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ORIGINAL RESEARCH PAPER

LYCOPENE AND β-CAROTENE RECOVERY FROM FERMENTED TOMATO WASTE AND THEIR ANTIOXIDANT ACTIVITY

JOHN OWUSU[1, 3], *HAILE MA[1, 2], NEWLOVE AKOWUAH AFOAKWAH[1, 4], AGNES AMISSAH[3], JOSEPH AHIMA[3]

[1]School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China.
[2]Key Laboratory for Physical Processing of Agricultural Products, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, China.
[3]School of Applied Science and Technology, Koforidua Polytechnic, Ghana.
[4]School of Applied Science and Arts, Bolgatanga Polytechnic, Bolagatanga, Ghana.
*Corresponding author information:
Name: Haile Ma, Tel: +86-511-88790958, Fax: +86 511 88 78 0201, Email: mhl@ujs.edu.cn

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Waste generated from tomato processing poses disposal challenge even though it is a potential source of bioactive compounds. Acetone/ethanol/hexane mixture was used to recover lycopene and β-carotene from tomato pomace obtained from tomato must fermentation (pH of 4.11, 3.40, 3.20; temperature of 15, 20°C). Tomato must pH and temperature influenced ($P<0.05$) both lycopene and β-carotene recovery from the pomace. The highest total antioxidant activity and reducing power values were obtained from the tomato pomace of must pH 3.20, and fermented at 20°C. Tomato pomace from winemaking is a rich source of the bioactive compounds, lycopene and β-carotene, and possess substantial levels of antioxidant activity.
Keywords: fermented tomato waste, fermentation temperature, bioactive compounds, lycopene, β-carotene

Introduction

Bioactive compounds have recently been a major subject of research. The interest in bioactive compounds probably stems from their ability to scavenge free radicals, to act as antibacterial and antiviral agents, immune system stimulators, and cell proliferation and apoptosis regulators (Kris-Etherton *et al.*, 2002). Fruits and vegetables are a major source of bioactive compounds (Steinmetz and Potter, 1991). During processing of fruits and vegetables, wastes are generated, and their disposal poses a major challenge to the environment. As a result, bioactive compound recovery from food waste have been studied (Nagarajaiah and Prakash, 2011; Xu *et al*., 2012; Fărcaş *et al*., 2013).

Tomato has many bioactive compounds, including lycopene, β-carotene, vitamins E and C, phenolics and flavonoids (Kaur *et al*., 2002; Periago and Garcia-Alonso, 2009). Lycopene is known to protect humans against prostate and other cancers (Kris-Etherton *et al.*, 2002). Significant correlation between the lycopene content

of tomato and antioxidant activity was reported (Martínez-Valverde et al., 2002). Tomato processing generates waste, mainly in the form of seeds and peels. The skin and the seeds are collectively known as pomace. When juice is extracted from tomato the waste generated is about 20-30% of the total tomato fruit (Haddadin *et al*., 2001). Studies on tomato fruits have indicated that the principal source of its bioactive compounds is the peel (George *et al.*, 2004; Chandra and Ramalingam, 2011).

Bioactive compound recovery from grape pomace (Hogan *et al.*, 2010; Ghafoor *et al*., 2011), apple pomace (Grigoraș *et al*., 2012), industrial tomato waste (Riggi and Avola, 2008; Naviglio *et al.*, 2008**)** and mango peels (Tunchaiyaphum *et al*., 2013) have been studied. Since tomato waste poses disposal challenges, and is a source of bioactive compounds, the objective of this research was to evaluate the amount of lycopene and β-carotene recovered from the waste generated from tomato after winemaking at different fermentation temperatures and different tomato must pH levels, and, in addition, to evaluate their antioxidant activity.

Materials and methods

Source of material and material preparation

Tomato (*Lycopersicon esculentum* var. Hong Xia Hybrid No.2) fruits were purchased from the Zhenjiang local market in China. Spoiled tomatoes were removed from the lot. They were thoroughly washed several times with tap water, sterilized with 2% potassium metabisulphite, rinsed several times with distilled water, and the water dried with napkin paper. They were then cut into smaller pieces with a sterilized knife and blended with a sterilized Kenwood blender (Philips HR 2006, China) to obtain tomato must.

Fermentation

The initial pH of the tomato must was 4.11, and this was changed to two other levels, 3.40, and 3.20 using tartaric acid. The experiments conducted with the musts with pH level 4.11 were designated as the Control. Triplicate tomato musts were fermented at temperatures 15°C and 20°C, using the yeast culture (*Saccharomyces bayanus*, BV 818) of concentration 0.03 g/100 mL prepared as described by Owusu *et al*. (2014). Tomato must of volume 4.5 L was inoculated with 0.18 L of inoculum. During fermentation, which lasted for 12 and 15 days at 20^{o}C and 15^{o}C respectively (Results not shown), the must was in contact with the wine produced. After fermentation, the tomato wine was separated from the pomace, and stored at -20^{o}C until needed for further experiment.

Determination of moisture content of tomato pomace

The moisture content of the tomato pomace was determined using AOAC (2000) and the value obtained ranged from 78.02 ±2.43 to 79.43 ±2.43%.

Determination of pH, titratable acidity and ethanol content of tomato wine

The pH of the tomato wine was measured with a pH Meter (PHS-2C Precision pH/mV meter, China). Briefly, the pH Meter was calibrated with buffer solutions of pH 7 and 4. After this, the electrode of the pH Meter was placed in the wine and the pH value was recorded. Titratable acidity (TA) was determined as described by

Sadler and Mulphy (2004), and the ethanol content was measured by the method described by Caputi *et al*. (1968). All measurements were triplicated.

Lycopene and beta-carotene determination

Total lycopene and ß-carotene content of the wines/tomato pomace were extracted following the method described by Fish *et al.* (2002). Triplicate samples (0.5 g) were weighed into vials. A mixture of hexane: acetone: ethanol [10: 5: 5 (v/v/v)] was used for the extraction. Five milliliters of acetone containing 0.05% (w/v) butylated hydroxytoluene (BHT), 5 mL of 95% ethanol, 10 mL hexane and 0.1 g $CaCO_3$ were added to the sample. The mixture obtained was centrifuged at 5000 rpm for 10 min and afterwards combined with 3 mL distilled water. In the following procedure, the mixture was shaken for phase separation using an incubator shaker (QYC 211 Incubator Shaker, Shanghai Test Equipment Co. Ltd) at a speed of 160 rpm for 15 min. After shaking, the hexane layer (non-polar) containing carotenoids was transferred into a separate test tube and shortly thereafter measured spectrophotometrically at absorbances 505, 453 and 663 nm blanked with hexane and calculated as described by Nagata and Yamashita (1992):

Lycopene (mg/100 gFW) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$

ß-carotene (mg/100 gFW) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$,

where FW means fresh weight. The values obtained were then expressed as mg/100 g DW (dry weight).

Determination of total antioxidant activity

The total antioxidant activity (TAA) was determined by the phosphomolybdate assay (Prieto *et al.*, 1999). Briefly, an aliquot of diluted sample (0.3 mL), obtained as in the lycopene and ß-carotene determination, was combined in a vial with 3 mL of molybdate reagent solution (0.6 M sulphuric acid, 28 Mm sodium phosphate and 4 Mm ammonium molybdate). The vials were capped and incubated in a water bath at 95^{o}C for 90 min. The sample mixture was cooled to room temperature, and the absorbance was measured at 695 nm against a blank. Ascorbic acid (diluted in 13% ethanol v/v) was used to prepare a standard curve in the range 20-100 mg/L, and the total antioxidant activity was expressed in terms of milligram per liter ascorbic acid equivalent (AAE mg/L). All measurements were triplicated.

Reducing power

The reducing power (RP) of the sample was determined according to the method of Oyaizu (1986). Diluted sample of volume 0.6 mL (obtained as in the lycopene and ß-carotene determination) was mixed with 1.5 mL phosphate buffer (0.2 M, pH 6.6) and 1.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min and 1.5 mL of 10% trichloracetic acid was added to the mixture to terminate the reaction. It was then centrifuged at 5000 rpm for 10 min and 1.5 mL of the supernatant solution was mixed with 1.5 mL of distilled water. Freshly prepared 0.1% ferric chloride solution of volume 0.3 mL was added and the absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates elevated reducing power. Reducing power was expressed as ascorbic acid equivalents in mg/L (AAE mg/L). Determinations were made in triplicates.

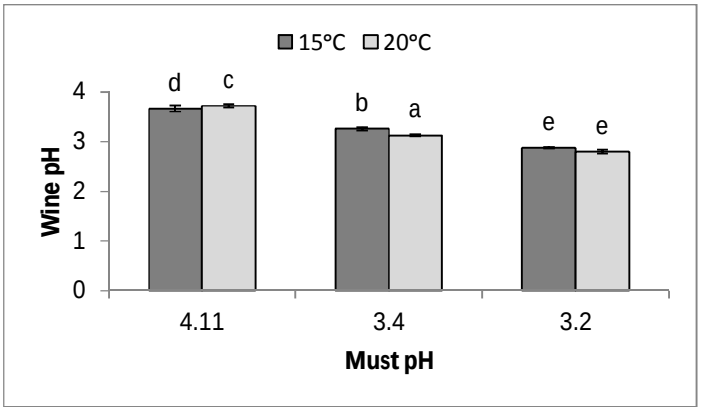
Statistical analysis

Experimental results were reported as mean ± standard deviation. The data was analyzed using ANOVA, and differences in means were determined by using the Duncan's Multiple Range Test. Differences were deemed to be significant at $P<0.01$.

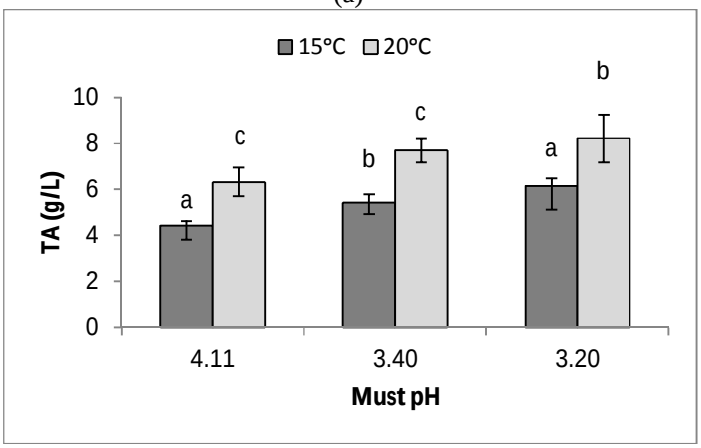
Results and discussion

Compositional attributes of tomato wine

The tomato wines recorded compositional attribute shown in Figures 1, 2, and 3. Must pH had effect ($P<0.01$) on the pH of the wines produced.



(a)



(b)

Figure 1. (a) Tomato wine pH (b) Tomato wine TA

With the exception of the Control, the effect of fermentation temperature on the wine pH was also significant. The TA values of the tomato wines were higher ($P<0.01$) at the higher fermentation temperature. This may be due to the higher production of organic acids by higher yeast population at 20°C than at 15°C (Jackson, 2008). The effect of must pH and the fermentation temperature on ethanol content of the wine was not significant ($P>0.01$) even though there were some slight variations. The lycopene and β-carotene contents of the tomato wines are shown in Figure 3. The effect of both must pH and the fermentation temperature on the contents of both lycopene and β-carotene of the wines was significant ($P<0.01$).

The effect of pH on the concentration of lycopene and β-carotene in the tomato wines is similar to the results reported for phenolic compounds extracted from mango peels (Tunchaiyaphum *et al*., 2013). There was a general reduction in the contents of both bioactive compounds when fermentation temperature increased from 15°C to 20°C.

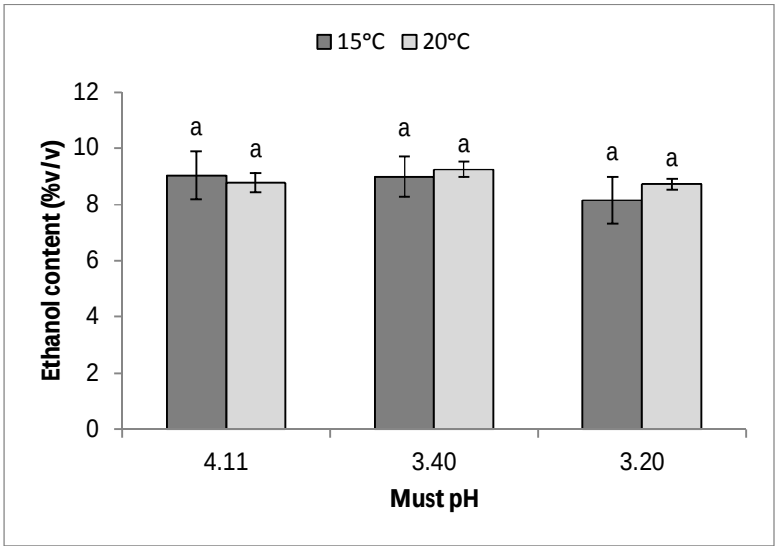


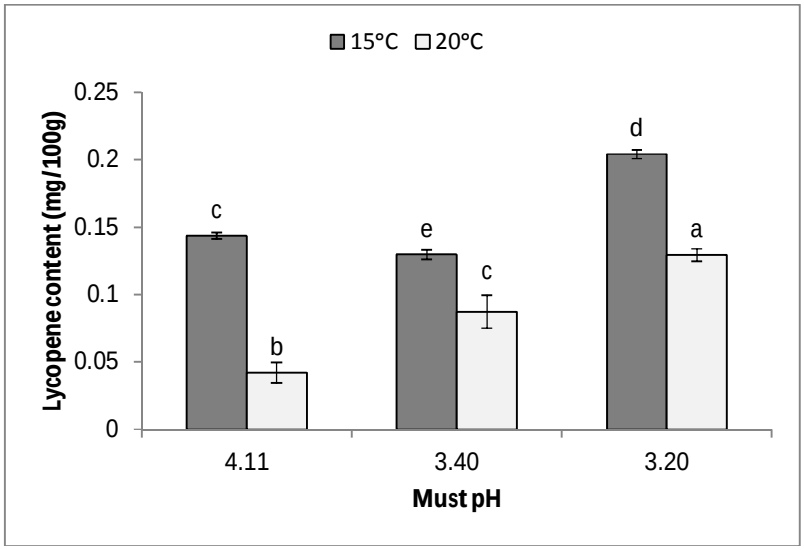
Figure 2. Ethanol content of tomato wine

This may be due to the higher adsorption of bioactive compounds by the yeast at the higher temperature which produced higher yeast population (Balík, 2006).

Lycopene and β-carotene recovery from tomato pomace

Lycopene and β-carotene were extracted from the tomato pomace using the solvent extraction method. The solvent mixture (hexane: acetone: ethanol) used in this study was in the ratio [10: 5: 5 (v/v/v)]. Extraction of lycopene with the same solvent mixture as the one used in this study was found to be more efficient

than the one where either hexane or ethyl acetate alone was used (Kumar et al., 2013).



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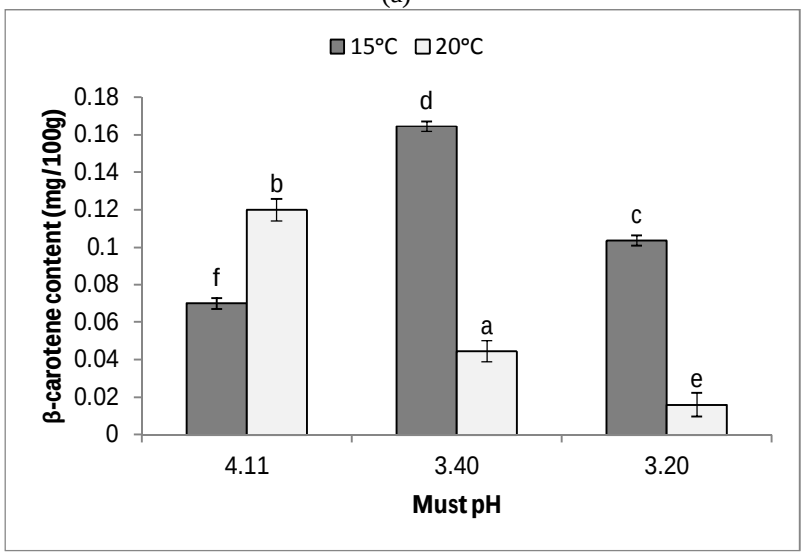


(b)

Figure 3. (a) Lycopene and (b) β-carotene contents of tomato wine

Lycopene and β-carotene recovered from the tomato pomace are shown in Table 1. The lycopene concentration of the tomato pomace ranged from 2.48-3.06 mg/100 g DW (representing 2.25-2.78% of the tomato pomace, Table 2), and this is

comparable to the results reported earlier **(**Naviglio *et al*., 2008), but lower than the result of Chun *et al.* (2009). This may be due to the fact that the method used in the previous work was supercritical fluid extraction, which is different from the one used in the present study. The β-carotene concentration of the tomato pomace, 0.78-0.93 mg/100 g DW (representing 0.71-0.85%, Table 2) is lower than the results reported for tomato waste (Riggi and Avola, 2008), and this may be due to the differences in the source of waste and solvent ratios used for the two experiments. The results are similar to what was reported previously where the lycopene content of tomato was higher than β-carotene (Chérif *et al*., 2010). The recovery of both bioactive compounds from the pomace were significantly influenced by both must pH and fermentation temperature.

Table1. Amount of lycopene and β-carotene recovered (mg/100g) from tomato pomace

Fermentation temperature	Bioactive compound	Must pH		
		4.11	3.40	3.20
15	Lycopene	$2.86\pm0.02^{b\alpha}$	$2.53\pm0.04^{a\alpha}$	$3.06\pm0.03^{c\alpha}$
	β-carotene	$0.80\pm0.02^{a\alpha}$	$0.93\pm0.03^{c\alpha}$	$0.91\pm0.03^{b\alpha}$
20	Lycopene	$2.48\pm0.03^{b\beta}$	$2.48\pm0.05^{a\beta}$	$2.58\pm0.05^{c\beta}$
	β-carotene	$0.78\pm0.03^{a\beta}$	$0.79\pm0.04^{b\beta}$	$0.93\pm0.05^{c\beta}$

Different alphabets in the same row is significant at P<0.01. Different Greek letters in the same column is significant at P<0.01.

Table 2. Yield (%) of lycopene and β-carotene recovered from tomato pomace

Fermentation temperature	Bioactive compound	Must pH		
		4.11	3.40	3.20
15	Lycopene (%)	2.60	2.30	2.78
	β-carotene (%)	0.73	0.85	0.83
20	Lycopene (%)	2.25	2.25	2.35
	β-carotene (%)	0.71	0.72	0.85

The lycopene recovery was higher (P<0.01) in the pomace obtained from the fermentation conducted at 15°C than that at 20°C for all levels of pH. Higher yeast population at higher fermentation temperature (Results not shown) might have led to higher adsorption of bioactive compounds by the yeast (Balík, 2006). The pomace obtained from must pH 3.20 recorded the highest content (P<0.01) of lycopene at both fermentation temperatures. In a previous work on extraction of carotenoids from tomato waste, pulsed electric field treatment (5 kV/cm) was reported to have enhanced the permeability and extraction of carotenoids (Luengo *et al*., 2014).

In this study, low pH could enhance the porosity of the tomato pomace and diffusivity of lycopene from the pomace, and hence lead to increased recovery. β-carotene also showed the same trend as lycopene, however, the exception was the content obtained from the fermentation conducted with must pH 3.20. Similar results on the effect of pH on the extraction yield of phenolics from mango peels were reported (Tunchaiyaphum *et al.*, 2013). The combined effect of must pH and

fermentation temperature on the amount of lycopene and β-carotene recovered from the pomace was also highly significant (P<0.01). The amount of dependent variable variance accounted for by the corrected model was measured by the R^2 value. The variance accounted for by the two main effects, must pH and fermentation temperature as well as their interaction was 100 and 98.8% for lycopene and β-carotene contents of the tomato pomace respectively (Tables 3 and 4). This means that must pH, fermentation temperature as well as their interaction accounted for 100 and 98.8% of the variation in lycopene and β-carotene contents, respectively, of the tomato pomace. During fermentation, the pomace was in contact with the wine, and this was separated after the fermentation. Thus, the different pH and temperature conditions in the fermenting media may influence the composition of bioactive compounds in the pomace significantly. Ethanol can influence the porosity of the pomace to enhance the diffusivity and extractability of the carotenoids. However, the content of ethanol was not significantly different, so the ethanol content of the wines may not have any significant effect on the contents of the lycopene and carotene extracted.

The lycopene content of the tomato waste was expressed as a percentage of lycopene in tomato wine (Table 5), and the values obtained ranged from 1.70-6.67%. This indicates that higher concentration of lycopene was found in the tomato pomace than the tomato wine (George *et al.*, 2004; Chandra and Ramalingam, 2011). In terms of the percentages, the values obtained for fermentation temperature 20°C were higher than those for 15°C. β-carotene in the tomato pomace was also expressed as a percentage of that in the tomato wine (Table 5), and the values obtained were in the range 1.71-17.68%.
Higher concentrations of β-carotene were also obtained from the pomace than in the wine (George *et al.*, 2004; Chandra and Ramalingam, 2011).

Table 3. Tests of Between-Subjects Effects for lycopene content of the pomace (Dependent Variable:Lycopene content of tomato pomace)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4.119[a]	5	0.824	6047.493	.000
Intercept	617.750	1	617.750	4534866.069	.000
TEMP	1.985	1	1.985	14569.547	.000
PH	1.444	2	0.722	5300.288	.000
TEMP * PH	0.690	2	0.345	2533.672	.000
Error	0.002	12	0.000		
Total	621.870	18			
Corrected Total	4.121	17			

a. R Squared = 1.000 (Adjusted R Squared = 0.999), p = 0.01

Table 4. Tests of Between-Subjects Effects for β-carotene content of the pomace (Dependent Variable: β-carotene content of tomato pomace)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.393[a]	5	0.079	203.316	.000
Intercept	63.879	1	63.879	165085.467	.000
TEMP	0.044	1	0.044	113.981	.000
PH	0.239	2	0.120	308.843	.000
TEMP * PH	0.110	2	0.055	142.455	.000
Error	0.005	12	0.000		
Total	64.277	18			
Corrected Total	0.398	17			

a. R squared = 0.988 (Adjusted R squared = 0.983), p = 0.01

Table 5. Amount of lycopene and β-carotene in tomato wine expressed as a percentage of lycopene and β-carotene recovered from tomato pomace

Fermentation temperature	Bioactive compound	Must pH		
		4.11	3.40	3.20
15	Lycopene (%)	5.02	5.12	6.67
	β-carotene (%)	8.75	17.68	11.38
20	Lycopene (%)	1.70	3.52	5.01
	β-carotene (%)	15.38	5.62	1.71

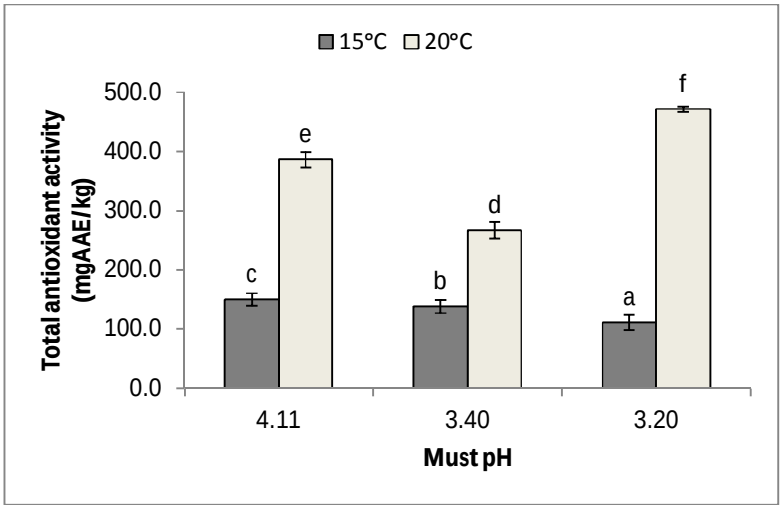
Antioxidant properties of the extract from the tomato pomace

The antioxidant properties of the extract from the tomato pomace were determined using total antioxidant activity (TAA) and ferric reducing power (RP) methods.

The TAA of the wines was determined using the phosphomolybdenum assay in which the antioxidant reduces Mo (VI) to Mo (V) leading to the formation of green phosphate/Mo (V) complex under acidic conditions, which has a maximum absorbance at 695 nm (Shajiselvin and Muthu, 2011). A compound's RP is linked to its ability to transfer electrons and hence an indicator of its potential antioxidant activity. The results on TAA and RP are shown in Fig. 4. The different values of the RP of the extracts indicate that they may have different abilities to react with free radicals to stabilize and terminate radical chain reaction (Shimada et al., 1992).

The TAA and the RP of the tomato pomace extract were influenced by both must pH and fermentation temperature ($P<0.05$). The results are similar to those of the bioactive compounds, lycopene and β-carotene. The acidity level and the fermentation temperature may influence the porosity of the pomace, increase the permeability of the extracting solvent, and hence diffusivity of the lycopene and β-carotene. In a previous work on extraction of carotenoids from tomato waste, pulsed electric field treatment (5 kV/cm) was reported to have enhanced the permeability and extraction of carotenoids (Luengo *et al.*, 2014). The highest TAA and RP were obtained from the tomato pomace which had must pH 3.20 and

fermentation temperature 20°C. Higher temperature may encourage the extraction of the bioactive compounds more than the lower one. The highest TAA and RP for the tomato pomace obtained from the must of pH and fermentation temperature 20°C may be due to higher lycopene and β-carotene contents than the others. This agrees with the results of a previous study where higher carotenoid content of tomato waste was found to have the highest antioxidant capacity (Luengo *et al.*, 2014). The fermented pomace exhibited substantial levels of antioxidant activity.



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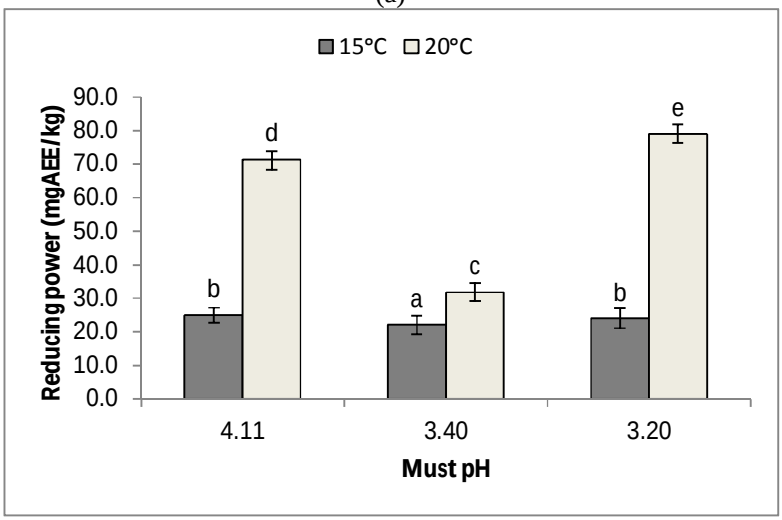

(b)

Figure 4. (a) Total antioxidant activity and (b) reducing power of tomato extract from pomace

Conclusion

The recovery of lycopene and β-carotene from tomato pomace obtained after fermentation was studied. Both must pH and fermentation temperature influenced the concentrations of lycopene and β-carotene recovered from the pomace. The tomato pomace was found to be a rich source of lycopene and β-carotene. The best fermentation conditions which gave the highest TAA and RP were must pH 3.20, and fermentation temperature 20°C. Fermented tomato pomace exhibited substantial level of antioxidant activity.

Acknowledgements

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Conflict of interest

None of the authors has any conflict of interest.

Ethical statement

This article does not contain any studies with human or animal subjects performed by any of the authors.

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