

1 Chromium speciation in foodstuffs: a review

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7 **Keywords**

8 Chromium, speciation, foodstuffs, review, hexavalent

9 **Highlights**

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

14 **Abstract**

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

26 **1.0 Introduction**

27 1.1 Background

28 Chromium (Cr) is a transition metal that exists in the environment as Cr(III) (trivalent) and Cr(VI)
29 (hexavalent) forms. These naturally occurring oxidation states differ significantly in their mobility,
30 bioavailability and toxicity (Oliveira, 2012). Trivalent Cr is present as cationic species and is
31 considered to be essential for insulin regulation and glucose metabolism (Office of Dietary
32 Supplements: National Institute of Health, 2013), whereas Cr(VI), which is largely anthropogenic in
33 origin and exists as anionic species, is toxic and a known carcinogen through inhalation (McCarroll,
34 Keshava, Chen, Akerman, Kligerman, & Rinde, 2010). The EH40/2005 Workplace Exposure Limit
35 (WEP) assigned for Cr(VI) is 0.05 milligrams per cubic metre of air averaged over an 8-hour period
36 (Health and Safety Executive, 2013). However, there is evidence of genotoxicity of Cr(III) compounds

37 (Fang, Zhao, Zhen, Chen, Shi, & Huang, 2014); their use in nutritional supplements has been the
38 subject of a number of research articles (Bailey, 2014).

39 The main applications of Cr are in the metallurgical and tanning industries (Stasicka, 2000). Owing to
40 its hardness and resistance to corrosion, Cr is used to anodise aluminium (Kendig, Jeanjaquet,
41 Addison, & Waldrop, 2001), as an additive in primer paint for aerospace and automobile refinishing
42 (Basketter, Slodovnik, Merimes, Trattner, & Ingber, 2000), and in the production of steel and alloys
43 (Dhal, Das, Thatoi, & Pandey, 2013). Trivalent Cr salts are used to tan leather by cross-linking
44 collagen fibres (Brown, 1997). In the United States, the mineral crocoite (PbCrO_4) was used as a
45 yellow pigment for school buses because it does not degrade in light and has a strong colour, but
46 this has declined due to environmental concerns over its disposal (Gettens, 1966). Lead chromate
47 was historically used as a food adulterant to bulk flour and enhance the yellow colour in turmeric
48 (Ellis, Brewster, Dunn, Allwood, Golovanov, & Goodacre, 2012). Chromium-containing pesticides,
49 such as chromated copper arsenate, were utilised in the timber industry (Hingston, Collins, Murphy,
50 & Lester, 2001), although their use in the UK has been highly restricted since 2004 (Mercer &
51 Frostick, 2012). This widespread use has led to concern over Cr(VI) pollution of the environment (A.
52 M. Zayed & Terry, 2003). Chromium is one of four priority soil-metal contaminants for the U.S.
53 Department of Defense, owing to the use of Cr(VI) compounds for aircraft fuselages (Salatas,
54 Lowney, Pastorok, Nelson, & Ruby, 2004).

55 1.2 Chromium in soil-water systems

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

63 Chromium in water originates from natural sources such as the weathering of rocks and
64 precipitation, with elevated concentrations attributed to wastewater from industrial sources
65 (Stasicka, 2000). Chromium has a strong affinity with ultrabasic and basic rocks (peridotite,
66 serpentinite, gabbro, dolerite and basalt), because some pyroxenes (e.g. kosmochlor) have
67 undergone isomorphic substitution of Cr(III) for Al (Oze, Fendorf, Bird, & Coleman, 2004). Chromium
68 in soils typically originates from fallout and washout of Cr-containing particles (Stasicka, 2000)
69 through geochemical processes such as weathering, diagenetic reactions and volcanic eruptions
70 (Prado, Hilal, Chocobar-Ponce, Pagano, Rosa, & Prado, 2016). Agricultural use of sewage sludge as a
71 fertiliser can also introduce Cr into soils (Loubna, Hafidi, Silvestre, Kallerhoff, Merlina, & Pinelli,
72 2015). Background concentrations of Cr in European soils are typically between 5 and 68 mg kg^{-1}
73 (Utermann, Düwel, & Nagel, 2006). Disposal of industrial waste can lead to percent levels of Cr in
74 soil, with the highest concentrations reported in India, East Africa, South America and China (Shahid,
75 Shamshad, Rafiq, Khalid, Bibi, Niazi, et al., 2017).

76 Due to its presence as cationic species such as Cr^{3+} , $\text{Cr}(\text{OH})^{2+}$ and $\text{Cr}(\text{OH})_2^+$, Cr(III) tends to form
77 strong complexes with alumino-silicate clays, soil organic matter and Fe/Al hydrous oxides in soil,

78 which restricts the mobility and bioavailability of Cr(III). The affinity of soil for Cr(III) increases with
79 pH due to the increased negative charge on soil particles (Ritchie & Sposito, 2007). By contrast, the
80 negative charge of Cr(VI) repels the predominantly-negatively charged clay minerals and humus in
81 soil, accounting for the increased mobility and bioavailability of Cr(VI) as pH rises (Jardine, Stewart,
82 Barnett, Basta, Brooks, Fendorf, et al., 2013). In the form of CrO_4^{2-} or HCrO_4^- , Cr(VI) is adsorbed by
83 Fe/Al hydrous oxides (James & Bartlett, 1983). The oxidation of Cr(III) and reduction of Cr(VI) in soils
84 is dependent on pH, oxygen concentration and the presence of appropriate redox coupling agents.
85 Hexavalent Cr as CrO_4^{2-} and HCrO_4^- can be reduced by Fe(II) and sulphide (James & Bartlett, 1983),
86 with Fe(II) dominating at $\text{pH} > 5.5$ and sulphide dominating at lower pH (Fendorf, Wielinga, & Hansel,
87 2000). Oxidation of Cr(III) to Cr(VI) can occur in the presence of oxidised manganese as Mn(IV)O_2
88 (Bartlett & James, 1979).

89 The toxicity of Cr to plants has been extensively covered in a number of reviews (Shanker, Cervantes,
90 Loza-Tavera, & Avudainayagam, 2005). Chromium has no essential role in plant metabolism (Mertz,
91 1969) and is poorly translocated within plants (Singh, Mahajan, Kaur, Batish, & Kohli, 2013). This is
92 likely due to Cr(III) binding to cell walls (A. Zayed, Lytle, Qian, & Terry, 1998), leading to increased
93 concentrations of Cr in the roots (Kabata-Pendias, 2010). Both Cr(III) and Cr(VI) can be absorbed,
94 although preferential uptake of Cr(VI) through sulphate carriers has been observed (Banerjee,
95 Nayak, Chakraborty, & Lahiri, 2008). Ultimately Cr(VI) is reduced to Cr(III) in roots by Fe(III)
96 reductase enzymes (A. Zayed, Lytle, Qian, & Terry, 1998). Deleterious effects, including restricted
97 root growth, biomass reduction and distortion of leaf appearance have been recorded for plants
98 grown in media with excess Cr(III) and Cr(VI) (Dube, Tewari, Chatterjee, & Chatterjee, 2003).

99 The toxicity and greater mobility of Cr(VI) has led to a number of research articles concerned with
100 quantitatively evaluating the Cr(VI) content in a variety of foodstuffs, including milk (Lameiras, Elisa
101 Soares, Lourdes Bastos, & Ferreira, 1998), mushrooms (Figueiredo, Soares, Baptista, Castro, &
102 Bastos, 2007), tea (Chen, Zhu, He, & Lu, 2014), bread (Soares, Vieira, & Bastos, 2010) and beer
103 (Vieira, Soares, Kozior, Krejpcio, Ferreira, & Bastos, 2014), utilising different sample preparation
104 techniques and analytical methodologies. Meat, dairy products, bread and tea are the most studied
105 as they constitute the main sources of Cr in the human diet (Lendinez, Lorenzo, Cabrera, & López,
106 2001). The key objective is to assess the oral genotoxicity of Cr(VI) from foodstuffs to reduce the risk
107 to human health from this pathway (Proctor, Otani, Finley, Paustenbach, Bland, Speizer, et al., 2002).
108 It is likely that any ingested Cr(VI) will be reduced to Cr(III) in the acidic conditions of the stomach
109 (Milacic & Stupar, 1994), and any Cr(VI) transferred from soil to plant will also be reduced. However,
110 there are other sources of Cr(VI) introduced during the manufacture and processing of foodstuffs
111 (e.g. stainless steel vats) that justify the need to undertake Cr speciation. It is therefore vital to
112 understand the limitations and interferences of each speciation technique. To the author's
113 knowledge, this article represents the first literature report to compile and critically review the
114 speciation of Cr in foodstuffs over the last twenty years.

115 **2.0 Analytical methods for chromium speciation in foodstuffs**

116 The approach to Cr speciation in foodstuffs can be broken down into two categories: off-line
117 determination of Cr species following sample pre-treatment and on-line speciation of the pre-
118 treated sample using hyphenated techniques. The pre-treatment step is designed to solubilise all
119 forms of Cr(VI) in the sample without inducing oxidation of Cr(III) or reduction of Cr(VI) (Unceta,

120 Séby, Malherbe, & Donard, 2010). This is particularly important because, depending on the sample
121 matrix, the concentration of Cr(III) can be between 10 and 1000 times greater than the concentration
122 of Cr(VI) (Ndung'u, Djane, Malcus, & Mathiasson, 1999). The use of solid-state speciation techniques
123 for foodstuffs has not been reported in the literature and is therefore outside the scope of this
124 review. Table 1 is a summary of the reported literature for the speciation of Cr in foodstuffs, with
125 analytical merits indicated; the table is arranged according to foodstuff. A number of additional
126 articles have summarised the speciation of Cr in other solid matrices (Unceta, Séby, Malherbe, &
127 Donard, 2010). Table 2 is a summary of the reported Cr(VI) concentrations for the literature in Table 1.

128 2.1 Sample Pretreatment

129 The extraction of Cr(VI) is most commonly undertaken in an alkaline medium due to the stability of
130 Cr(VI) in high-pH matrices (Zhao, Sobecky, Zhao, Crawford, & Li, 2016). The U.S. Environmental
131 Protection Agency (EPA) developed a number of extraction methods that are in common use; for
132 example (1) Method 3060A Alkaline Digestion for Hexavalent Chromium (United States
133 Environmental Protection Agency, 1996) and (2) Method 7196A Hexavalent Chromium by
134 Colorimetry (United States Environmental Protection Agency, 1992). The latter is based on the
135 reaction of Cr(VI) with diphenylcarbazide (DPC) to form a Cr-diphenylcarbazone complex which can
136 be detected using UV-Vis Spectrophotometry at 540 nm. The main disadvantages of this technique
137 are comparatively high detection limits (typically 0.5 mg L^{-1}) and susceptibility to interfering species
138 including molybdenum, vanadium, mercury and iron. The release of humic compounds during the
139 extraction can also bias determination of Cr(VI), as these also absorb at 540 nm (Pettine & Capri,
140 2005).

141 To account for this, and the possibility of Cr(III) oxidation under alkaline conditions, Method 3060A
142 was developed; this involves extracting the sample using $0.28\text{M Na}_2\text{CO}_3/0.5\text{M NaOH}$ at $90\text{-}95^\circ\text{C}$ for
143 60 minutes. In cases of high Cr(III) concentrations the addition of Mg^{2+} can suppress oxidation of
144 Cr(III) to Cr(VI). The resulting solution can then be analysed using a suitable off-line or on-line
145 analytical technique (United States Environmental Protection Agency, 1996). The majority of Cr(III) in
146 the sample precipitates out in the form of hydroxides, oxides and carbonates (Rahman, Kingston,
147 Towns, Vitale, & Clay, 2005) which can be filtered out of the solution prior to analysis. The species
148 present in the insoluble solid can then be determined and a Cr mass balance employed to confirm
149 the efficiency of the entire procedure (Wolle, Rahman, Skip Kingston, & Pamuku, 2014).

150 Ammonium nitrate (NH_4NO_3) (Soares, Vieira, & Bastos, 2010) and ammonium hydroxide (NH_4OH)
151 (Vacchina, de la Calle, & Séby, 2015) have also been used for selective extraction of Cr(VI). Aside
152 from alkaline extraction, solid phase extraction (SPE) columns can be used to selectively retain Cr(III)
153 or Cr(VI); this method also enables pre-concentration of the retained species for improved detection
154 limits (Ahmad, Murthy, & Chandra, 1990), (Narin, Kars, & Soylak, 2008). Solid phase extraction can
155 utilize alumina columns (Mahmoud, Yakout, Ahmed, & Osman, 2008) or nanotubes (Chen, Zhu, He,
156 & Lu, 2014), occasionally with a reduction step to convert Cr(VI) to Cr(III) for retention on the SPE
157 medium.

158 Cloud point extraction (CPE) has recently been used by Tiwari *et al.* for speciation of Cr in turmeric to
159 investigate food adulteration (Tiwari, Deb, & Sen, 2017). Cloud point extraction has been used for
160 speciation of Cr in waters (Kiran, Kumar, Prasad, Suvadhan, Lekkala, & Janardhanam, 2008), (Zhu,
161 Hu, Jiang, & Li, 2005) and is considered to be a “greener” method of pre-concentration and
162 speciation owing to the use of fewer harmful chemicals (Samaddar & Sen, 2014). Tiwari *et al.* used
163 diffuse reflectance-Fourier transform infrared spectroscopy (DRS-FTIR) to analyse Cr(VI) complexed
164 with 1,5-diphenylcarbazide, offering a lower cost alternative to other techniques at the expense of
165 higher detection limits.

166 2.2 Off-Line Analytical Techniques

167 Off-line analytical techniques can be described as instrumentation with no separation technique at
168 the point of analysis, requiring the separation of chemical species through selective extraction prior
169 to determination of Cr concentration. Off-line techniques require minimal sample preparation and
170 post-analysis processing. Up until c. 2013, the speciation of Cr in foodstuffs was undertaken using
171 off-line techniques, with the most common detection method being electrothermal atomic
172 absorption spectrometry (ETAAS) (Figueiredo, Soares, Baptista, Castro, & Bastos, 2007; Lameiras,
173 Elisa Soares, Lourdes Bastos, & Ferreira, 1998; Panichev, Mandiwana, Kataeva, & Siebert, 2005;
174 Soares, Vieira, & Bastos, 2010), followed by inductively coupled plasma mass spectrometry (ICP-MS)
175 (Ambushe, McCrindle, & McCrindle, 2009) and flame atomic absorption spectroscopy (FAAS)
176 (Uluozlu, Tuzen, & Soylak, 2009). ETAAS is a mono-elemental technique capable of detection limits
177 lower than $1 \mu\text{g L}^{-1}$ using sample volumes less than $100 \mu\text{l}$ (Daftsis & Zachariadis, 2007), making it
178 suitable for the sensitive determination of Cr. The larger linear dynamic range of FAAS is applicable
179 to samples with high concentrations of Cr(VI) (Marqués, Salvador, Morales-Rubio, & Guardia, 2000).

180 Aside from spectrometric techniques, electroanalytical speciation of Cr in foodstuffs was reported by
181 Abbasi *et al.* (Abbasi & Bahiraei, 2012) using catalytic adsorptive stripping voltammetry. The
182 speciation of Cr(VI) in vinegar was achieved with a detection limit of $0.002 \mu\text{g L}^{-1}$. Electroanalytical
183 speciation has also been used for speciation of Cr in soil samples (Grabarczyk, Korolczuk, & Tyszczyk,
184 2006). The main issue with this form of speciation is the underlying assumption that all of the Cr
185 measured in the extract is in the form of Cr(VI), and that no species interconversions have taken
186 place during and/or after the extraction procedure.

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

193 2.3 On-Line Analytical Techniques

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

205 The enriched Cr isotopes ^{50}Cr (III) and ^{53}Cr (VI) are most commonly used as isotopic spikes due to their
206 ease of preparation, with a few laboratories offering bespoke products designed for SIDMS
207 applications (Applied Isotope Technologies). Single-spike isotope dilution mass spectrometry (IDMS)

208 has also been explored, where samples are spiked with only one isotope corresponding to the
209 species of interest. Guidotti *et al.* demonstrated reliable extraction and quantification of Cr(VI) in
210 contaminated soils using $^{53}\text{Cr(VI)}$ and ethylenediaminetetraacetic acid (EDTA) extraction, with
211 minimal reduction of Cr(VI) even in soils with high Fe and organic matter content (Guidotti, Abad,
212 Rodríguez-González, Alonso, & Beone, 2015).

213 Anion-exchange columns are most frequently used to separate Cr species (Sabty-Daily, Luk, &
214 Froines, 2002). If the sample pre-treatment step does not include a chelating agent, such as EDTA,
215 Cr(VI) will be the only species attracted to the stationary phase of the column. The presence of
216 polyatomic interferences associated with the extraction matrix, such as $^{40}\text{Ar}^{12}\text{C}^+$ and $^{35}\text{Cl}^{16}\text{O}^1\text{H}^+$, can
217 be controlled using the collision or reaction cell of the ICP-MS; this is usually achieved by
218 pressurising with NH_3 (Chang & Jiang, 2001) or He (Hagendorfer & Goessler, 2008).

219 2.4 Reference Materials- Validation of Speciation Methodology

220 One of the main limitations associated with elemental speciation is the verification of the accuracy
221 of the speciation methodology and sample preparation procedures. Non-aqueous matrix-specific
222 certified reference materials (CRMs) are difficult to produce due to potential species instability and
223 interconversions during the extraction procedure (Oliveira, 2012). Commonly the accuracy of these
224 methodologies is determined through spike recoveries, but these fail to take into account the
225 possibility for species interactions with matrix components (James, Petura, Vitale, & Mussoline,
226 1995). Although currently no CRM exists for speciation of Cr in foodstuffs, the National Institute of
227 Standards and Technology (NIST) have produced the only CRM for Cr speciation in contaminated
228 soils, Standard Reference Material[®] SRM 2701 (Nagourney, Wilson, Buckley, Kingston, Yang, & Long,
229 2008). Data from EPA Method 6800 were used to produce a certified value for Cr(VI) in this material,
230 with the conclusion that this CRM should be included as an external quality control sample to
231 confirm the accuracy of Cr(VI) analyses in Cr-containing waste. The development of CRMs for Cr(VI)
232 in a range of foodstuffs, particularly bread, dairy products and tea that may be adulterated by
233 manufacturing and processing methods, is an important consideration for the validation of future
234 work.

235 2.5 Disparity of results between off-line and on-line analytical techniques

236 As mentioned previously, the results obtained from off-line and on-line analytical techniques have
237 led to conflicting conclusions regarding the content of Cr(VI) in foodstuffs. One particular example is
238 the presence of Cr(VI) in bread. Prior to the implementation of SIDMS, Soares *et al.* investigated
239 bread samples using alkaline extraction and ETAAS, reporting Cr(VI) as representing 12% of the total
240 Cr concentration ($5.65 \pm 5.44 \mu\text{g kg}^{-1}$ for white bread and $6.82 \pm 4.88 \mu\text{g kg}^{-1}$ for wholegrain bread)
241 (Soares, Vieira, & Bastos, 2010). Novotnik *et al.* repeated the experiment using SIDMS, showing that
242 Cr(VI) is not stable in bread extracts and is partially reduced to Cr(III) by organic matter within the
243 sample, with the extent of reduction greater in wholegrain bread compared to white (Novotnik,
244 Zuliani, Scancar, & Milacic, 2013). Cr(VI) values could not be reported for alkaline extracts of bread in
245 this work. This highlights the issue of reporting species concentrations without a speciation step;
246 selective extraction alone cannot account for poor extraction efficiency or interconversions, and any
247 assessments of dietary intake could be significantly skewed as a result. More recently, Mathebula *et al.*
248 reported that between 33 and 73% ($58.17 \pm 5.12 \mu\text{g kg}^{-1}$ to $156.1 \pm 6.66 \mu\text{g kg}^{-1}$) of total Cr in

249 bread samples from South Africa was Cr(VI) using selective extraction and high resolution continuum
250 source atomic absorption spectrometry, although it was concluded that average consumption would
251 not exceed maximum acceptable concentrations of Cr(VI) of $0.003 \text{ mg kg}^{-1} \text{ bw}^{-1} \text{ day}^{-1}$ according to
252 the US EPA (Mathebula, Mandiwana, & Panichev, 2017). Therefore, the lack of an on-line speciation
253 procedure and the proven issues with Cr(VI) reduction in bread extracts casts doubt on results and
254 conclusions for Cr(VI) concentrations in bread determined using off-line analytical techniques.

255 Another example is the Cr(VI) content of tea, namely tea leaves and tea infusions. Mandiwana *et al.*
256 used alkaline extraction and ETAAS to carry out Cr speciation in black, green and herbal teas
257 (Mandiwana, Panichev, & Panicheva, 2011). Cr(VI) concentrations were reported between 0.03 and
258 $3.15 \mu\text{g g}^{-1}$ for black tea and 0.03 and $0.14 \mu\text{g g}^{-1}$ for green tea; Cr(VI) in herbal tea was below the
259 detection limit. Based on the preparation of 200 ml of black tea using 2.0 g tea material, it was
260 determined that $17.5 \mu\text{g L}^{-1}$ of Cr(VI) could be consumed per unit cup. Novotnik *et al.* repeated this
261 experiment using on-line speciation techniques with isotopic tracers ($^{53}\text{Cr(III)}$ and $^{50}\text{Cr(VI)}$), proving
262 that Cr(VI) is reduced to Cr(III) by antioxidants in tea below the detection limit of $0.06 \mu\text{g L}^{-1}$. In highly
263 alkaline extracts Cr(III) can be partially extracted as Cr(OH)_4^- , and if no on-line speciation procedure is
264 used then this will be interpreted as Cr(VI) during the measurement step. Chen *et al.* used titanium
265 dioxide nanotubes (TDNTs) to carry out solid phase extraction on tea infusions, reporting Cr(VI)
266 concentrations of $0.12 \pm 0.011 \mu\text{g g}^{-1}$. Cr(VI) was determined as the difference between total Cr and
267 Cr(III) retained on the TDNTs. However, Cr(III) in tea infusions can form neutral and negatively-
268 charged complexes with organic ligands which will not be retained on the TDNTs, leading to
269 erroneous results as no on-line speciation technique or isotopic tracing was performed to identify
270 the formation of these complexes (Novotnik, Zuliani, Ščančar, & Milačič, 2015). These issues of
271 analytical artefacts can only be prevented through the use of on-line analytical techniques;
272 widespread adoption of these speciation methods will therefore lead to more reliable assessment of
273 the Cr(VI) content in foodstuffs.

274 2.6 Measurement of Cr(III)

275 The majority of the analytical methods reviewed in this article prioritise measurement of Cr(VI) over
276 Cr(III) due to the known genotoxicity of Cr(VI). However, the use of Cr(III) compounds in nutritional
277 supplements has generated a number of research articles assessing the biological importance and
278 potential toxicity of Cr(III) (Lukaski, 2007). The use of Cr(III)-containing supplements has increased
279 over the last 15 years (Prado, Hilal, Chocobar-Ponce, Pagano, Rosa, & Prado, 2016), with emphasis
280 being placed on the putative ability of the supplements to control diabetes and aid in weight loss.
281 There are a number of Cr(III) compounds used in nutritional supplements, with Cr picolinate being
282 the most common due to its relatively high bioavailability (A. M. Zayed & Terry, 2003). Sales of
283 Cr(III)-containing supplements are approximately \$100 million per year (U.S. Department of Health
284 and Human Services), accounting for 6% of all supplement sales (Chen, Zhu, He, & Lu, 2014). Despite
285 this large market it has been shown that, while Cr(III) may play a role in the management of type II
286 diabetes (Bailey, 2014), there is no significant improvement or acceleration of weight loss that can
287 be attributed solely to supplementation of Cr(III) (Dhal, Das, Thatoi, & Pandey, 2013). Recently, Wu
288 *et al.* found that adipocytes treated with Cr(III) displayed localised regions or “hotspots” of Cr(V) and
289 Cr(VI), likely formed during cell signalling where oxidising agents such as hydrogen peroxide are
290 created with the potential to oxidise Cr(III) (Wu, Levina, Harris, Cai, Lai, Vogt, et al., 2016).

291 Concerning the genotoxicity of Cr(III), a recent article by Fang *et al.* (Fang, Zhao, Zhen, Chen, Shi, &
292 Huang, 2014) reported that both Cr(III) and Cr(VI) induce genetic mutations and DNA damage in
293 yeast cells, with the effect of Cr(III) being greater than that of Cr(VI). The mechanism of DNA damage
294 is also different; Cr(III) interferes with base pair stacking whilst Cr(VI) intercalates within DNA. As the
295 research focus shifts to Cr(III) genotoxicity, and the potential for oxidation to Cr(VI) following
296 ingestion, the need to measure Cr(III) as well as Cr(VI) is likely to become more commonplace. The
297 likelihood of Cr(VI) reducing to Cr(III) during transfer into staple crops could increase the
298 concentration of Cr(III) within the foodstuff (Lytle, Lytle, Yang, Qian, Hansen, Zayed, et al., 1998),
299 potentially leading to an increased risk of exposure to Cr(VI) following Cr(III) re-oxidation in cells.
300 Therefore, more research is needed to explore this possible route for Cr(VI) exposure, with future
301 speciation methods incorporating measurement of both Cr(III) and Cr(VI).

302 **3.0 Conclusion**

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

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321 **Conflict of Interest**

322 The author declares no conflict of interest.

323 **References**

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