Chapter 5

Assessment of Bioaccessibility: A Vital Aspect for Determining the Efficacy of Superfoods

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Additional information is available at the end of the chapter

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Abstract

Bioaccessibility is a vital aspect when qualifying food products to be superfoods. It could be defined as the amount of a food constituent which is present in the gut, as a consequence of its release from the solid food matrix. This chapter highlights the evaluation of the bioaccessibility of three studies using an *in vitro* model of digestion, involving potential superfoods which are as follows: (1) Five Sri Lankan endemic fruits (2) ten spices which are commonly used in culinary preparations in Sri Lanka as well as throughout the world, and (3) three Kombucha 'tea fungus' fermented beverages obtained through different microbial cultures. In all three studies, the antioxidant and starch hydrolase activities of the food products were evaluated, given the therapeutic importance of these characteristics. In the first study, it was observed that the antioxidant and activities had decreased, although the starch hydrolase inhibitory activities had been sustained. The remaining two studies demonstrated that both these functional properties had statistically significantly increased ($P < 0.05$), or sustained. Overall, the studies emphasise the need for evaluating the bioaccessibility of functional properties of potential superfoods before they can be properly advocated for consumption to the general public.

Keywords: antioxidants, bioaccessibility, bioavailability, functional food, starch hydrolase inhibitory activity

1. Introduction

Superfoods have been thought to possess many health benefits mostly owing to the existence of bioactive compounds. Most of the superfoods which are believed to possess disease preventive properties have demonstrated superior antioxidant activity. This property itself is one of the most coveted mechanisms touted to prevent the occurrence of non-communicable diseases such as cardiovascular disease (CVD), diabetes and cancer. Carotenoids and phenolic compounds are the

two major categories of dietary antioxidants where over a hundred member compounds have been identified per each class to date. Carotenoids are fat-soluble, mostly occurring in the form of colouring agents and pigments [1]. On the other hand, phenolic compounds exist as free, esterified, etherified and insoluble-bound forms and are commonly found in edible fruits and vegetables, leafy vegetables, roots, tubers, bulbs, herbs, spices and legumes [1]. A recent trait of these compounds which is of relevance to superfoods is their ability to inhibit starch hydrolases – in particular, α-amylase and α-glucosidase. Starch hydrolase inhibitory activity prevents the sudden release of glucose into the physiological system, thereby preventing the triggering of biochemical pathways which produce free radicals inside the mitochondria as a result of glucose metabolism. Since the digested products of α-amylase act as substrates for α-glucosidase, inhibition of α-amylase is believed to be more important in preventing the commencement of the biochemical pathway of starch digestion and thereby, curbing the release of glucose to the physiological system.

When it comes to bioactive compounds in superfoods – antioxidants in particular, their release and availability from the food matrix into the digestive tract is of paramount importance. This is technically referred to as bioavailability. One step prior to bioavailability is bioaccessibility. This is the amount of a food constituent which is present in the gut, as a consequence of its release from the solid food matrix, and thus, which may be able to pass through the intestinal barrier into the circulatory system. Considering both bioaccessibility and bioavailability, only bioactive constituents released from the food matrix by the digestive enzymes are bioaccessible in the gut, and therefore are potentially bioavailable. Digestive enzymes play an important role when it comes to bioaccessibility, since their action may essentially increase or decrease their release into the digestive system. As a consequence of this phenomenon, it is evident that the amount of bioaccessible food antioxidants and other therapeutic compounds of interest may differ quantitatively and qualitatively from the values which have been included in food databases.

Several *in vitro* and *in vivo* methodologies have been developed over the past few years to assess the bioaccessibility of bioactive compounds in many of the superfoods [1, 2]. *In vivo* methods using animal models or in the form of clinical trials, usually provide the most accurate results. However, the two major shortcomings of *in vivo* assessments is that they are time consuming and costly. Thus, much effort has been devoted to the development of *in vitro* procedures, where nearly accurate outcomes can be achieved within a short span of experimental time. However, it has to be borne in mind that any *in vitro* method would eventually fall short in matching the precision which can be achieved by actually studying the behaviour of a food *in vivo* due to the natural complexity of the release and digestive process itself [2]. As a result of this, some compromise is understandably needed between the accuracy and ease of utilisation of any *in vitro* digestion model which aims at assessing bioaccessibility. During the past few years, food and animal scientists have utilised several *in vitro* digestion models to test the structural and chemical changes occurring in different food under simulated gastrointestinal (GI) conditions. However, none of these methods have yet been widely accepted and no method has been singularly standardised in terms of assessment.

This chapter aims at giving examples demonstrating the importance of assessing bioaccessibility of the antioxidant and starch hydrolase inhibitory through three types of food products subjected to pancreatic and duodenal digestion: (1) Five Sri Lankan endemic fruits (2) ten

spices which are commonly used in culinary preparations in Sri Lanka as well as throughout the world, and (3) three Kombucha 'tea fungus' fermented beverages obtained through different microbial cultures. Although not entirely confirmed, all these food products have demonstrated to possess significant health benefits, mostly owing to the phenolic compounds present in them and their corresponding antioxidant activities. The starch hydrolase inhibitory activities of some of these food products have not been systematically explored before given the native nature of the plant products. The results enclosed herewith show the importance of measuring the total antioxidant capacity and starch hydrolase inhibitory properties before and after pancreatic and duodenal digestion using an *in vitro* model, where further studies based on the outcomes can be conducted on some of the products.

2. Stability of the antioxidant and starch hydrolase inhibitory activities of five Sri Lankan fruits subjected to pancreatic and duodenal digestion

As a tropical country, Sri Lanka has been gifted with a myriad of fruits some of which are rare and endemic and consumed by locals for generations for their associated therapeutic properties. Given their extensive and historical usage as traditional medicines, it is to be expected that many of these fruits are superfoods, although this aspect has not been systematically evaluated and confirmed to date.

In order to verify these functional properties, the stability of the antioxidant and starch hydrolase inhibitory activities of the following fruits were evaluated when subjected to pancreatic and duodenal digestion: *Elaeocarpus serratus* (ES), *Flacourtia indica* (FInd), *Flacourtia inermis* (FIne), *Pouteria campechiana* (PC), *Solanum nigrum* (SN). The digestion model consisted of exposure of the macerated pulps of these fruits to the enzymatic action of pepsin and pancreatin following the model of Wootton-Beard et al. [2]. The total phenolics content (TPC), Oxygen Radical Absorbance Capacity (ORAC), di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) radical scavenging and α-amylase & α-glucosidase inhibitory activities of the fruit pulps, prior to digestion as well as following pancreatic and duodenal digestion were evaluated. The TPC was determined according to Singleton et al. [3] and expressed in milligrammes gallic acid equivalents per gram (mg GAE/g). The ORAC and DPPH radical scavenging activities were determined according to Prior et al. [3] and Brand-Williams et al. [5] respectively, and expressed in μmol trolox equivalents per gram (μmol TE/g) and EC_{50} (mg/kg), respectively. The starch hydrolase inhibitory activities – both α-amylase and α-glucosidase, were determined according to Liu et al. [6]. The results were expressed for this particular parameter in terms of acarbose equivalents per gram (AE/g), where acarbose is a known starch hydrolase inhibitor prescribed to diabetic patients.

Changes to the antioxidant activities are shown in **Figure 1**, while the starch hydrolase inhibitory activities are shown in **Figure 2**. ES was observed to possess the highest TPC, ORAC and DPPH radical scavenging activities prior to digestion. Following both digestion phases, all antioxidant activities had statistically significantly decreased ($P < 0.05$) in the fruit pulps. A higher correlation was observed between TPC and ORAC ($R^2 = 0.872$), as compared with TPC

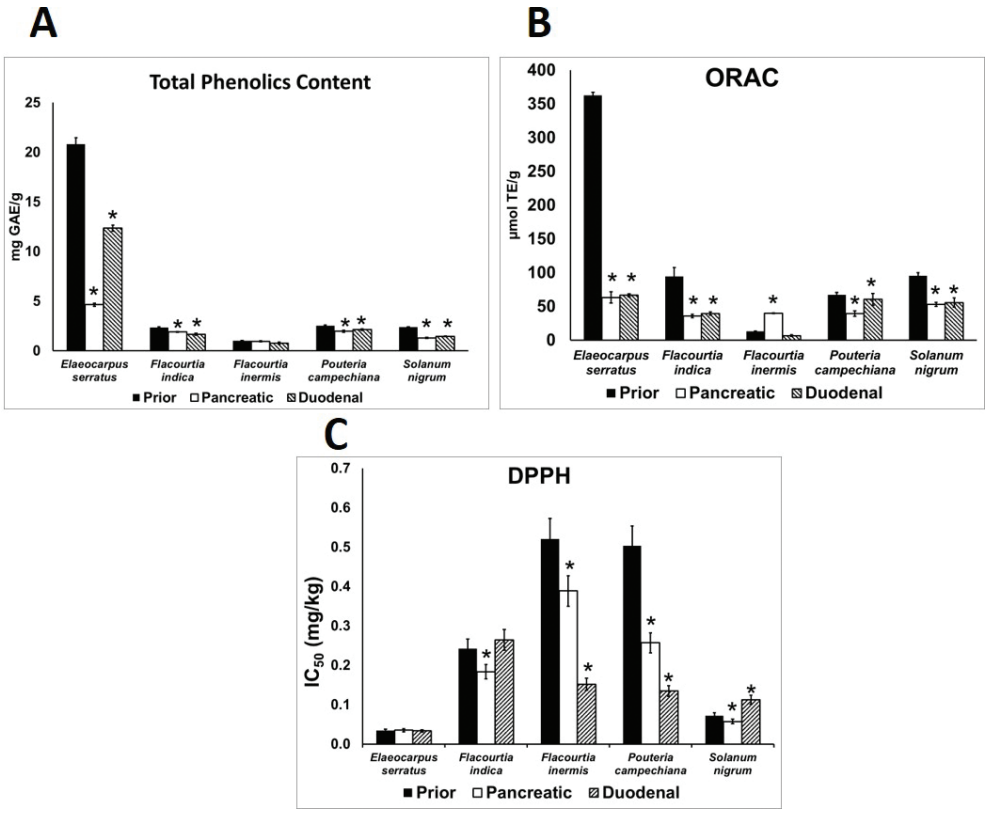

Figure 1. The (A) TPC, (B) ORAC and (C) DPPH radical scavenging activities of the fruits, prior to digestion as well as following exposure to pepsin and pancreatin enzymatic action. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

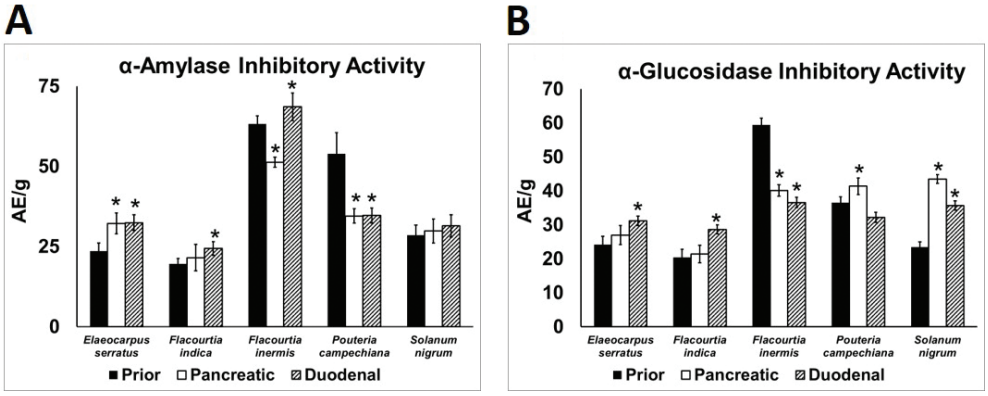

Figure 2. The (A) α-amylase and (B) α-glucosidase inhibitory activities of the fruits, prior to digestion as well as following exposure to pepsin and pancreatin enzymatic action. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

and DPPH radical scavenging activities ($R^2 = 0.341$), indicating that the phenolic compounds of the fruits were better scavengers of hydroxyl radicals which are generated during the assay. FIne was observed to possess the highest starch hydrolase inhibitory activities prior to digestion. Statistically significant ($P < 0.05$) enhancements in this characteristic were observed in all samples following duodenal digestion. There was no observable correlation between the TPC and starch hydrolase inhibitory activities, which implied that this particular characteristic may not have been necessarily extended from the phenolic compounds.

The mechanistic action behind the reduction in TPC, ORAC and DPPH radical scavenging activities would have to be further explored based on these outcomes. In fact, many of the previous researches examining fruit juices in a similar nature have consistently shown large decreases in total antioxidant capacities (TAC) post digestion [7–9]. One study which demonstrates otherwise is that of Wootton-Beard et al. [2]. With reference to the TPC, it was suggested by Ryan et al. [10] that this may have been due to a structural transformation of polyphenols which render them undetectable to be assessed via the assay methodology. However, the reduction in the antioxidant activities strongly suggests that the functional properties of the fruits maybe hampered when subjected to the digestion process.

Overall, the study highlights the necessity of obtaining biologically relevant information on antioxidants by providing data concerning the bioaccessibility and bioavailability as well for that matter, in a human system. The model by Wootton-Beard et al. [2] could essentially be a key methodology in this aspect, in order to obtain preliminary data, prior to embarking on *in vivo* and clinical studies. It must also be mentioned that the antioxidant and starch hydrolase inhibitory activities of the five endemic Sri Lankan fruits are reported herein for the first time. There are many more fruits which are native to the country which have not been studied in similar manner, and given the outcome of this study, it appears to be imperative that they be investigated for their potentials as superfoods.

3. Evaluation of the antioxidant and starch hydrolase inhibitory activity of 10 commonly used household spices subjected to *in vitro* digestion

Spices and herbs are generally used for flavouring and colouring purposes when it comes to culinary preparations. However, they are also known for their medical or antiseptic properties – characteristics which have been owed to the presence of immense amounts of antioxidant compounds. They have been particularly touted as superfoods based on their antioxidant properties. For this particular study, the following spices were selected for assessing the stability of their antioxidant and starch hydrolase inhibitory properties based on their frequent usage in Sri Lanka households as well as throughout the world – any previous studies carried out on their therapeutic potentials of these spices were taken into account during the selection as well: Cardamom (CA – *Elettaria cardamomum*), cloves (CV – *Syzygium aromaticum*), coriander (CO – *Coriandrum sativum*), cumin seeds (CS – *Cuminum cyminum*), curry leaves (CL – *Murraya koenigii*), fenugreek (FG – *Trigonella foenum*), mustard (MU – *Brassica nigra*), nutmeg (NM – *Myristica fragrans*), sweet cumin (SC – *Pimpinella anisum*), and star anise (SA – *Illicium verum*). In order to reduce the content of moisture, powders of these spices were obtained in dried form from the Ayurvedic Medicinal Hall in Kandy, Sri Lanka. The methodology by Wu et al. [11] was followed for the preparation

of the herbal extracts. For the digestion procedure, the same methodology as Wootton-Beard et al. [2] was used. In addition to the TPC, DPPH and starch hydrolase inhibitory activities mentioned in the previous study, quantification of the water-soluble as well as oil-soluble ORAC values was carried out. The water-soluble ORAC ($ORAC_{FL}$) was carried out according to the method by Prior et al. [4], while the lipophilic ORAC ($ORAC_{oil}$) was carried out according to the method by Hay et al. [12]. Both parameters were expressed as μmol TE/g. The rationale for using both these ORAC methodologies for this study was because spices are known to possess many oil-soluble antioxidant pigments – in particular, carotenoids, along with phenolic compounds. Thus, it could be deemed necessary to assess the antioxidant activity extending from these oil-soluble as well as water-soluble antioxidant compounds. Also, the FRAP assay was carried out as described by Benzie and Strain [13], while the $ABTS^+$ radical scavenging activity was evaluated using a methodology previously reported by Ozgen et al. [14]. These two assays were added to this study as compared with the previous one, in order to capture the antioxidant activities extending from the various methods of scavenging radicals. Results from these two particular assays were reported as μmol TE/g. Similar to the previous study, the starch hydrolase inhibitory activities were determined according to Liu et al. [6], where the results were expressed as AE/g.

Changes to the TPC, $ORAC_{FL}$, $ORAC_{oil}$, FRAP, DPPH and $ABTS^+$ radical scavenging activities are shown in **Figure 3**, while the starch hydrolase inhibitory activities are shown in **Figure 4**. When taking the TPC values prior to digestion, CV had the highest amount (22.83 ± 0.20 mg GAE/g) followed by CL (21.94 ± 0.19 mg GAE/g). NM had the lowest TPC (0.80 ± 0.03 mg GAE/g). Following the gastric phase of digestion, all spice extracts had statistically significant increases ($P < 0.05$) in terms of the TPC. This trend was observed in the duodenal phase of digestion as well ($P < 0.05$). As compared with the previous study on the endemic fruits, it could be said that the digestion process released more phenolic compounds, thus increasing their bioaccessibility.

Despite the increases in the TPC in both digestion phases, the $ORAC_{FL}$, ABTS, DPPH and FRAP results did not indicate similar trends, although a few exceptions were observed. Nevertheless, it was heartening to observe that the antioxidant activity values coming from these assays had either been maintained during the digestion phases or statistically significantly increased ($P < 0.05$). This was another difference as compared with the study on the endemic fruits. Similar to the $ORAC_{FL}$ values, the $ORAC_{oil}$ values did not display any statistically significant increases or decreases ($P < 0.05$) as compared with the values prior to the gastric and duodenal digestion phases. The only exception in this instance was CA, where a statistically significant increase ($P < 0.05$) was observed in the duodenal digestion phase. Although the $ABTS^+$, DPPH and FRAP assay values followed an almost similar trend as the $ORAC_{FL}$ values, their correlation with the TPC was comparatively less. A clear correlation between each of the antioxidant assays used to evaluate the TAC was also not observed.

When it comes to the starch hydrolase inhibitory activities, FG had the highest α-amylase and α-glucosidase inhibitory activities, while CS had the lowest for both enzymes. With the exception of CS, none of the spice extracts showed statistically significant changes ($P < 0.05$) to the initial enzyme inhibitory values prior to being exposed to gastric and duodenal digestion. This observation was of significance since the initial starch hydrolase inhibitory activities of the spice extracts were maintained even though they were exposed to the digestive enzymes. The spices were observed to inhibit α-amylase better than α-glucosidase, based on the mean inhibitory values. This characteristic is of significance as well. Inhibition of α-amylase is considered

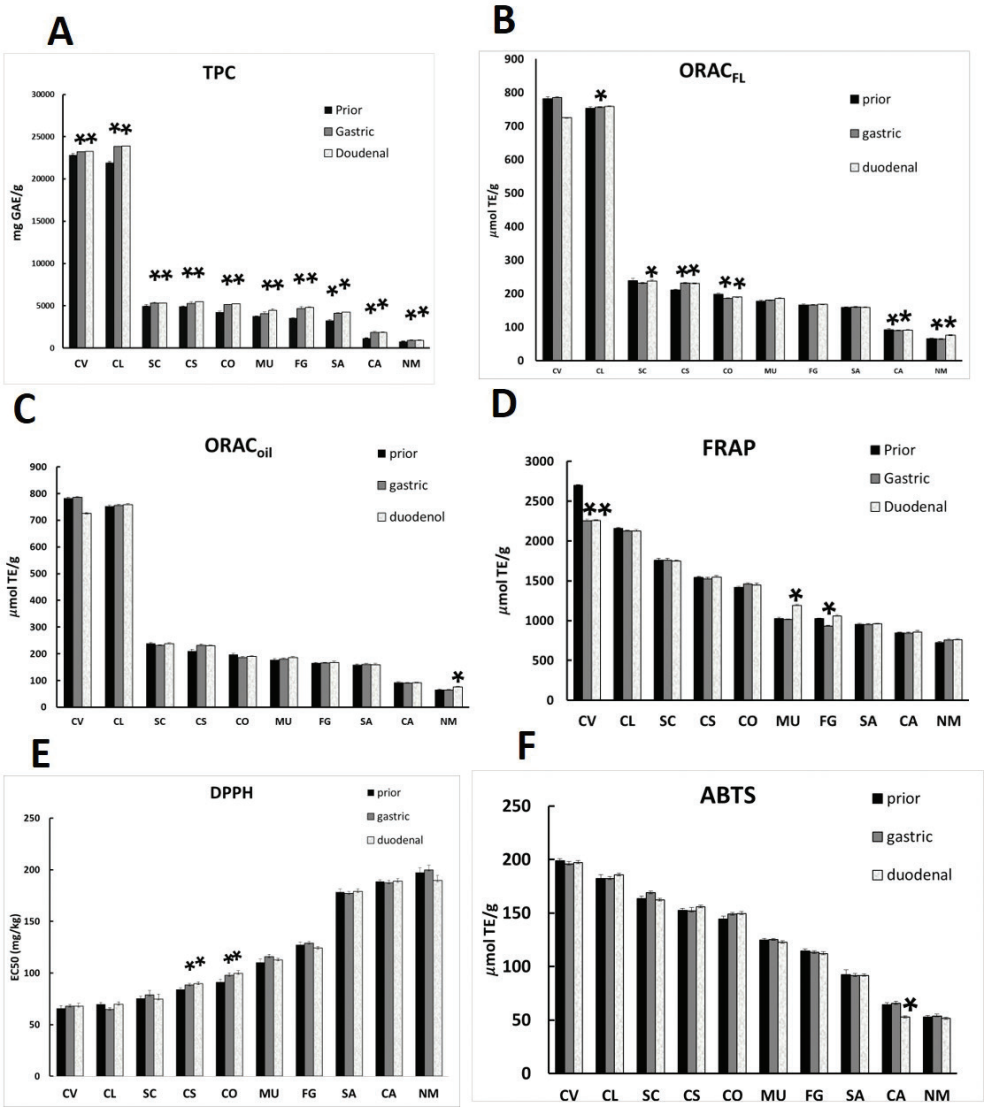

Figure 3. Changes to the (A) TPC, (B) ORAC$_{FL}$, (C) ORAC$_{oil}$, (D) FRAP, (E) DPPH and (F) ABTS^{+} radical scavenging activities of the 10 spices when subjected to pancreatic and duodenal digestion. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

to be more important when it comes to reducing the breakdown of starch, since as explained before, it triggers the production of the substrate for the subsequent action of α-glucosidase. Thus, the spices demonstrated the capability to inhibit the cascade of enzymatic starch breakdown as a whole. Similar to the study on the endemic fruits of Sri Lanka, a clear correlation between the starch hydrolase inhibitory activities and TPC was not observed in this study.

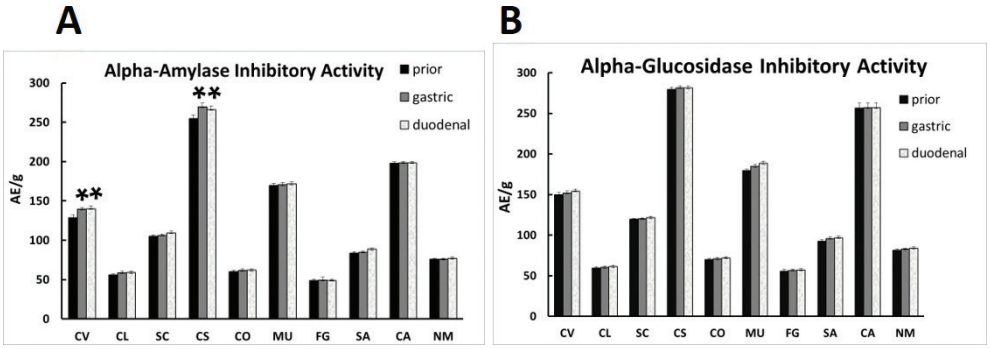

Figure 4. Changes to the (A) α-amylase and (B) α-glucosidase inhibitory activities of the 10 spices when subjected to pancreatic and duodenal digestion. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

Thus, in this instance as well, it may be concluded that the starch hydrolase inhibitory potential may not have been necessarily drawn from the phenolic compounds present in the spices.

Similar to the study on the endemic fruits of Sri Lanka, this investigation was able to provide the first measurement concerning the stability of the antioxidant and starch hydrolase inhibitory potential of these 10 spices when exposed to an *in vitro* model of digestion involving pepsin and pancreatin. Additionally, in contrast to the previous study, it may be preliminarily concluded that the spices investigated in this instance were more resistant to gastric and duodenal digestion, thus maintaining their superfood effects in terms of the antioxidant and starch hydrolase inhibitory properties.

4. Stability of the antioxidant and starch hydrolase inhibitory activities of Kombucha teas prepared from three microbial cultures

Kombucha is a well-known functional fermented tea beverage which has gained immense popularity throughout the world in recent times. This is more so in Western and Mediterranean regions, primarily due to various health benefits associated with its consumption such as anticancer, anti-diabetic, anti-inflammatory, hepatoprotective and detoxification properties as well as its ability to act as a probiotic [15]. The studies focusing on the antioxidant activity of the beverage have also demonstrated the possession of various bioactive ingredients of therapeutic interest, mainly polyphenols and other categories of secondary metabolites which are generated as a result of the fermentation process itself [16]. The bacterial component of the Kombucha culture consists of strains such as *Acetobacter xylinum*, *A. xylinoides*, *A. aceti*, *A. pausterianus,* and *Bacterium gluconicum*, while the dominant yeast strains are *Zygosaccharomyces bailii*, *Schizosaccharomyces pombe*, *Saccharomyces ludwigii*, *S. cerevisiae*, *Kloeckera* spp., *Torulaspora* spp., and *Pichia* species [17]. Given its purported health benefits, it could be easily seen that Kombucha is a potential superfood and thus, its bioaccessibility should be evaluated.

This study evaluated the changes of the antioxidant activity, TPC and the starch hydrolase inhibitory activity in sugared black tea fermented with three Kombucha cultures possessing differed microbial compositions, when subjected to pancreatic and duodenal digestion. The bioaccessible antioxidant capacity and starch hydrolase inhibitory activities were evaluated, similar to the two previous studies on the endemic fruits of Sri Lanka and the spice extracts. Whether sufficient antioxidant and starch hydrolase inhibitory properties were released from the fermented beverage and subsequently released for absorption during the intestinal and duodenal digestion processes was of interest and worth investigating. Study of such phenomenon would be of value, especially to demonstrate the applicability of *in vitro* digestion models for fermented functional beverages, and also to observe whether the functional properties can be sustained during the pancreatic and duodenal digestion phases.

Three Kombucha tea fungal mats were used in this study and their microbial compositions in terms of the dominant species were determined as follows:

- K1: *Acetobacter aceti*, *Zygosaccharomyces bailii*, *Brettanomyces claussenii*
- K2: *A. aceti*, Saccharomyces ludwigii, *Zygosaccharomyces rouxii*
- K3: *A. aceti*, *Lactobacillus* spp., *Leuconostoc* spp., *S. ludwigii*

Three separate portions of sugared black tea were inoculated with 3% (w/v) of each of the cultures above aseptically for 7 days at 24 ± 3°C. This fermentation time has been identified as the ideal duration prior to unwanted metabolites being formed as a result of extended periods of microbial activity. The fermented broth was subjected to the *in vitro* digestion process detailed by Wootton-Beard et al. [2]. The TPC, ORAC and DPPH radical scavenging activities were measured using the same methods mentioned in the two previous studies on the endemic fruits and spice extracts. However, for this study, the superoxide radical scavenging activity was used. This was because previous studies had used this assay for this particular beverage with noteworthy outcomes, indicating that it has a superior ability to scavenge superoxide radicals [15, 16]. The value was expressed in terms of percentage inhibition (%). The starch hydrolase inhibitory activities were also evaluated in this study using the same methodology described by Hay et al. [12]. However, given that the samples in this study contained ongoing microbial activities, the α-amylase and α-glucosidase inhibitory activities were expressed as IC_{50} (μg/mL) instead of AE/g.

Changes to the TPC, ORAC, DPPH and superoxide radical scavenging activities are shown in **Figure 5**, while the starch hydrolase inhibitory activities are shown in **Figure 6**. Statistical comparisons were done with the fermented beverage at day 7, prior to being subjected to the *in vitro* digestion process. When the three fermented teas were subjected to the *in vitro* digestion phases, statistically significant increases ($P < 0.05$) were observed in all after intestinal digestion. In addition, statistically significant increases ($P < 0.05$) were observed in the TPC following the duodenal digestion phase as compared with the undigested sample at day 7 of the fermentation as well. As for the antioxidant values, ORAC value for all teas prior to digestion was within a range of 2460–2640 μmol TE/mL. K3 had the highest ORAC value among all three fermented samples by the end of the fermentation process. In comparing the values during the digestion processes, statistically

significant increases ($P < 0.05$) were observed following both phases in all three beverages. Similar to the study on the spices, an increase in the ORAC value represents the sustenance of the antioxidant potential when exposed to pancreatic and duodenal digestion and this could be of therapeutic importance. The increase in the ORAC value of K3 was less than K1 and K2 in the duodenal digestion phases as compared with the undigested counterparts. Nevertheless, the final ORAC values of all three teas were within a comparable range at the end of the two digestion phases.

The DPPH EC_{50} values of the three fermented Kombucha teas remained within the range of 56–59 mg/kg prior to the digestion phases. In contrast to the ORAC assay values, there were no statistically significant changes ($P < 0.05$) to the DPPH EC_{50} values and the superoxide scavenging activities when subjected to the digestion process. This was noteworthy, considering the instance of the study on endemic fruits. It was apparent that the fermented beverages had retained its antioxidant potential in all three beverages, despite the enzymatic activities of pepsin and pancreatin. A better correlation between the TPC and the ORAC values were observed in comparison to the correlation between DPPH EC_{50} and superoxide scavenging values. This

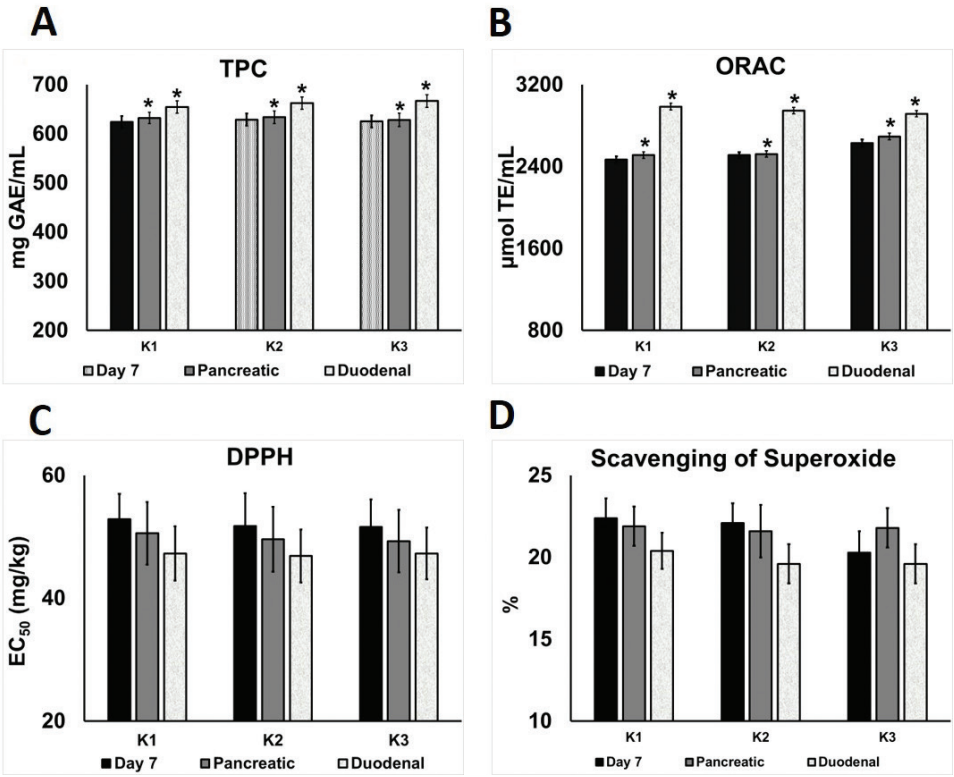

Figure 5. Changes to the (A) TPC, (B) ORAC, (C) DPPH scavenging activity and (D) superoxide scavenging activity of the three fermented beverages. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

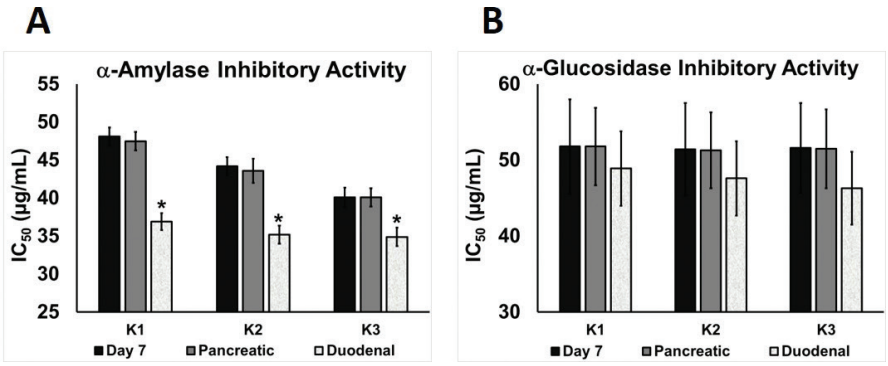

Figure 6. Changes to the (A) α-amylase and (B) α-glucosidase inhibitory activities of the three fermented Kombucha samples when subjected to pancreatic and duodenal digestion. $P < 0.05$ denotes statistically significant difference as compared with the respective values prior to *in vitro* digestion. Values represent mean ± SEM of 3 ≤ independent experiments.

maybe because the phenolic compounds present in all three types of tea samples possess a better scavenging activity of peroxide radicals which are generated during the ORAC assay.

There were notable observations in terms of the starch hydrolase inhibitory activities. When considering the results obtained for the three Kombucha strains used in this study, it was observed that the α-glucosidase inhibitory activity of K3 after duodenal digestion was higher than the other two beverages. Additionally, when the three beverages were subjected to pancreatic digestion, the α-amylase inhibitory activity remains more so the same compared with the undigested counterpart for all three teas. However, after duodenal digestion the α-amylase inhibitory activity displayed statistically significant increases ($P < 0.05$) compared with the control sample (i.e. undigested beverage at 7 days of fermentation). Statistically significant changes ($P < 0.05$) in the α-glucosidase inhibitory activity were not observed in any of the teas. This was of importance, since the starch hydrolase inhibitory activities demonstrated stability against the enzymatic activity of pepsin and pancreatin.

In conclusion, the tea fermented with the K3 pellicle was discovered to be the better Kombucha beverage in terms of having the highest antioxidant and starch hydrolase inhibitory activities following fermentation, as well as its resistance to enzymatic activity of pepsin and pancreatin. Since the K3 Kombucha sample contains *Lactobacillus* spp., this beverage can be used as a potentially good probiotic supplement and could also be considered as a prospective superfood being superior in terms of antioxidant potential and probiotic effects.

5. Conclusions

In conclusion, this chapter details the application of an *in vitro* digestion system to a variety of food samples – mostly of plant origin, and measurement parameters. It is obvious from the outcomes that despite superior antioxidant and starch hydrolase activities

being displayed in the samples, whether they can be sustained when subjected to digestive enzymes needs proper examination. In terms of the results included in this chapter, it needs to be highlighted that the antioxidant and starch hydrolase inhibitory activities mentioned in some of the food products are reported herein for the first time. Thus, it highlights the necessity to explore for superfoods in countries such as Sri Lanka which have a plethora of natural resources with bioactives of value, which can even be developed into nutraceuticals.

The efficacy of the plant material mentioned in this chapter needs to be commented on as well. Some of the items showed superior antioxidant and starch hydrolase inhibitory activities, for instance, *Syzygium aromaticum* and *Murraya koenigii*. These spices have been traditionally used for many medicinal purposes such as diabetes. The antioxidant activities displayed by these spices only affirmed their potential in combating hyperglycemia-induced oxidative stress. Nevertheless, the actual efficacy of these plant material and the fermented beverages could only be confirmed if they were subjected to clinical evaluations.

Several studies have utilised *in vitro* digestion methods to analyse structural changes, bioavailability, bioaccessibility and digestibility of foods, indicating that *in vitro* digestion systems are common useful tools for analyses of the efficacy of foods and drugs. In terms of bioaccessibility, there is clearly an urgent need for more research into *in vitro–in vivo* correlations with well-defined systems, so that more realistic *in vitro* models can be developed for screening purposes. Finally, between bioavailability and bioaccessibility, bioavailability has been given more attention; however, from the studies highlighted in this chapter, it is apparent that bioaccessibility deserves equal attention, since it is a precursor to bioavailability, and also an important aspect which determines whether a food product is indeed a superfood.

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