Orange Heirloom Tomato: anti-cancer properties

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INTRODUCTION

Solanum lycopersicum (tomato) originated from the Andean region of South America. It was later domesticated in Mexico and introduced to Europe in the 16th century, where it was used only for decoration as it was believed to be toxic. In Italy these newly arrived exotic beauties were referred to as *pomodoro*, meaning "Golden Apple." This leads us to believe that the early tomatoes were yellow/orange, referred to nowadays as tangerine.

Carotenoids are produced by plants and the type of carotenoid that is dominant determines the colour of the fruit. One such carotenoid, namely lycopene, exists in various isoforms. Tetra-cis lycopene (TCL) gives tomatoes a yellow to orange colour, whilst tomatoes containing all-trans lycopene (ATL) are always red. The carotenoid ß-carotene (ßC) is found in some tomato varieties, and imparts a yellow-orange colour.

Due to the increasing incidence of non-communicable diseases, of which cancers form one group, there are efforts to provide clear information to the layman with respect to risk factors, and how one might improve diet to negate risk. In this context the Heritage Food Crops Research Trust (HFCRT) have endeavoured to make food crops, high in bioactives, more readily available to their local community, and internationally. The HFCRT have a number of heirloom tomato varieties that are high in TCL and ßC, and we wished to investigate these varieties in the context of the hallmarks and enabling characteristics of cancer.

Investigations into the health properties of ATL and extracts from red tomatoes have been documented, particularly in the context of prostate cancer [1, 2], but very few investigations have been carried out on TCL. Based on work carried out by Plant and Food Research Ltd, TCL is more bioavailable than ATL in humans [3]. For this reason, tangerine tomatoes rich in TCL are of particular interest.

AIM

The aim of this study was to investigate the *in vitro* biological activities of tangerine tomato extracts rich in TCL and compare them to that of common red tomato extracts rich in ATL and high βC tomato extracts rich in βC.

Specific objectives:

- To determine the carotenoid compositions of extracts from four tomato varieties
- To determine the *in vitro* antioxidant capacity of the tomato extracts
- To determine the *in vitro* anti-proliferation activity of the tomato extracts in prostate cancer cells
- To determine the *in vitro* anti-inflammation activity of the tomato extracts

METHODS

- Four varieties
  - Olga's Round Golden Chicken Egg Tomato
  - Golden Green
  - Golden Eye
  - Alfred (control)
- Extraction
  - Tomatoes were freeze dried
  - The following solvents were used for extraction:
    - Hexane
    - Hexane:Ethanol (3:4)
    - Hexane:Acetone (1:1)
    - Hexane:Acetone:Ethanol (2:1:1)
  - Concentrated using nitrogen gas in the dark
- UltiMate™ 3000 HPLC to determine carotenoid composition.
- Antioxidant activity was assessed using the FRAP and ABST assays on all four varieties and extracts.
- Anti-proliferative assays using all four varieties and extracts were tested on prostate cancer cell lines, namely LNCaP, DU145 and PC3.
- Anti-inflammatory assay:
  - Cell lines were dosed for 24hrs with selected extracts.
  - Stimulated with appropriate ligands for 24 hrs: HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells - PAM3CSK4 and LPS respectively, NOD2-WT and NOD2-G908R (Single Nucleotide Polymorphism (SNP)) cells were stimulated with MDP.
  - Quanti-blue was used to measure SEAP quantities.
  - The higher the SEAP concentration, the lower the anti-inflammatory activity.
  - Extract concentration was optimised based on extract cytotoxicity/cell viability.

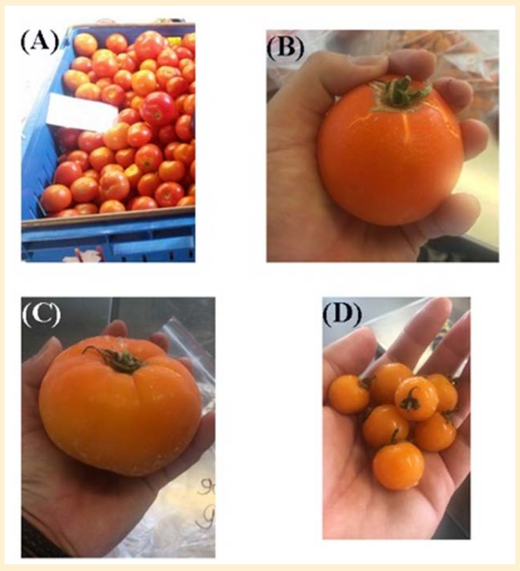


Figure 1. Physical appearances of the tomato varieties used in this study. A: Alfred; B: Olga's Round Golden Chicken Egg; C: Golden Green; D: Golden Eye. A: Alfred; B: Olga's Round Golden Chicken Egg; C: Golden Green; D: Golden Eye.

RESULTS

- Extracts were obtained from four varieties of tomato, and four solvent combinations (16 extracts).
- Although the FRAP and ABST assays gave different results, the trends are clear (Figure 2).
- The hexane extracts gave significantly higher antioxidant capacity than the other extracts.
- Carotenoid content: GE>Alfred>Olga>GG; βC content: GE>Alfred; Lutein: Alfred>GE>Olga>GG; TCL: Olga>GG; ATL: Alfred
- The hexane extract from Olga's was found to have the greatest anti-proliferative activity in the LNCaP cell line, versus DU145 and PC3 (Table 1).
- Based on the anti-proliferative activity, extracts were selected for testing in anti-inflammatory assays, specifically used to determine activity in the TLR2, TLR4 and NOD pathways. The results from the TLR2, TLR4 and NOD2 anti-inflammatory assays showed that the HA extracts of GG, Olga and Alfred had the greatest anti-inflammatory action on the TLR2 and 4 pathways. Although many extracts appeared to have anti-inflammatory activity (Table 2), the positive control (ibuprofen) was a great deal more anti-inflammatory than any of the extracts. Olga Hexane and HA, GG HA and Alfred Hexane and HA extracts had the greatest impact on the NOD2 pathway. As seen in Figure 3, Olga's hexane extract had an effect on SEAP production in the NOD2 cell lines.

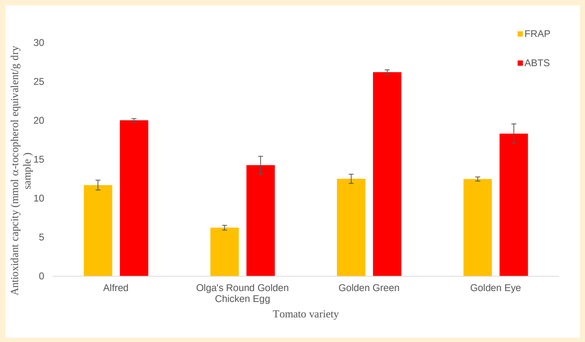
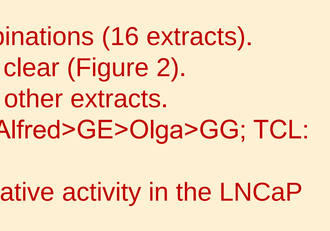


Figure 2. A comparison of the antioxidant capacity of hexane extracts of different tomato varieties based on FRAP and ABTS assays

Tomato Variety	Extraction Solvent			
	Hexane	Hexane-Acetone-Ethanol	Hexane-Acetone	Hexane-Ethanol
LNCaP				
Alfred	14.46	X	11.76†‡	X
Olga's Round Golden Chicken Egg	**5.62†‡**	16.93	10.1	X
Golden Green	8.08†‡	15.45	10.19	X
Golden Eye	+	+	+	+
DU145				
Alfred	X	X	-†‡	X
Olga's Round Golden Chicken Egg	**11.73†‡**	X	-	X
Golden Green	17.84†‡	X	18.01	X
Golden Eye	+	+	+	+
PC3				
Alfred	X	X	-†‡	X
Olga's Round Golden Chicken Egg	**15.64†‡**	X	-	X
Golden Green	-†	X	X	X
Golden Eye	+‡	+	+	+

Table 1. IC50 of prostate cancer cells treated with different tomato extracts (dry weight mg/ml)



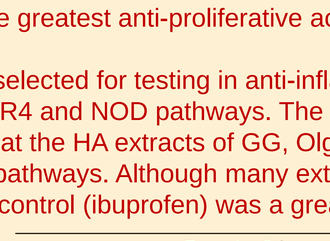


Figure 3. SEAP concentration of NOD2-WT (left) and NOD2-G908R (right) cell lines treated with Olga's Round Golden Chicken Egg Hexane extracts. Note: red circles represent values obtained from each biological replicate; ** and * indicate significant difference p< 0.01 and p< 0.001 respectively.**

	Dry Weight Equivalent of Tomato Extract (mg/mL)						
	Alfred Hexane	Alfred HA	Olga Hexane	Olga HA	GG Hexane	GG HA	Ibuprofen (mg/mL)
HEK-Blue™ hTLR2							
IC30	60.24	59.35	**53.69**	39.96	**50.01**	32.83	0.27
IC50	88.46	74.73	**66.22**	55.62	**61.16**	50.41	0.40
IC70	N/A	90.10	**78.75**	71.28	**72.30**	67.99	N/A
HEK-Blue™ hTLR4							
IC30	**84.56**	64.47	**52.20**	51.51	**56.69**	42.76	0.15
IC50	N/A	76.01	**61.44**	62.53	**62.89**	53.08	0.27
IC70	N/A	87.55	**70.68**	73.55	**69.09**	63.40	0.38

Table 2. Inhibition of SEAP expression by tomato extracts and ibuprofen on the HEK-Blue™ cells

CONCLUSIONS

- Although the FRAP and ABST assays gave different results, the trend is consistent. Hexane extracts had a significantly higher antioxidant capacity than other extracts with Golden Green having the highest antioxidant capacity of the varieties tested, followed by Alfred, Golden Eye and Olga's. The antioxidant capacity of TCL vs ATL is therefore unclear.
- Olga's hexane extract was the most anti-proliferative extract among all extracts obtained from the four varieties, followed by the GG hexane, and Alfred HA. These findings can be interpreted to mean that the TCL-containing extracts (Olga hexane and GG hexane) have superior anti-proliferative effects on PCa cells compared to the ATL-containing extracts (Alfred HA). The extracts were shown to be most anti-proliferative to LNCaP, and are least anti-proliferative to PC3.
- By combining the results from both the WT-NOD2 and the NOD2 G908R SNP related inflammatory pathways, Olga hexane, Olga HA, and GG HA were found to be the better extracts in combating *NOD2*-related inflammation *in vitro.*
- It therefore seems likely that TCL has a greater anti-proliferative and anti-inflammatory effect than ATL, although it is a little premature to draw definitive conclusions.

FUTURE WORK

- Accurately quantification the carotenoids found in the tomato extracts and assess compound stability.
- With respect to the anti-inflammatory assay, there may be an overlap with regards to the wavelength used for measurement of WST-1 and that of the carotenoids in question. This is worth checking.
- Further investigates into the TLR2 and NOD2 pathways should be carried out.
- Observe the effect of extracts on stimulated cells…..

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