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 **Nutrient, fibre, sorbitol and chlorogenic acid content of prunes (***Prunus domestica***): an updated analysis and comparison of different countries of origin and database values Samantha K Gill\*, Ellen Lever\*, Peter W Emery, Kevin Whelan**  King's College London, Department of Nutritional Sciences, London, United Kingdom Corresponding author: Professor Kevin Whelan, King's College London, Department of Nutritional Sciences, 150 Stamford Street, London, SE1 9NH, United Kingdom, + 44 20 78 48 38 58, kevin.whelan@kcl.ac.uk Short running head: Prune composition Key words: dried fruit, dried plums, prunes, gastrointestinal health, fibre, polyphenols \*Joint first authors **Disclosure of interest:** This study was funded by the California Dried Plum Board. The funders played no role in study conception, design, analysis, interpretation or writing of the manuscript. KW has served as a consultant for Danone, has received speaker fees from Alpro and the National Dried Fruit Trade Association UK Ltd and has received research funding from Almond Board of California, California Dried Plum Board, Clasado Biosciences, Nestec Ltd, International Nut and Dried Fruit Council, and receives royalties from FoodMaestro. The

remaining authors report no conflicts of interest.

## **Abstract**

 Current prune composition data is outdated and requires a comprehensive and comparative re-analysis. This novel study aimed to: (i) analyse and compare prune composition from major countries of origin; and (ii) provide a comprehensive compositional analysis of prunes of USA origin and compare this with UK and USA database data. Prune samples were analysed for major nutrients and bioactive compounds and compared between countries of origin. Total fibre was higher in prunes from the USA (12.0 g/100g) and Chile (11.5 g/100g) compared with France (8.4 g/100g) and Argentina (8.9 g/100g), while prunes from all countries contained high levels of sorbitol (11.2-15.5 g/100g). Differences of energy and starch values compared with national databases reflected different approaches to sampling and analysis. In conclusion, prunes contain high levels of fibre and other bioactive compounds. Variations between country of origin and database values highlight the importance of transparency in documenting sampling and analysis methods.

#### **Introduction**

 Studies have highlighted the potential benefits of dried fruits on a variety of health outcomes (Chang et al. 2016). In particular their high fibre content has led to investigation of the role of dried fruit in the maintenance and promotion of gastrointestinal health (Lever et al. 2015) which is considered of major public health importance (DuBois 2004; Wald et al. 2007). The 42 impact of dietary fibre on health is affected by variations in its chemical composition (e.g. distribution of different fibre fractions) and physical structure (e.g. degree of polymerisation, molecular weight and linkages) that alter its solubility, viscosity and fermentability. Given that dried fruits are nutritionally comparable to whole fresh fruits, only provided in a smaller and more concentrated form, they may be a convenient and versatile option for increasing fruit consumption across population groups (Sadler et al. 2019).

 Plums are taxonomically diverse stone fruits of *Prunus domestica L.* and are commonly consumed in their dried form, termed prunes. Data from various sources, including the United Kingdom (Finglas 2015) and the United States of America (USA) (US Department of Agriculture 2018), indicate that prunes are naturally high in a variety of poorly-fermented and readily- fermented dietary fibres (>6 g/100g including hemicellulose, pectin, cellulose). In addition, prunes contain other bioactive compounds such as polyphenols, which may stimulate colonic proliferation of microorganisms such as Bifidobacteria and Lactobacilli. Furthermore, prunes contain high amounts of sorbitol (~12 g/100g) which is known to have laxative effects (Yao et al. 2014). Indeed, a systematic review concluded that prunes may play a role in gastrointestinal health by increasing stool frequency and improving stool consistency (Lever et al. 2014).

 Data on prune composition require updating for several reasons. Firstly, existing databases, such as McCance and Widdowson (UK) and USDA (USA) databases, do not report a wide range of components relevant to gut health (e.g. different fibre fractions and sorbitol content), and secondly the current data were compiled between 1980 and 2001 and therefore may no longer accurately reflect present-day prune composition. Thirdly, the USDA data calculates total carbohydrate 'by difference', which does not account for the lower energy contributions from unavailable carbohydrates. Fourthly, the composition of prunes may vary depending on  a variety of factors including growing and harvesting conditions and post-harvest processes (e.g. drying, dehydration and rehydration, storage conditions). Given that the vast majority of global supply of prunes originates from four countries: the USA (largely California, 43%), Chile (24%), France (16%) and Argentina (15%) (Buncher 2012), currently-available prune composition data may be confounded by variations in origin. For example, standard yellow plums have been shown to contain higher vitamin and phenolic compound content than organically grown plums (Lombardi-Boccia et al. 2004), while prunes from Australia have been shown to contain higher iron and folate contents than prunes from USA and Chile (Bennett et al. 2011). Finally, variations in the nutrient composition of prunes of different countries of origin will impact the database values in each country. For example, databases in the USA (USDA) and France (CIQUAL), both of whom are large producers of prunes, will reflect the composition of prunes from those countries, whereas the database in the UK (McCance and Widdowson), which does not grow large supplies of prunes, will reflect the composite of prunes from different countries of origin.

83 With this in mind, we aimed to investigate the energy, macronutrient, micronutrient, fibre, sorbitol and polyphenol composition of prunes by: (i) analysing and comparing the composition of prunes from major countries of origin (USA, Chile, France and Argentina); and (ii) undertaking an in-depth analysis of prunes of USA (Californian) origin and comparing this with data from food composition databases.

# **Materials and Methods**

#### *Sample collection*

 Prune samples grown by the four largest producers of prunes were collected in order that composition could be both globally representative and compared between country of origin (USA, Chile, France and Argentina). Prune samples were purchased from major population centres in five countries across Europe (France, Germany, Italy, Spain, United Kingdom) as these are five major European markets for prunes and thus data would reflect the composition of prunes available across Europe, as well as meeting the Food Information to Consumers Legislation (European Commission 2011). Prune samples were purchased as sold to the customer from major retail outlets including supermarkets, department stores and health food stores and including a range of brands (where available) to ensure purchase of  prunes representative of those most frequently consumed, i.e. with the highest volume of sales. Prune samples were purchased at the same time of year and within use-by-dates. Samples were required to be in unopened packets of ≥100 g with a remaining shelf life of ≥6 months. Prunes were stored unopened until analysis to minimise drying, water absorption and contamination. Prunes were purchased pitted (stone removed) or whole. If purchased whole, stones were removed prior to compositional analysis.

 In total, the goal was to purchase 10 to 12 samples from each of the four countries of origin, with at least 3 samples from each sampling country.This number is recommended for nutrient composition database data and based upon guidance from Greenfield and Southgate (2003), though this depends on the variability of the nutrients being measured.

 Prune samples from each country of origin were pooled prior to analysis. Funding restrictions meant that the study could either: (i) individually analyse a number of prune samples from a single country, thus allowing measurement of within-country variation but not between- country variation; or (ii) analyse a pooled sample from a number of sampling countries, thus allowing measurement of between-country variation albeit without statistical comparison.

 Given the wide geographic difference in countries of origin (USA, France, Chile, Argentina), it was felt that between-country variations, rather than within-country variations, were likely to be larger and therefore of greater nutritional relevance.

## *Sample preparation and analysis*

 Samples were pooled according to country of origin (**Table 1**). This pooled sample comprised an equal weight of 500 g (i.e. 100 g adjusted weight from each sampling country), of prunes from each of the five sampling countries. Pooled samples were homogenised using a hand mincer, divided into aliquots, stored frozen at -80°C and defrosted prior to analysis.

 Prune samples were analysed at Leatherhead Food Research, Surrey, UK. The pooled sample from each of the four countries of origin was analysed using standard methods for energy (calculated from macronutrient data), protein (total nitrogen), fat (Soxhlet), carbohydrate (calculated by difference), sugars and sorbitol (ion-exchange chromatography), a range of fibre classifications (AOAC methods, Englyst), and chlorogenic and neochlorogenic acid (ultra performance liquid chromatography tandem mass spectrometry, UPLC with MS-MS) (**Table 2)**. In addition, further in-depth analyses were performed on the pooled sample of prunes from the largest global producer (California, USA) including fatty acids (gas chromatography with flame ionisation detection), sugars (ion-exchange chromatography) and major micronutrients (inductively coupled plasma optical emission spectrometry ICP-OES, high performance liquid chromatography HPLC) (**Table 2)**.

 In terms of the chromatographic methods, for sugar and sorbitol, extraction from the prune samples was performed by sonication in hot water and treatment with Carrez reagents. The filtered solution was then analysed using high-performance anion-exchange chromatography coupled with pulsed electrochemical detection (HPAEC-PED) using a Dionex PA20 column (Corradini et al, 2012). For chlorogenic and neochlorogenic acids, extraction from the prune 144 samples was performed in hot water and methanol and the solution analysed using UPLC with 145 MS-MS equipped with an ethylene bridged hybrid column (C18 2.1 x 50 mm, 1.7 µm). For fatty acids, transmethylation was undertaken to form methyl esters which were analysed using gas-liquid chromatography with a flame ionisation detection (Seppänen-Laakso, et al, 2002). For vitamin B analysis, extraction was performed using HCl and the solution analysed using HPLC with fluorescence detection using a C18 conventional column (250 x 4.6 mm, 5 µm).

 Duplicate analyses were carried out for analytes that were not routinely measured at the research centre. However, routine analyses were not performed in duplicate as these had criteria defining the limits of repeatability.

# **Results**

#### *Sample purchases*

 Sample purchases were made in France (Normandy), Germany (Bonn), Italy (Milan, Novara), Spain (Madrid), and the UK (London) between March and June 2013. The pack sizes of the purchased prune samples varied between 120 g and 1000 g. **Table 1** shows the number of prune samples purchased and analysed from each sampling country and by country of origin. One sample was excluded as it exceeded the use-by-date by the time of analysis and four 163 samples were excluded because the country of origin was unclear. Eighteen different samples were pooled and analysed for USA and French prunes, fifteen for Chilean prunes, but only five for Argentinian prunes (all purchased from Spain) due to their lack of availability in Europe at that time. The amount analysed from each individual sample was weighted so that an equal amount from each sampling country was included and pooled to make up a total of 500g from each country of origin (**Table 1**).

### *Composition Data*

 The composition of prunes from the pooled samples originating from USA, Chile, France and Argentina are shown in **Table 2.** In general there were few major differences in nutrients and fibre fractions between prunes of different countries of origin.

 Differences in starch content were observed between countries, being lower in prunes of French origin (1.9 g/100 g) compared with others (5.7-6.6 g/100g). Total fibre (measured using AOAC 2011.25) was higher in prunes from the USA (12.0g/100g) and Chile (11.5g/100g) compared with those from France (8.4g/100g) and Argentina (8.9g/100g).

 Prunes contained high levels of sorbitol (11.2-15.5g/100g) with broadly similar values across the countries of origin. High levels of the phenolic compounds chlorogenic acid and neochlorogenic acid were also found in prunes, however, in general these were higher in prunes from the USA (3.6 and 89.3 g/100g) and France (3.9 and 92.0 g/100g) compared with prunes from Chile (1.3 and 39.8 g/100g) and Argentina (1.3 and 40.3 g/100g) (**Table 2**).

 The composition of prunes from the USA pooled sample compared with data from the USDA nutrient database and McCance and Widdowson's the composition of foods isshown in **Table 3**. Concentrations per wet weight are presented in order to be consistent with these databases. Energy and starch values (230 kcals/100g and 6.6g/100g, respectively) were closer to values published by the USDA database (240 kcals/100g and 5.1 g/100g, respectively), than McCance and Widdowson (141 kcals/100g and 0.0 g/100g, respectively).

## **Discussion**

 The current study aimed to investigate the energy, macronutrient, micronutrient, fibre, sorbitol and polyphenol composition of prunes by: (i) analysing and comparing the composition of prunes from major countries of origin (USA, Chile, France and Argentina); and (ii) undertaking an in-depth analysis of prunes of USA (Californian) origin and comparing this with data from food composition databases.

200 In regards to the measured composition of prunes from different countries of origin, while the pooling of samples precluded statistical comparisons, at face value there were few 202 differences in energy and macronutrient content between them, apart from starch which was lower in prunes of French origin (**Table 2**). Given that the same methods of analysis were used for prunes of all countries of origin and analysis occurred at the same time and in the same run, these differences likely reflect true compositional differences in prune samples between countries.

 There were no major differences in dietary fibre content when measured using the AOAC 991.43 method (which excludes low molecular weight fibres and most types of resistant starch) nor using the Englyst method (non-starch polysaccharides). However, when measured using the AOAC 2011.25 method, fibre was higher in prunes from USA and Chile compared with France and Argentina. The AOAC 2011.25 method includes all categories of dietary fibre, high and low molecular weight fibres and all types of resistant starch. Taken together, this suggests that US and Chilean prunes likely contain greater low molecular weight fibres and resistant starch than French and Argentinian prunes. Low molecular weight fibres are soluble, 216 explaining the higher soluble fibre content in USA (7.6 g/100 g) and Chilean (6.3 g/100 g) 217 prunes compared with others  $(4.4-4.6 \text{ g}/100 \text{ g})$ . In addition, French prunes contained less sorbitol, while Chilean and Argentinian prunes had lower chlorogenic and neochlorogenic acid content than the prunes of other origins. As previously mentioned, these differences may be due to variations in soil management, plum ripeness or storage conditions (Donen 1939; Piga et al. 2003), although the reasons for any differences was not investigated here.

 In regards to prunes of USA (Californian) origin (**Table 3**), there were minor differences between the current analytical data and those published by USDA, though these were small and likely negligible from a nutritional perspective. Given that the UDSA database is updated regularly through submission of independent analyses from food manufacturers, the minor 227 differences observed may simply reflect seasonal variation in composition. However, there were larger differences in both the current analytical data and the USDA data compared with 229 the UK data provided by McCance and Widdowson, the latter reporting lower energy and starch values. Notably, little information is provided on the sourcing of prune samples reported in McCance and Widdowson and so it is unknown whether prune origin could be responsible for differences in starch content. Water content was comparable between the data (30.9-31.1%), therefore any differences were not due to variation in water content. Rather, the difference in energy content is explained by differences in the components included in the energy calculation and different conversion factors used.

 In the current study, energy content is calculated based upon the contribution of 'available carbohydrate', fat, protein, fibre and polyols, as per European Union labelling regulations (EC, 239 2011). The USDA data includes 'total carbohydrate' in the energy calculation (rather than 'available carbohydrate'), and therefore does not take into account the lower energy contribution from fibre and polyols, and this is reflected in the slightly higher energy value published by USDA (240 kcal/100 g) compared with the current analytical data (230 kcal/100 g). In stark contrast, the UK data from McCance and Widdowson (141 kcal/100 g) excludes fibre and polyols from the energy calculation.

246 Prunes contained high levels of sorbitol (11.2-15.5 g/100g), these values being similar to USDA 247 values (12.0  $g/100g$ ) and other studies in the scientific literature (10.8  $g/100g$ ) (Yao et al. 2014). The sorbitol content of prunes is therefore higher than that of its non-dried counterpart plums (2.4 g/100g), as well as other non-dried stone fruits such as cherries (0.7 250 g/100g) and dried fruits such as dried apricots ( $6g/100g$ ), dried pear (8.1 g/100g) and dried apple (1.9g/100g) (Yao et al. 2014). Some polyols have been shown to induce increases in small intestinal water, although this has not been confirmed for sorbitol. For example, a fourfold increase in small intestinal water was observed in healthy individuals 60 minutes following ingestion of 17.5 g of mannitol (Marciani et al. 2010).

 Prunes also contained high levels of chlorogenic acid (1.3-3.9 g/100g) and neochlorogenic acid (39.8-92.0 g/100g), particularly those from the USA and France. This reflects data from previous studies reporting high levels of phenolic compounds in prunes (Donovan et al. 1998; Stacewicz-Sapuntzakis 2013). These phenolic compounds are partially absorbed in the small intestine and the remainder enter the colon where they undergo biotransformation by the microbiota into caffeic acid and quinic acid (Olthof et al. 2001). A recent systematic review suggests that polyphenols and their degradation products can modulate the gut microbiota and have prebiotic effects (Nash et al. 2018). Chlorogenic acid has been shown to inhibit the growth and adhesion of selected gut pathogens to a human gut cell line and to enhance the proliferation and adhesion of the probiotic *L. rhamnosus* (Parkar et al. 2008). Taken together, it is plausible that the combination of different dietary fibres, sorbitol and polyphenols naturally abundant in prunes create a synergistic effect, which in part, may be the reason why prunes are considered beneficial for gastrointestinal health (European Food Safety Authority 2014).

 The current analytical data and the USDA data calculate carbohydrate values by difference (subtracting amounts of the other proximates from the total weight), while McCance and Widdowson calculate available carbohydrate using monosaccharide equivalents of each measured component. In the McCance and Widdowson UK data, available carbohydrate is 275 equal to total sugars since no polyols, oligosaccharides or starch are reported. Though the 276 reason for the lack of starch in prunes (0.0  $g/100 g$ ) reported in the McCance and Widdowson UK data is unclear, it is possible that when analysis was undertaken, prunes of French origin (which in the current analysis contained less starch) were more readily available and accessible. Notably, the current analytical data and the data from the USDA database may considerably overestimate the available carbohydrate content by including unmeasured components which are not absorbed or not metabolised in the body to produce energy (e.g. 282 sorbitol). In the present analytical data the sum of starch and sugars is 12  $g/100g$  less than the value for available carbohydrate by difference, while in the USDA data the sum of starch 284 and sugars is around 20 g/100g less than the value for total carbohydrate by difference that has been used to calculate energy content. True energy values (kcals) for prunes appear to be between 230-240 kcals/100g in accordance with the present analytical data (230 kcals/100g)  and the USDA database (240 kcals/100g). This is in contrast to McCance and Widdowson that presents noticeably lower energy values (141 kcals/100g).

#### **Limitations and strengths**

 The major limitation of this study was that, due to financial constraints, we did not analyse multiple prune samples from each country of origin that would have enabled both within- country variation and between-country statistical comparisons to be performed. In contrast, our approach enabled only between-country variation to be analysed, albeit not statistically compared. However, this approach allowed for a wide range of important nutrients and other compounds relevant to health to be included, which we felt outweighed the limitations of pooling samples. Despite the limitation of pooling prune samples from each country of origin, a robust sampling methodology was adopted based upon standards used for food composition databases to ensure high levels of representativeness in each pooled sample, including sourcing from a range of major retail centres in numerous sampling countries.

 Further limitations include the small number of samples from Argentina, which may therefore not be fully representative of Argentinian prunes available across Europe. Any differences attributable to country of origin can only be ascertained by controlling other factors that can influence variation in nutrient composition. The sampling protocol attempted to account for seasonal variation and storage conditions by purchasing samples at the same time of year and within use by dates, and minimised changes in composition between purchase and analysis. However, given that all prunes were sampled at point of sale, pre-purchase confounding variables such as exposure to heat, light and humidity could not be controlled for. This might be relevant if these factors influence nutrient composition as some previous data suggests, however, although such analyses are of important academic and commercial interest, from a practical perspective the consumer cannot currently impact post-harvest/pre-purchasing processing.

#### **Conclusion**

 The current study provides evidence that small differences in dietary fibre, sorbitol and phenolic content may exist between prunes of different countries of origin. To our knowledge, this is the first study to provide a comprehensive and comparable compositional analysis of



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**Table 1:** Number of prune samples purchased, pooled and analysed, by sampling country and country of origin.

\* One sample exceeded the best before date and was excluded

\*\* Only 8 samples required

\*\*\* Samples all labelled Spanish/French origin and thus excluded



**Table 2:** Composition of prunes from the pooled samples from USA, Chile, France and Argentina, purchased in Europe



UPLC with MS-MS: Ultra-performance liquid-chromatography tandem mass spectrometry

**Table 3:** Composition of prunes originating from the USA (California), as analysed in the current study, compared with data from the USDA nutrient database and McCance and Widdowson's The Composition of Foods. Values are units per 100 g wet weight.







NR = Not reported

Tr = trace

ICP-OES Inductively Coupled Plasma Optical Emission Spectrometry

HPAEC-PED High-Performance Anion-Exchange Chromatography Coupled with Pulsed Electrochemical Detection

HPLC High Performance Liquid Chromatography