

# 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 is  
25 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 considered  
31 to be essential for insulin regulation and glucose metabolism (Office of Dietary Supplements: National  
32 Institute of Health, 2013), whereas Cr(VI), which is largely anthropogenic in origin and exists as anionic  
33 species, is toxic and a known carcinogen through inhalation (McCarroll, Keshava, Chen, Akerman,  
34 Kligerman, & Rinde, 2010). The EH40/2005 Workplace Exposure Limit (WEP) assigned for Cr(VI) is 0.05  
35 milligrams per cubic metre of air averaged over an 8-hour period (Health and Safety Executive, 2013).  
36 However, there is evidence of genotoxicity of Cr(III) compounds (Fang, Zhao, Zhen, Chen, Shi, & Huang,

37 2014); their use in nutritional supplements has been the subject of a number of research articles  
38 (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, Addison,  
41 & Waldrop, 2001), as an additive in primer paint for aerospace and automobile refinishing (Basketter,  
42 Slodovnik, Merimes, Trattner, & Ingber, 2000), and in the production of steel and alloys (Dhal, Das,  
43 Thatoi, & Pandey, 2013). Trivalent Cr salts are used to tan leather by cross-linking collagen fibres  
44 (Brown, 1997). In the United States, the mineral crocoite ( $\text{PbCrO}_4$ ) was used as a yellow pigment for  
45 school buses because it does not degrade in light and has a strong colour, but this has declined due to  
46 environmental concerns over its disposal (Gettens, 1966). Lead chromate was historically used as a  
47 food adulterant to bulk flour and enhance the yellow colour in turmeric (Ellis, Brewster, Dunn,  
48 Allwood, Golovanov, & Goodacre, 2012). Chromium-containing pesticides, such as chromated copper  
49 arsenate, were utilised in the timber industry (Hingston, Collins, Murphy, & Lester, 2001), although  
50 their use in the UK has been highly restricted since 2004 (Mercer & Frostick, 2012). This widespread  
51 use has led to concern over Cr(VI) pollution of the environment (A. M. Zayed & Terry, 2003). Chromium  
52 is one of four priority soil-metal contaminants for the U.S. Department of Defense, owing to the use  
53 of Cr(VI) compounds for aircraft fuselages (Salatas, Lowney, Pastorok, Nelson, & Ruby, 2004).

## 54 1.2 Chromium in soil-water systems

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

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

75 Due to its presence as cationic species such as  $\text{Cr}^{3+}$ ,  $\text{Cr}(\text{OH})^{2+}$  and  $\text{Cr}(\text{OH})_2^+$ , Cr(III) tends to form strong  
76 complexes with alumino-silicate clays, soil organic matter and Fe/Al hydrous oxides in soil, which  
77 restricts the mobility and bioavailability of Cr(III). The affinity of soil for Cr(III) increases with pH due

78 to the increased negative charge on soil particles (Ritchie & Sposito, 2007). By contrast, the negative  
79 charge of Cr(VI) repels the predominantly-negatively charged clay minerals and humus in soil,  
80 accounting for the increased mobility and bioavailability of Cr(VI) as pH rises (Jardine, Stewart, Barnett,  
81 Basta, Brooks, Fendorf, et al., 2013). In the form of  $\text{CrO}_4^{2-}$  or  $\text{HCrO}_4^-$ , Cr(VI) is adsorbed by Fe/Al hydrous  
82 oxides (James & Bartlett, 1983). The oxidation of Cr(III) and reduction of Cr(VI) in soils is dependent  
83 on pH, oxygen concentration and the presence of appropriate redox coupling agents. Hexavalent Cr  
84 as  $\text{CrO}_4^{2-}$  and  $\text{HCrO}_4^-$  can be reduced by Fe(II) and sulphide (James & Bartlett, 1983), with Fe(II)  
85 dominating at  $\text{pH} > 5.5$  and sulphide dominating at lower pH (Fendorf, Wielinga, & Hansel, 2000).  
86 Oxidation of Cr(III) to Cr(VI) can occur in the presence of oxidised manganese as  $\text{Mn(IV)O}_2$  (Bartlett &  
87 James, 1979).

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

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

## 114 **2.0 Analytical methods for chromium speciation in foodstuffs**

115 The approach to Cr speciation in foodstuffs can be broken down into two categories: off-line  
116 determination of Cr species following sample pre-treatment and on-line speciation of the pre-treated  
117 sample using hyphenated techniques. The pre-treatment step is designed to solubilise all forms of  
118 Cr(VI) in the sample without inducing oxidation of Cr(III) or reduction of Cr(VI) (Unceta, Séby,  
119 Malherbe, & Donard, 2010). This is particularly important because, depending on the sample matrix,

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

## 127 2.1 Sample Pretreatment

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

139 To account for this, and the possibility of Cr(III) oxidation under alkaline conditions, Method 3060A  
140 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 60  
141 minutes. In cases of high Cr(III) concentrations the addition of  $\text{Mg}^{2+}$  can suppress oxidation of Cr(III) to  
142 Cr(VI). The resulting solution can then be analysed using a suitable off-line or on-line analytical  
143 technique (United States Environmental Protection Agency, 1996). The majority of Cr(III) in the sample  
144 precipitates out in the form of hydroxides, oxides and carbonates (Rahman, Kingston, Towns, Vitale,  
145 & Clay, 2005) which can be filtered out of the solution prior to analysis. The species present in the  
146 insoluble solid can then be determined and a Cr mass balance employed to confirm the efficiency of  
147 the entire procedure (Wolle, Rahman, Skip Kingston, & Pamuku, 2014).

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

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

## 164 2.2 Off-Line Analytical Techniques

165 Off-line analytical techniques can be described as instrumentation with no separation technique at  
166 the point of analysis, requiring the separation of chemical species through selective extraction prior

167 to determination of Cr concentration. Off-line techniques require minimal sample preparation and  
168 post-analysis processing. Up until c. 2013, the speciation of Cr in foodstuffs was undertaken using off-  
169 line techniques, with the most common detection method being electrothermal atomic absorption  
170 spectrometry (ETAAS) (Figueiredo, Soares, Baptista, Castro, & Bastos, 2007; Lameiras, Elisa Soares,  
171 Lourdes Bastos, & Ferreira, 1998; Panichev, Mandiwana, Kataeva, & Siebert, 2005; Soares, Vieira, &  
172 Bastos, 2010), followed by inductively coupled plasma mass spectrometry (ICP-MS) (Ambushe,  
173 McCrindle, & McCrindle, 2009) and flame atomic absorption spectroscopy (FAAS) (Uluozlu, Tuzen, &  
174 Soylak, 2009). ETAAS is a mono-elemental technique capable of detection limits lower than  $1 \mu\text{g L}^{-1}$   
175 using sample volumes less than  $100 \mu\text{l}$  (Daftsis & Zachariadis, 2007), making it suitable for the sensitive  
176 determination of Cr. The larger linear dynamic range of FAAS is applicable to samples with high  
177 concentrations of Cr(VI) (Marqués, Salvador, Morales-Rubio, & Guardia, 2000).

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

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

### 191 2.3 On-Line Analytical Techniques

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

203 The enriched Cr isotopes  $^{50}\text{Cr(III)}$  and  $^{53}\text{Cr(VI)}$  are most commonly used as isotopic spikes due to their  
204 ease of preparation, with a few laboratories offering bespoke products designed for SIDMS  
205 applications (Applied Isotope Technologies). Single-spike isotope dilution mass spectrometry (IDMS)  
206 has also been explored, where samples are spiked with only one isotope corresponding to the species  
207 of interest. Guidotti *et al.* demonstrated reliable extraction and quantification of Cr(VI) in

208 contaminated soils using  $^{53}\text{Cr(VI)}$  and ethylenediaminetetraacetic acid (EDTA) extraction, with minimal  
209 reduction of Cr(VI) even in soils with high Fe and organic matter content (Guidotti, Abad, Rodríguez-  
210 González, Alonso, & Beone, 2015).

211 Anion-exchange columns are most frequently used to separate Cr species (Sabty-Daily, Luk, & Froines,  
212 2002). If the sample pre-treatment step does not include a chelating agent, such as EDTA, Cr(VI) will  
213 be the only species attracted to the stationary phase of the column. The presence of polyatomic  
214 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 be controlled  
215 using the collision or reaction cell of the ICP-MS; this is usually achieved by pressurising with  $\text{NH}_3$   
216 (Chang & Jiang, 2001) or He (Hagendorfer & Goessler, 2008).

#### 217 2.4 Reference Materials- Validation of Speciation Methodology

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

#### 232 2.5 Disparity of results between off-line and on-line analytical techniques

233 As mentioned previously, the results obtained from off-line and on-line analytical techniques have led  
234 to conflicting conclusions regarding the content of Cr(VI) in foodstuffs. One particular example is the  
235 presence of Cr(VI) in bread. Prior to the implementation of SIDMS, Soares *et al.* investigated bread  
236 samples using alkaline extraction and ETAAS, reporting Cr(VI) as representing 12% of the total Cr  
237 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)  
238 (Soares, Vieira, & Bastos, 2010). Novotnik *et al.* repeated the experiment using SIDMS, showing that  
239 Cr(VI) is not stable in bread extracts and is partially reduced to Cr(III) by organic matter within the  
240 sample, with the extent of reduction greater in wholegrain bread compared to white (Novotnik,  
241 Zuliani, Scancar, & Milacic, 2013). Cr(VI) values could not be reported for alkaline extracts of bread in  
242 this work. This highlights the issue of reporting species concentrations without a speciation step;  
243 selective extraction alone cannot account for poor extraction efficiency or interconversions, and any  
244 assessments of dietary intake could be significantly skewed as a result. More recently, Mathebula *et*  
245 *al.* 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 bread  
246 samples from South Africa was Cr(VI) using selective extraction and high resolution continuum source  
247 atomic absorption spectrometry, although it was concluded that average consumption would not  
248 exceed maximum acceptable concentrations of Cr(VI) of  $0.003 \text{ mg kg}^{-1} \text{ bw}^{-1} \text{ day}^{-1}$  according to the US

249 EPA (Mathebula, Mandiwana, & Panichev, 2017). Therefore, the lack of an on-line speciation  
250 procedure and the proven issues with Cr(VI) reduction in bread extracts casts doubt on results and  
251 conclusions for Cr(VI) concentrations in bread determined using off-line analytical techniques.

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

## 270 2.6 Measurement of Cr(III)

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

287 Concerning the genotoxicity of Cr(III), a recent article by Fang *et al.* (Fang, Zhao, Zhen, Chen, Shi, &  
288 Huang, 2014) reported that both Cr(III) and Cr(VI) induce genetic mutations and DNA damage in yeast  
289 cells, with the effect of Cr(III) being greater than that of Cr(VI). The mechanism of DNA damage is also  
290 different; Cr(III) interferes with base pair stacking whilst Cr(VI) intercalates within DNA. As the research



291 focus shifts to Cr(III) genotoxicity, and the potential for oxidation to Cr(VI) following ingestion, the  
292 need to measure Cr(III) as well as Cr(VI) is likely to become more commonplace. The likelihood of Cr(VI)  
293 reducing to Cr(III) during transfer into staple crops could increase the concentration of Cr(III) within  
294 the foodstuff (Lytle, Lytle, Yang, Qian, Hansen, Zayed, et al., 1998), potentially leading to an increased  
295 risk of exposure to Cr(VI) following Cr(III) re-oxidation in cells. Therefore, more research is needed to  
296 explore this possible route for Cr(VI) exposure, with future speciation methods incorporating  
297 measurement of both Cr(III) and Cr(VI).

### 298 **3.0 Conclusion**

299 This article presented a review of the analytical techniques used to undertake Cr speciation in  
300 foodstuffs over the last twenty years. Selective extraction using an alkaline medium, commonly a  
301 mixture of NaOH/Na<sub>2</sub>CO<sub>3</sub> or NaOH/NH<sub>4</sub>NO<sub>3</sub>, is the most widely used technique for liberating Cr(VI)  
302 from the foodstuff prior to determination using off-line (FAAS, ETAAS, ICP-MS) or on-line (HPLC-ICP-  
303 MS) analytical methods. For all techniques the main goal is to maximise the amount of Cr(VI) extracted  
304 from the foodstuff whilst minimising species interconversions. The introduction of SIDMS has  
305 highlighted problems with studies that utilised selective extraction with no on-line speciation step;  
306 despite this, researchers are continuing to publish data for Cr(VI) in foodstuffs and estimating daily  
307 intakes of Cr(VI) using off-line techniques. Without applying adequate speciation analysis using on-  
308 line techniques, there is a risk of introducing analytical artefacts that could lead to incorrect  
309 conclusions regarding the Cr(VI) content of complex food matrices. The only way to mitigate this issue  
310 is through the use of SIDMS to reliably determine Cr(VI) in foodstuffs and account for species  
311 interconversions during the extraction procedure or the formation of complexes due to the presence  
312 of available ligands in the sample matrix. In light of recent toxicological studies, future research should  
313 incorporate measurement of Cr(III) to better evaluate the health risks from consuming foodstuffs high  
314 in Cr.

### 315 **Acknowledgments**

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### 317 **Conflict of Interest**

318 The author declares no conflict of interest.

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530

**Table 1** Extraction and quantification techniques for chromium speciation in foodstuffs. Table is ordered according to foodstuff.

Foodstuff	Analytical Technique	Sample Preparation	Analytical Merits	Reference
<b>Milk and Dairy Products</b>				
UHT Milk	ETAAS	Selective extraction using Chromabond NH <sub>2</sub> column following precipitation of proteins.	Cr(VI) concentrations in milk approximately 2-4 times lower than total Cr. Detection limit of 0.15 µg L <sup>-1</sup> Cr(VI).	(Lameiras, Elisa Soares, Lourdes Bastos, & Ferreira, 1998)
Cow milk	ICP-MS	Selective extraction using Chromabond NH <sub>2</sub> column following precipitation of proteins.	Detection limit of 0.085 µg L <sup>-1</sup> Cr(VI). Reported Cr(VI) range of 1.31-3.28% compared to total Cr.	(Ambushe, McCrindle, & McCrindle, 2009)
Powdered milk formula	ETAAS	Reconstituted milk selectively extracted using Chromabond NH <sub>2</sub> .	Cr(VI) detection limit of 1.8 µg L <sup>-1</sup> . Cr(VI) concentrations in commercial brands ranging between 10 and 75 µg kg <sup>-1</sup> .	(Maria E. Soares, Bastos, & Ferreira, 2000)
Dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, beverages	HPLC-ICP-MS	Alkaline extraction using NH <sub>4</sub> OH (pH 11.5).	No Cr(VI) found in any samples. Stability of Cr(VI) in cow milk investigated, organic matter found to induce reduction of Cr(VI).	(Vacchina, de la Calle, & Séby, 2015)
<b>Fungi</b>				
Mushrooms	ETAAS	Cap and stalk removed, sample consisted of several portions of either cap or stalk dried at 30-35°C for 3 days. Alkaline extraction using 0.01 M NaOH/1.0 M NH <sub>4</sub> NO <sub>3</sub> .	Detection limit of 0.17 µg L <sup>-1</sup> Cr(VI). Mushrooms accumulate Cr(VI), potential use as bioindicators for Cr(VI) soil pollution.	(Figueiredo, Soares, Baptista, Castro, & Bastos, 2007)
<b>Pulses</b>				
Red lentils	FAAS	Selective extraction using Amberlite XAD-1180 resin.	Detection limit of 7.7 µg L <sup>-1</sup> Cr(VI). Preconcentration factor of 75.	(Narin, Kars, & Soylak, 2008)

<b>Bread and Cereals</b>				
Bread	ETAAS	Melting durum flour and Cr(III) solution to simulate bread toasting and investigate possible oxidation of Cr(III) to Cr(VI) during this process.	No oxidation of Cr(III) to Cr(VI) occurred during simulated toasting procedure.	(Kovács, Béni, Karosi, Sógor, & Posta, 2007)
Bread	ETAAS	Small portions dried at 30-35°C for 1 hour, approximately 10 g ground in agate mortar by hand. Alkaline extraction using 0.01 M NaOH/1.0 M NH <sub>4</sub> NO <sub>3</sub> .	On average, 12% of total Cr in bread is Cr(VI). Estimated average daily intake of 0.57 µg/day and 0.69 µg/day for white and whole bread respectively.	(Maria Elisa Soares, Vieira, & Bastos, 2010)
Bread, breakfast cereals	HR-CS AAS	Alkaline extraction using 0.1 M Na <sub>2</sub> CO <sub>3</sub> .	Between 33-73% of total Cr in bread found to be Cr(VI). Demonstrated oxidation of Cr(III) to Cr(VI) during toasting. Daily consumption of cereal and bread does not exceed EPA maximum acceptable concentration of 0.003 mg kg <sup>-1</sup> bw day <sup>-1</sup> .	(Mathebula, Mandiwana, & Panichev, 2017)
<b>Beverages</b>				
Tea leaves, bread	SIDMS	Tea samples: alkaline extraction using 0.1 M Na <sub>2</sub> CO <sub>3</sub> . Double spiking using <sup>50</sup> Cr(VI) and <sup>53</sup> Cr(III) to monitor species interconversions. Bread samples: alkaline extraction using 0.01 M NaOH/1.0 M NH <sub>4</sub> NO <sub>3</sub> , double spiking.	No Cr(VI) detected in any extracts. No evidence of Cr(III) oxidation during sample preparation, evidence of Cr(VI) reduction in tea due to antioxidants and in bread due to high organic matter content. Emphasising requirement of speciation analysis.	(Novotnik, Zuliani, Scancar, & Milacic, 2013)

Black, green and herbal teas	ETAAS	Alkaline extraction using 0.1 M Na <sub>2</sub> CO <sub>3</sub> .	Up to 17.5 µg L <sup>-1</sup> Cr(VI) could be consumed per 200 ml cup of black tea.	(Mandiwana, Panichev, & Panicheva, 2011)
Tea leaves, tea infusions	ICP-MS	Selective solid phase extraction of Cr(III) using titanium dioxide nanotubes (TDNTs).	Cr(III) retained in pH range 5.0-8.0, Cr(VI) remains in solution. Detection limit of 0.0075 µg L <sup>-1</sup> Cr(III).	(Chen, Zhu, He, & Lu, 2014)
Neem tea infusions	SIDMS	Alkaline extraction using 0.1 M Na <sub>2</sub> CO <sub>3</sub> /0.1 M MgCl <sub>2</sub> to induce precipitation of Cr(III). Double spiking using <sup>50</sup> Cr(VI) and <sup>53</sup> Cr(III) to monitor species interconversions.	Cr(VI) reduced by antioxidants, below LOD (0.06 µg L <sup>-1</sup> ).	(Novotnik, Zuliani, Ščančar, & Milačič, 2015)
Lager beer	ETAAS	Selective extraction using Chromabond NH <sub>2</sub> column following precipitation of proteins.	Cr(VI) only detected in pale lager and low alcohol lager, estimated daily intake of 0.47 µg/day and 1.26 µg/day respectively.	(Vieira, Soares, Kozior, Krejpcio, Ferreira, & Bastos, 2014)
<b>Miscellaneous</b>				
Tobacco, eggplant	Activated carbon from tea-industry waste (TIWAC), FAAS	Extraction of Cr(III) using TIWAC, determination using FAAS	Detection limit for Cr(III) of 0.27 µg L <sup>-1</sup> . Preconcentration factor of 50 when using sample volume of 200 ml.	(Duran, Ozdes, Gundogdu, Imamoglu, & Senturk, 2011)
Vinegar	Catalytic adsorptive stripping voltammetry	Reduction of Cr(VI) at electrode surface to Cr(III), complexation with rubeanic acid, voltammetric response measured.	Detection limit of 0.002 µg L <sup>-1</sup> .	(Abbasi & Bahiraei, 2012)
Edible animal oils (cattle, fish)	HPLC-ICP-MS	Microwave extraction with 0.4% v/v HF and 2% Triton X-100 in HPLC mobile phase (0.5 mmol L <sup>-1</sup> TBAP and 0.3 mmol	Detection limit of 0.045 µg L <sup>-1</sup> for Cr(III) and 0.052 µg L <sup>-1</sup> for Cr(VI). No Cr(VI) detected in any samples, unidentified peak observed during	(Lin, Jiang, Sahayam, & Huang, 2016)



		L <sup>-1</sup> EDTA in 1% methanol adjusted to pH 6.9).	chromatographic separation. Spike recoveries suggest reduction of Cr(VI) to Cr(III) in oil matrix.	
Turmeric	Cloud point extraction (CPE), diffuse reflectance-Fourier transform infrared spectroscopy (DRS-FTIR)	Complexation of Cr(VI) with 1,5-diphenylcarbazide, enrichment of complex using CPE, analysis of complex using DRS-FTIR.	Detection limit for Cr(VI) of 1.22 mg L <sup>-1</sup> . Between 3.31 and 5.41 mg L <sup>-1</sup> Cr(VI) found in turmeric samples.	(Tiwari, Deb, & Sen, 2017)
Plants ( <i>Alyssum obovatum</i> , <i>Cochlearia arctica</i> ( <i>Brassicaceae</i> ), <i>Armeria scabra</i> ( <i>Limoniaceae</i> ), <i>Salix arctica</i> ( <i>Salicaceae</i> ))	ETAAS	Alkaline extraction using 0.1 M Na <sub>2</sub> CO <sub>3</sub> .	Cr(VI) stable in extraction matrix for up to 6 months. Less than 10% of total Cr in samples found to be Cr(VI).	(Panichev, Mandiwana, Kataeva, & Siebert, 2005)
Fish, white cheese, cow meat, black tea, boiled wheat	FAAS	Coprecipitation of Cr(III) using Ni <sup>2+</sup> /2-Nitroso-1-naphthol-4-sulfonic acid, subtraction of total Cr and Cr(III) to determine Cr(VI).	Detection limit for Cr(III) of 1.33 µg L <sup>-1</sup> .	(Uluozlu, Tuzen, & Soylak, 2009)
Apple, parsley, wheat, marrow, quince	FAAS	Selective extraction of Cr(III) using synthesised chelating resin.	Detection limit of 1.11 µg L <sup>-1</sup> . Cr(VI) concentrations in foodstuffs investigated were below detection limit.	(Çimen, Tokalio lu, erife, Özentürk, & Soykan, 2013)

**Table 2** Reported Cr(VI) concentrations in foodstuffs

Foodstuff	Reported Cr(VI)	Reference
<b>Milk and Dairy Products</b>		
UHT Milk	0.15-1.20 $\mu\text{g L}^{-1}$	(Lameiras, Elisa Soares, Lourdes Bastos, & Ferreira, 1998)
Cow milk	0.61-1.44 $\mu\text{g L}^{-1}$	(Ambushe, McCrindle, & McCrindle, 2009)
Powdered milk formula	<10-75 $\mu\text{g kg}^{-1}$	(Maria E. Soares, Bastos, & Ferreira, 2000)
Dairy products, flour, chocolate, vegetables, fruits, meat, fish, eggs, beverages	Below detection limit	(Vacchina, de la Calle, & Séby, 2015)
<b>Fungi</b>		
Mushrooms	<0.0085-0.580 $\text{mg kg}^{-1}$ in cap, <0.0085-0.813 $\text{mg kg}^{-1}$ in stalk	(Figueiredo, Soares, Baptista, Castro, & Bastos, 2007)
<b>Pulses</b>		
Red lentils	Below detection limit	(Narin, Kars, & Soylak, 2008)
<b>Bread and Cereals</b>		
Bread	Below detection limit	(Kovács, Béni, Karosi, Sógor, & Posta, 2007)
Bread	<5.60-18.80 $\mu\text{g kg}^{-1}$ in white bread, <5.60-19.70 $\mu\text{g kg}^{-1}$ in whole bread	(Maria Elisa Soares, Vieira, & Bastos, 2010)
Bread, breakfast cereals	19.25-63.86 $\mu\text{g kg}^{-1}$ , 41.0-470.4 $\mu\text{g kg}^{-1}$ in breakfast cereal	(Mathebula, Mandiwana, & Panichev, 2017)
<b>Beverages</b>		
Tea leaves, bread	Below detection limit	(Novotnik, Zuliani, Scancar, & Milacic, 2013)

Black, green and herbal teas	0.03-3.15 mg kg <sup>-1</sup> total, 0.025-1.75 mg kg <sup>-1</sup> water soluble	(Mandiwana, Panichev, & Panicheva, 2011)
Tea leaves, tea infusions	3.39-4.41 µg L <sup>-1</sup>	(Chen, Zhu, He, & Lu, 2014)
Neem tea infusions	Below detection limit	(Novotnik, Zuliani, Ščančar, & Milačič, 2015)
Lager beer	<1.61-13.0 µg L <sup>-1</sup>	(Vieira, Soares, Kozior, Krejpcio, Ferreira, & Bastos, 2014)
<b>Miscellaneous</b>		
Tobacco, eggplant	Below detection limit	(Duran, Ozdes, Gundogdu, Imamoglu, & Senturk, 2011)
Vinegar	3.30 ± 0.11 µg L <sup>-1</sup>	(Abbasi & Bahiraei, 2012)
Edible animal oils (cattle, fish)	Below detection limit	(Lin, Jiang, Sahayam, & Huang, 2016)
Turmeric	3.19-5.41 mg L <sup>-1</sup>	(Tiwari, Deb, & Sen, 2017)
Plants ( <i>Alyssum obovatum</i> , <i>Cochlearia arctica</i> ( <i>Brassicaceae</i> ), <i>Armeria scabra</i> ( <i>Limoniaceae</i> ), <i>Salix arctica</i> ( <i>Salicaceae</i> ))	0.09-0.59 mg kg <sup>-1</sup>	(Panichev, Mandiwana, Kataeva, & Siebert, 2005)
Fish, white cheese, cow meat, black tea, boiled wheat	Below detection limit	(Uluozlu, Tuzen, & Soylak, 2009)
Apple, parsley, wheat, marrow, quince	Below detection limit	(Çimen, Tokalio lu, erife, Özentürk, & Soykan, 2013)