with no on-line speciation step; despite this, researchers are continuing to publish data for Cr(VI) in foodstuffs and estimating daily intakes of Cr(VI) using off-line techniques. Without applying adequate speciation analysis using on-line techniques, there is a risk of introducing analytical artefacts that could lead to incorrect conclusions regarding the Cr(VI) content of complex food matrices. The only way to mitigate this issue is through the use of SIDMS to reliably determine Cr(VI) in foodstuffs and account for species interconversions during the extraction procedure or the formation of complexes due to the presence of available ligands in the sample matrix. In light of recent toxicological studies, future research should incorporate measurement of Cr(III) to better evaluate the health risks from consuming foodstuffs high in Cr.

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Conflict of Interest

The author declares no conflict of interest.

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