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Changes in rocket salad phytochemicals within the commercial supply chain: Glucosinolates, isothiocyanates, amino acids and bacterial load increase significantly after processing

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ABSTRACT

Five cultivars of *Eruca sativa* and a commercial variety of *Diplotaxis tenuifolia* were grown in the UK (summer) and subjected to commercial growth, harvesting and processing, with subsequent shelf life storage. Glucosinolates (GSL), isothiocyanates (ITC), amino acids (AA), free sugars, and bacterial loads were analysed throughout the supply chain to determine the effects on phytochemical compositions.

Bacterial load of leaves increased significantly over time and peaked during shelf life storage. Significant correlations were observed with GSL and AA concentrations, suggesting a previously unknown relationship between plants and endemic leaf bacteria.

GSLs, ITCs and AAs increased significantly after processing and during shelf life. The supply chain did not significantly affect glucoraphanin concentrations, and its ITC sulforaphane significantly increased during shelf life in *E. sativa* cultivars. We hypothesise that commercial processing may increase the nutritional value of the crop, and have added health benefits for the consumer.

1. Introduction

The majority of rocket (*Eruca sativa* & *Diplotaxis tenuifolia*) consumed in the UK is imported from Italy (Bell, Spadafora, Müller, Wagstaff, & Rogers, 2016). In 2015 sales of bagged rocket salad in the UK increased 3.9% on the previous year (Dr. Lorraine Shaw, Bakkavor, Spalding, UK; personal communication, 2016) and this trend is expected to continue in future.

Leaves are typically harvested by machine from long, linear beds in open fields, polytunnels or glasshouses. Time from sowing to harvest can be between 20 and 40 days depending on the growing region and species (Bell, Oruna-Concha, & Wagstaff, 2015), and has been reported to extend up to 99 days in winter months (Hall, Jobling, & Rogers, 2012). Growing methods vary according to region and grower preference. Produce for the bagged salad market is generally processed in the same way; after harvesting, leaves are vacuum chilled and stored under cool-chain conditions (<5 °C) until processing. This may be at the site of harvest, a nearby facility, or after transport to the country where it will be sold and consumed. Leaves enter a 'low care' environment, and are typically washed in chlorinated water (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007) with mechanically induced water turbulence to remove detritus. Leaves are spin-dried in a high care environment to remove excess water, and then passed into a 'high care' environment, where it is weighed and bagged. Products use micro or laser perforated bags that contain modified or unmodified atmosphere to preserve and prolong self life (Hall, Jobling, & Rogers, 2013). Bags are shipped through a cold-chain to supermarkets and other vendors who store them in open-fronted chiller cabinets (Hall et al., 2013). Shelf life of rocket has been reported to range from seven to 14 days depending on environmental conditions (Martínez-Sánchez, Allende, Cortes-Galera, & Gil, 2008).

The stressful nature of the supply chain on leafy produce has led to questions regarding how nutritional value is affected (Verkerk et al., 2009). It is known that adverse storage conditions post harvest have a negative impact upon the appearance and odour of leaves (Lokke, Seefeldt, & Edelenbos, 2012). Cutting and processing material also makes it more perishable during storage (Watada, Ko, & Minott, 1996), and temperature is the predominant means by which degradation is controlled (Lokke et al., 2012). There has been little research into how nutritional traits are affected by the industrial supply chain in leafy salads. Studies have covered parts of the supply chain for different *Brassicaceae*, such as effects of cutting

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and washing (Martínez-Sánchez et al., 2008), post harvest storage (Bell et al., 2016), and packaging treatments (Rangkadilok et al., 2002).

In this study, a commercial supply chain was utilised to assess phytochemical profiles of rocket salads across multiple time points – immature leaves, harvest, processing, and throughout shelf life. Building upon previous phytochemical, sensory and consumer analyses (Bell et al., 2015; Bell et al., 2016; Bell, Methven, Signore, Oruna-Concha, & Wagstaff, 2017), six underutilised germplasm accessions and one commercial variety were tested for glucosinolate (GSL), isothiocyanate (ITC), free amino acid (AA), and free sugar concentrations. The aim of our work is to inform the breeding selections and practices of industrial collaborators to create new, sensorially and nutritively enhanced varieties of rocket. The accessions used throughout have been shown to vary significantly in phytochemical composition under controlled environmental conditions, but it is unknown how these might change under industrial circumstances.

Rocket is well known for accumulating GSL compounds, which are hydrolysed by myrosinase enzymes into ITCs, nitriles, and other degradation products (Bell & Wagstaff, 2014). ITCs such as erucin (4-(methylthio)-butyl-ITC) and sulforaphane (4-(methylsulfinyl)-butyl-ITC) are both present in rocket species, and their potential anticarcinogenic properties are well studied in the literature (Traka et al., 2013). Other ITCs present in rocket are not well understood. The GSLs DMB (dimeric 4-mercaptobutyl-GSL), glucosativin (4-mercaptobutyl-GSL), diglucothiobeinin (4-(β-D-glucopyranosyldisulfanyl)-butyl-GSL), and their respective myrosinase degradation products are poorly understood in terms of abundance and anti-cancer properties. As demonstrated in Bell et al. (2017) some of the volatile derivatives of the GSL-myrosinase reaction, infer significant associations with sensory attributes such as bitterness and pungency. Some GSLs such as glucoerucin and glucoraphanin have no significant sensory properties associated with them.

In Bell et al. (2017), total AA concentration was negatively correlated with the perceptions of bitterness and pungency, leading to the hypothesis that certain AAs contribute to sensory qualities of the crop (Solms, 1969). The way AAs respond to commercial processing may therefore impact upon sensory traits, and are an important indicator of senescence and tissue breakdown (Buchanan-Wollaston et al., 2003). Free sugars may also impact sensory attributes by masking bitter and pungent sensations, though it is unknown how they are affected by processing in rocket.

Another important aspect of rocket in the supply chain is the presence of bacteria (which are naturally present on leaves). Usually these are non-pathogenic strains and do not pose a health concern for humans, but can contribute to spoilage and shorten shelf life (Lokke et al., 2012). It has been known for over 20 years that chlorinated or chemically treated water does not erradicate bacterial populations from leaves, but does have a role to play in ensuring sanitation of recirculated water in processing facilities. Strict field technical control protocols are followed to prevent contamination with pathogenic strains (Dr. Lorraine Shaw, Bakkavor, Spalding, UK; personal communication, 2016), however native leaf bacteria reside within cells and crevaces on the leaf surface, making it impossible to fully remove them from fresh-cut produce (Watada et al., 1996). ITCs are known to have antibacterial effects (Vig, Rampal, Thind, & Arora, 2009) but this relationship has not been studied in the context of the commercial supply chain. Free sugars may also provide a food source for bacteria, and we question how natural populations respond to concentrations within leaves during commercial processing and shelf life.

With the aforementioned aspects in mind (Verkerk et al., 2009), we hypothesised that GSL and ITC content would decline significantly over time due to a combination of GSL hydrolysis and leaching into wash water. We theorised that this would lead to a reduction in the nutritive and health beneficial properties of leaves. We also hypothesised that with a decrease in potentially anti-microbial compounds (ITCs) bacterial populations would increase and peak during shelf-life. The results presented in this paper show however that these hypotheses could be rejected, and that processing of rocket leaves may add nutritional value to the crop.

2. Materials & methods

2.1. Plant material

The five non-commercial accessions used in this paper (*E. sativa*) were originally sourced from European germplasm collections. See Bell et al. (2015) for information regarding the supplying institutes. Due to the small amounts of seed given, each cultivar was individually bulked by open pollination in separate glasshouse compartments at Elsoms Seeds Ltd. (Spalding, UK) in the spring/-summer of 2014. The amount of seed produced for each cultivar weighed >500 g. The commercial variety *Torino* (*Diplotaxis tenuifolia*) used as a comparator to gene bank-sourced cultivars.

2.2. Growing & industrial supply chain conditions

Plants were grown in an open field at a Bakkavor supplier, (Dorchester, England) from the 3rd to the 25th of July 2014. Cultivars were sown using a tractor mounted air drill in parallel beds measuring approximately 50 m in length. *Torino* was sown as a guard crop surrounding the trial beds, and crop protection and irrigation of the trial was as per standard commercial practice.

Plants were harvested on the morning of 25th of July 2014 (22 days old) by machine. Due to the slower growth of *Torino*, plants drilled on the same date as the *E. sativa* cultivars were not harvested. Leaves were loaded into crates, which were placed into a waiting trailer. From harvest (H) onwards, five temperature data loggers (Tinytag Transit 2, −40 to +70 °C sensitivity range; Gemini Data Loggers Ltd., Chichester, UK) were added to crates and set to record one data point every five minutes for the remainder of the trial. See Fig. S1 for a temperature-time plot of averaged data. The temperature on the day of harvest was unusually hot for UK summer time, and the recorded average was 34.8 °C.

A tractor-trailer loaded with samples was driven approximately one mile to a storage facility. Crates were unloaded into a vacuum cooler, which removed field heat from the produce. Samples were stored in a 4 °C cold store, in the dark, for two days; the average temperature for this period was 4.9 °C. Samples were transported on the third day after harvest to a Bakkavor processing site via temperature-controlled lorry. Produce was stored in a 4 °C environment for the remainder of that day, but temperatures ranged between 2.2 °C and 8.6 °C during this time.

The following day, samples were processed using a commercial wash line with mild water chlorination. Each cultivar was entered into the line separately with a five-minute gap between to prevent mixing. Leaves were spin-dried, before being transferred by conveyor belt to be bagged in unmodified atmosphere, microperforated bags. Produce was stored overnight under controlled conditions; temperatures averaged 5.1 °C in the processing environment. The day after, samples were transported via courier in a temperature-controlled vehicle to the University of Reading (UoR), but temperatures as high as 14.3 °C were recorded during this time, representing a potential breach in the cold-chain (Fig. S1). The temperature upon arrival at UoR was 21.7 °C.