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Correlation of Cooking duration and Phytonutrient release in Vegetables

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Abstract

The culinary arts across continents have provided a whole range of variant practices that now concentrate to contain the essential components of the nutriment used. Among the disparate healthy cooking practices, boiling is considered as a quick and effortless way for both preserving and extracting the water soluble vitamins. In this study, an attempt was carried out to correlate the extent of phytochemical release and antioxidant activity in raw and cooked food items. Common vegetables that form any part of appetizer, entrée or main course of food were targeted for this swot. Extracts obtained after 20 min of cooking showed increased phytochemical contents with corresponding free radical scavenging activity. Based on the targeted food samples an ideal cooking duration for each is recommended in accordance to their scavenging property.

Keywords: Polyphenols, Flavonoids, Antioxidant activity, *Lactuca sativa*, *Solanumlycopersicum*, *Brassica oleracea*.

Introduction

A side track of modernization and advancements is an overgrowing need to satiate hunger contemplating every nutritious nuance of the given food. The very realization that "the groundwork of all happiness is good health" has led to the egregiousness of culinary arts. Apart from being the basic necessity, food and healthy food habits plays a significant role in curbing disease that range from cardiovascular diseases to cancer[1–3]. The "good effects" of this subsistence are majorly contributed by fruits and vegetables which are an excellent source of vitamins, minerals, nutrients and Phytochemicals[4,5]. The cooking methods are majorly categorized into water media based (steaming, boiling, poaching, stewing, braising);fat media based (roasting, frying); heat media based (grilling/ boiling, baking) and smoke media based (braising, roasting, grilling and smoking) of which the most preferred mode used for vegetables is boiling[6]. Phytonutrients or phytochemicals are mostly water soluble [7]and tend to leach out when vegetables are submerged in water for prolonged period[8,9]; their thermo labile nature also lead to the loss duringexposure toheat[10–12]. Apart from these detriments, bactericidal activity enabling food consumption without harmful microbes, improved digestibility and increased availability of nutrients are beneficial factors credited to cooking[13,14]. Conversely, cooking vegetables increase the availability of antioxidants by enabling their release from trapped fibrous part of the vegetables[15].

The present work aims at analyzing the effect of methods and cooking duration on the release of phytonutrients and antioxidants from 8 different vegetables belonging to *Lactuca*, *Brassica* and *Solanum*genus. The *modus operandii* was to compare pressure cooked approach and boiling approach of cooking against the raw usage of the same. The results of the phytonutrient release were used to decide an ideal and optimum approach for consuming food.

Materials and Methods

Plant material: Fresh high quality packaged samples of Lettuce Leaf (*Lactuca sativa* L. *crispa*), Lettuce Iceberg (*Lactuca sativa* L. *capitata*), Tomato (*Solanum lycopersicum*), Cherry Tomato (*Solanum lycopersicum* var. *cerasiforme*), Cabbage (*Brassica oleracea* var. *capitata* L. *alba*), Red Cabbage (*Brassica oleraceavar. capitata L. rubra*), Cauliflower (*Brassica oleracea* var. *botrytis*) and Broccoli (*Brassica oleracea* var. *italica*) were purchased from Godrej Nature's Basket Gourmet retail store, Thane, Maharashtra, India in the month of March, 2012. These set of vegetables were selected owing to their availability throughout the year, usage in multi-cuisines, widespread consumption in raw as well as cooked forms. Another important criterion that was decisive in targeting these vegetable were their antioxidant potential[16–25].

Preparation of extracts: Based on the framed methodology (Figure-1), two major types of extracts were used for this experiment; one being the raw extract and the other being the cooked extracts. Each of the collected vegetables were properly washed to remove dirt particles and dried for few minutes to remove traces of water. For raw extracts, 20 gm of edible portion of each vegetable was evenly chopped and dispensed into 200 mL of distilled water; after 6 hrs of soaking, the vegetable were crushed using a mortar and pestle, squeezed and filtered with muslin cloth (extract A). Three decoction were considered for cooked extract of which the first two were boiled and the last being pressure cooked. 20gm of edible portion of each vegetable was boiled in 200 mL of distilled water in an open vessel for 10mins (extract B) and 20mins (extract C);

whereas for the latter, the same quantity of vegetable was pressure cooker at high flame for 10 mins (extract D) in 200 mL distilled water. After cooling, the extracts were filtered using muslin cloth; the final concentration of the sample was made upto 200 mL with distilled water for further assay.

Estimation of Water Soluble Polyphenolic and Flavanoid Contents: Folin – Ciocalteau Method was employed for evaluating the polyphenol content[26] and aluminum chloride method was applied for flavanoid content[27]. For polyphenolic assay, to each of 20µL of 1% aqueous extracts (A, B, C and D), 1.58 mL of distilled water was dispensed followed by addition of 100µL Folin-Ciocalteau reagent. After thorough mixing, the solution was left undisturbed for few minutes; 300µL sodium carbonate solution was added and left for incubation at room temperature for 2 hrs. Sample absorbance was measured at 765 nm with gallic acid was used as a standard to obtain standard graph with the concentration range of 200-1000µg/mL. The results obtained were expressed in µg/mL of Gallic Acid Equivalent (GAE).

In the flavanoid estimation assay, 0.5 mL each of the 1% aqueous extracts (A, B, C and D) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubating at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Quercetin in 80% ethanol was used as the standard to obtain a standard graph with the concentration range of 20-100µg/ml and the results were expressed in µg/ml of Quercet in Equivalent (QE).

Antioxidant assay: 1,1-Diphenyl 2-picrylhydrazyl-Radical Scavenging Activity (DPPH-RSA) method[28] was followed to estimate the radical scavenging activity of extracts (A, B, C and D) and the results were compared against the RSA activity of standard L-Ascorbic Acid (Vitamin C), a control sample. DPPH being free radical shows a diminution of its optimum absorption at 517nm by getting reduced with an antioxidant compound. 3mL of DPPH solution was added to 1mL of each extracts respectively and onto the standard solution of Vit C (10-100 µg/ml).Absorbance was taken after a time span 30 min at 517 nm; percentage inhibition activity was calculated using the following formula:

$$\frac{Ao - A1}{Ao} * 100$$

where: *Ao* is the absorbance of the control and *A1* is the absorbance of extract/standard taken as Ascorbic acid. The results were expressed in percentage inhibition.

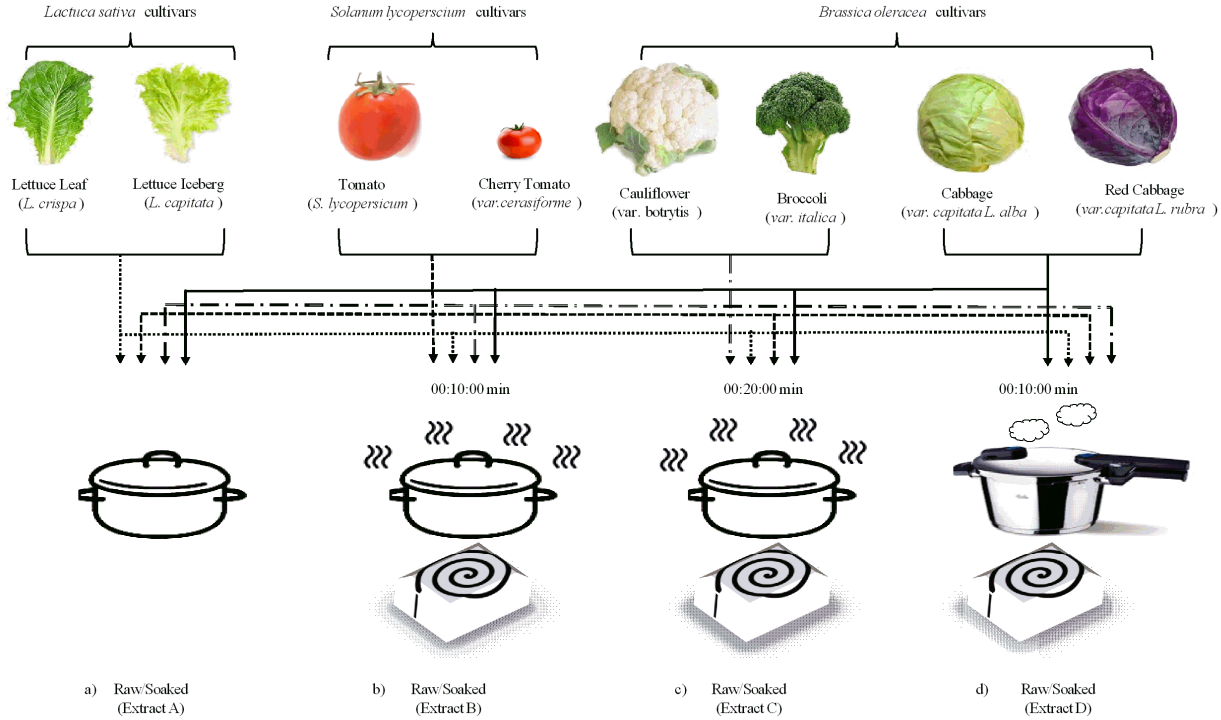

Figure-1
Cooking Trials a) Extract A: Raw; b) Extract B: 10 mins open vessel boiling; c) Extract C: 20 mins open vessel boiling; d) Extract D: Pressure Cooking for 10 mins

Statistical Analysis: One-way ANOVA method followed by Dunnett's multiple comparison test using Graph Pad Prism software (Version 6.03) was employed for statistical analysis; results were denoted as mean±SEM. Values of $p \leq 0.05$ were considered to be statistically significant.

Results and Discussion

Polyphenols found ubiquitously in fruits and vegetables[29] have various health benefits providing anti-ageing, anti-inflammatory, anti-microbial and anti-cancer properties[30–33]. Among these, the most common being the flavonoids (especially catechins) are relatively less toxic in comparison to other active plant compounds (for instance alkaloids)[34]. This is suggestive of former being suitable for consumption among both animals and humans. Studies implicate flavonoids as biological "response modifiers[35–41] with anti-allergic[42], anti-inflammatory[43] and antimicrobial capabilities[44–46]. Many of these phytochemicals also possess the antioxidant properties or "scavenger" effect that circumvents the negative impact of free radicals[47–50].

Lactuca sativa L. *crispa* (lettuce leaf) displayed significant release of polyphenols and flavonoids after 20 min of boiling; pressure cooked approach showed one third of this (Figure-2A and 2B). *Lactuca sativa* L. *capitata* (lettuce iceberg) showed similar amount of polyphenol release for both boiling and pressure cooked extracts whereas flavonoids were seen more in pressure cooked approach. The raw extracts of the same in comparison to *L. crispa* showed slightly more release of both the phytochemicals. These results could be complemented with highest antioxidant activity in extract C for both the vegetables. An unswerving correlation between the phytochemical release and antioxidant activity could not be deduced due to the values obtained (Figure-2C).

In *Solanum* cultivars, *S. lycopersicum* (tomato) showed maximum release of polyphenols after 10 min of boiling (Figure-3A) and that of flavonoids (Figure-3B) in pressure cooked method. For *S. lycopersicum* var. *cerasiforme* (cherry tomato), both polyphenol and flavanoid compounds were observed to be maximum at pressure cooked approach with 20 min boiling extracts succeeding it. The upper limit of antioxidant activities for both the cultivars were seen with extract B followed by extracts C and D (Figure-3C).

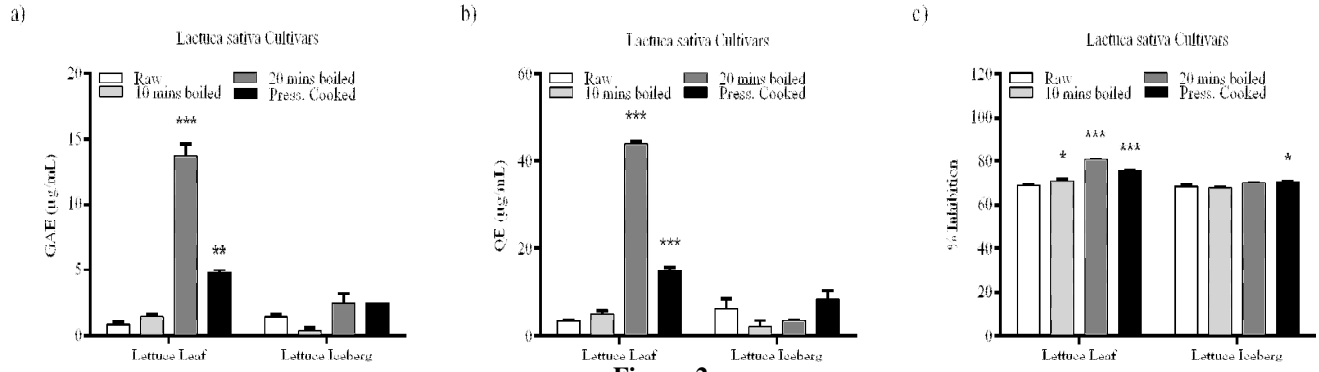

Figure-2

***Lactuca sativa* cultivars' phytonutrient release and antioxidant activity. a) Water Soluble Polyphenol Release; b) Water Soluble Flavonoid Release; c) Antioxidant Potential. Each bar represents Mean ± SEM. *P≤0.05, **P<0.01 and ***P<0.001; was considered significantly different compared to the raw group**

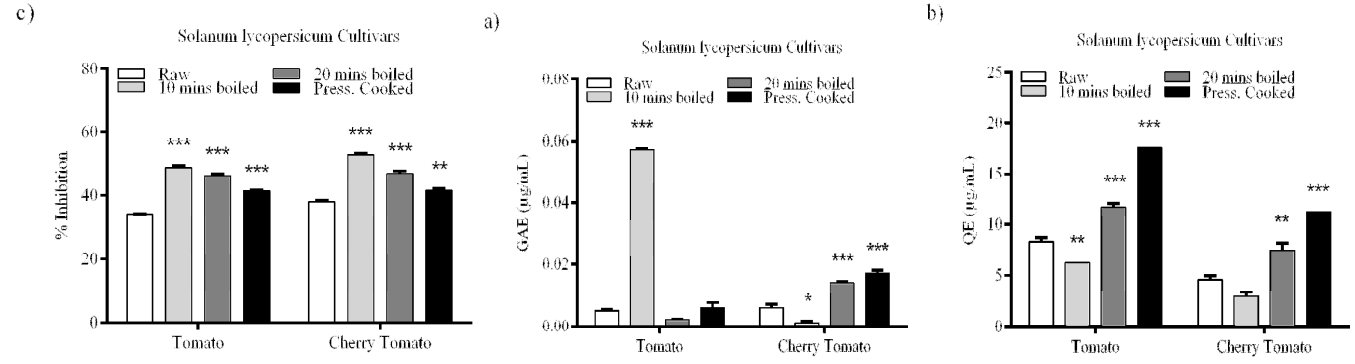

Figure-3

***Solanum lycopersicum* cultivars' phytonutrient release and antioxidant activity.a) Water Soluble Polyphenol Release; b) Water Soluble Flavonoid Release; c) Antioxidant Potential. Each bar represents Mean±SEM. *P≤0.05, **P<0.01 and ***P<0.001; was considered significantly different compared to the raw group**

In *B. oleracea*var. *capitata* cultivars, both *L. alba* (cabbage) and *L. rubra* (red cabbage) gave maximum polyphenol release in extracts C and that of flavonoids in extracts D (Figure-4A and B). Releases of flavonoids were accounted more than polyphenols in both the cases; pressure cooked approach showed twice the flavonoids discharge than in 20 min of boiling. Red cabbage exhibited an increased release of polyphenols and flavonoids from raw to boiling (Figure-4B); whereas in cabbage the release of flavonoids were found to be more in 10 min of cooking than 20 min. All the cooking processes showed similar antioxidant values with slight increase in the extract D of red cabbage. Among the *Brassica oleracea* varieties, var. *botrytis* gave maximum release of polyphenols and flavonoids in extracts A (Figure-5A) with var. *italica* giving the similar results in extracts D (Figure-5B). Antioxidant values were similar in both the varieties showing 75% activity (Figure-5C).

Conclusion

From the above results, considerable variation (Table-1) in the release of polyphenols and flavonoids could be correlated to the cooking duration and nature and initial content of the phytochemicals[16–25]. Most of vegetables under study showed release of phytochemicals on cooking with exception to cauliflower. In this study flavonoids were shown to be less thermo liable than polyphenols with the former exhibiting higher values even after cooking. Common varieties of the selected vegetables (lettuce leaf, tomato, cabbage and cauliflower) showed a direct correlation between increase in antioxidant potential and cooking duration in contrast to their exotic counterparts (lettuce iceberg, cherry tomato, red cabbage and broccoli). Increased antioxidant activities in *Lactuca* and *Brassica* cultivars were linked to flavanoids whereas for *solanum* the same was linked to polyphenolic content. In case of the exotic varieties, phytonutrients like sulforaphane in broccoli[51], high levels of lycopene in cherry tomato[52]and anthocyanin like isomers of cyanidin 3-O-(acyl) diglucoside-5-O-glucoside, cyanidin 3-O-(acyl1) (acyl2) diglucoside-5-O-glucoside, and cyanidin 3-O-(acyl1) (acyl2) diglucoside-5-O-(malonyl) glucosidein red cabbage[23,53] can be attributed to antioxidant potential of these vegetables apart from the targeted phytochemicals.

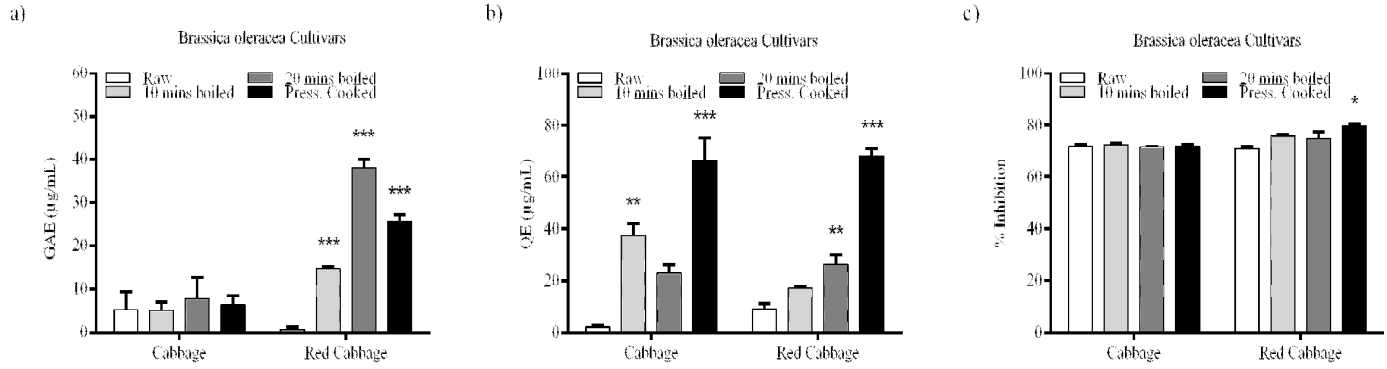

Figure-4

***Brassica oleracea* cultivars' phytonutrient release and antioxidant activity.a) Water Soluble Polyphenol Release; b) Water Soluble Flavonoid Release; c) Antioxidant Potential. Each bar represents Mean±SEM. *P≤0.05, **P<0.01 and ***P<0.001; was considered significantly different compared to the raw group**

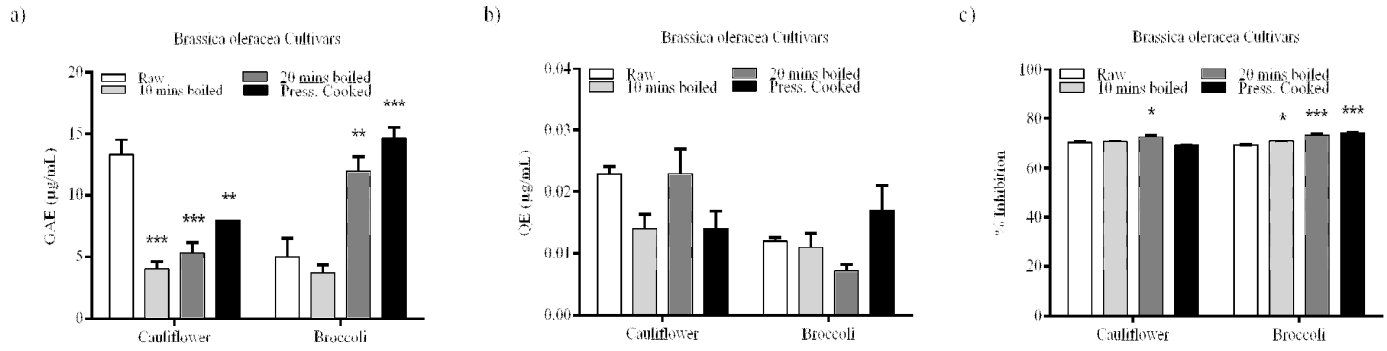

Figure-5

Brassica oleracea cultivars' phytonutrient release and antioxidant activity. a) Water Soluble Polyphenol Release; b) Water Soluble Flavonoid Release; c) Antioxidant Potential. Each bar represents Mean±SEM. *P≤0.05, **P<0.01 and ***P<0.001; was considered significantly different compared to the raw group

Table-1
Polyphenol, Flavanoid release and antioxidant values across the extracts of the selected vegetables

Vegetables and its Cultivars Phyto-chemicals	*Lactuca sativa*		*Solanum lycopersicum*		*Brassica oleracea*			
	L. crispa	*L. capitata*	-	*cereas-iforme*	*L. alba*	*L. rubra*	*botrytis*	*italica*
	Lettuce Leaf	Lettuce Iceberg	Tomato	Cherry Tomato	Cabbage	Red Cabbage	Cauliflower	Broccoli
Polyphenols	C (~14µg/ml)	C/D (>5µg/ml)	B (0.06µg/ml)	D>C (~0.02µg/ml)	C (>10µg/ml)	C (~45µg/ml)	A (~14µg/ml)	D (~15µg/ml)
Flavanoids	C (~45µg/ml)	D (>5µg/ml)	D (~20µg/ml)	D (~15µg/ml)	D (>70µg/ml)	D (>70µg/ml)	A/C (>0.03µg/ml)	D (~0.02µg/ml)
Antioxidant activity	C (80%) >D>B>A (60-70%)	Same (~70%)	B(50%) C>D (40-50%)	B (55%) C>D (40-50%)	Same (~75%)	D (~80%) Rest (~75%)	C/B/A>D (~75%)	D/C>B/A (~75%)

Extract A (raw), Extract B (10 min boiling), C (20 min boiling), D (pressure cooked for 10 min). Polyphenol concentration is expressed in Gallic Acid Equivalent (GAE) and flavanoids are expressed in µg/ml of Quercetin Equivalent (QE).

Boiling of *Solanum* cultivars for 10 min and *Brassica* and *Lactuca* vegetables for 20 min to is recommended as a healthier cooking practice to avail maximum benefit of their antioxidant potential.

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