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Physico-chemical Properties of Corn Extrudates Enriched with Tomato Powder and Ascorbic Acid

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The aim of this research was to investigate the influence of the addition of tomato powder (TP) to corn grits at levels 4, 6 or 8 % and the addition of ascorbic acid (AA) at levels 0.5 and 1 %, on total polyphenol content (PF), and antioxidant activity of the extrudates. The hardness and the expansion ratio of the extruded products were also tested. Mathematical models that describe the influence of additives on the mentioned properties were also determined. Extrusion was performed at two temperature regimes: 135/170/170 °C and 100/150/150 °C. Lower temperature regime led to increased hardness and the expansion of extrudates. The addition of tomato and AA led to decreased hardness and the expansion at both temperature regimes. The addition of tomato increased PF and AA compared with pure corn extrudates. Greater degradation of PF and AA was at lower temperature regime. High correlation between PF and AA was demonstrated at both extrusion temperatures.

Key words:
extrusion, tomato powder, ascorbic acid, hardness, expansion ratio, polyphenols, antioxidant activity

Introduction

The demand for highly nutritional quality food is increasing because of (a) the commercial opportunities offered by such products due to their visual and functional properties, (b) increasing consumer awareness of the relationship between food and health, and (c) the widespread industrial use for nutrient supplementation, pharmaceutical purposes, food additives and animal feeds.[1] The tomato is the second most consumed vegetable in the world, after the potato, and approximately 30 % is consumed as transformed products. Among them, tomato powder is a common product widely used by food processors. Whereas numerous studies on the micronutrient content of fresh tomato have been conducted, very little is known about the effects of processing on its nutritional quality, and controversial results can be found in the literature.[2] Tomatoes are a major source of antioxidants and contribute to the daily intake of a significant amount of these molecules. These compounds may play an important role in inhibiting reactive oxygen species responsible for many serious diseases. Along with carotenoids, other antioxidant compounds present in tomatoes, including ascorbic acid, tocopherols and phenols, play a determinant role in disease prevention.[1,3] In recent years, extrusion has become one of the fastest growing food processing operations. Due to the reduction of microbial loads, the prevention of endogenous enzymes, extrusion technology improves the safety and quality of intermediate and final products.[4] Extrusion is particularly interesting in the context of functional food production, because it belongs to HTST (high temperature short time) processes, so it restricts the unwanted effects on proteins, amino acids, vitamins, starch and enzymes.[5] Besides, it can be used in the production of a wide range of products, such as snack-foods, baby foods, breakfast cereals or pasta.[6] Enrichment of the extruded snacks with nutritionally valuable ingredients is increasingly practised by many studies, wherein the leading is the addition of protein and fibre-rich ingredients, like legumes or whey protein, while the addition of fruit and vegetables has been studied to a lesser extent.[7] Unfortunately, the production of nutritionally fortified snack products, with the acceptable physical properties which are crucial for their actual acceptance, is not easy. The addition of high-fibre, high-protein alternate ingredients to starch significantly affects the texture, expansion and overall acceptability of extruded

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snacks.[8] The aim of this research was to investigate the influence of adding tomato powder to corn grits on the hardness and expansion of the extrudates, since it is crucial for actual consumers acceptance. As previously mentioned, tomatoes are considered a rich source of antioxidants, so we also studied polyphenols and antioxidant activity in the raw samples and extrudates. The addition of tomato products to snacks has been investigated in several studies,[9–11] mainly focused on the physical properties of the extrudates. Besides tomatoes, in this research, ascorbic acid was also added to the extrusion mixture, with the aim of protecting the antioxidants originating from tomatoes. Previous research has shown retention of ascorbic acid in the extruded mixtures to a certain extent,[12,13] but there are no data on the influence of the ascorbic acid on dry systems like extrusion mixtures.

Materials and methods

Materials

Corn grits (particle size > 500 μm) were obtained from "Žito" Ltd., Osijek, Croatia. Spray-dried tomato powder (proteins 15.25 %, crude fibre 6.14 %, ash 8.43 %) was purchased from Cofco Tunhe Co. Ltd. China. Ascorbic acid was purchased from T.T.T. Ltd., Sveta Nedjelja, Croatia.

Sample preparation

Levels of tomato addition were selected according to the preliminary studies, in order to achieve extrusion process continuity and products with satisfying characteristics. Corn grits and tomato powder were mixed in 96:4, 94:6 and 92:8 ratios (dry to dry weight), and ascorbic acid was also added to each mixture at 0 %, 0.5 % and 1 % (dry basis). Total moisture of the mixtures was set to 15 %, the mixtures were put in plastic bags, sealed and left in the dark at 4 °C for 24 hours before extrusion.

Extrusion

Extrusion experiments were performed using a laboratory single-screw extruder (model Do-Coder, Brabender 19/20 DN, Duisburg, Germany). Extrusion parameters were as follows: temperature profiles 135/170/170 °C and 100/150/150 °C; 4:1 screw compression ratio; screw speed 100 rpm; feed rate 15 rpm. The obtained extrudates were air-dried at ambient temperature overnight, put in plastic bags, vacuum sealed, and stored in darkness until analysis.

Physical properties

Hardness: The hardness analysis was performed on a texturometer TA.XT2 Plus, Stable Microsystem using the method "Measurement of the hardness and fracturability of pretzel sticks" with the following settings: Pre-Test speed: 1.0 mm s^{-1}; Test speed: 1.0 mm s^{-1}; Post-Test speed: 10.0 mm s^{-1}; Distance 3 mm; Trigger Type: Auto – 5 kg.[14] The results were expressed as the mean of 10 replications. Hardness is the peak force required for a probe of parallel blades to penetrate the extrudate. The higher the value of maximum peak force required, the higher the hardness of the sample.[15]

Expansion ratio: The expansion ratio was measured according to Brnčić *et al.* (2008)[16] where the expansion ratio (ER) was calculated as follows (Eq. 1):

$$\text{ER} = \text{extrudate diameter (mm)/die diameter (mm)} \quad (1)$$

Total polyphenols determination

Polyphenols were determined according to the Folin-Ciocalteu method[17] with modifications. An amount of 0.5 g of ground extruded samples were extracted with 10 mL acidified methanol (methanol/2 % HCl, 95:5) at room temperature for 60 minutes with constant mixing on magnetic stirrer. The glasses were covered with aluminium folium to prevent evaporation of the solvent. An aliquot of the extract (200 μL) was mixed with 2 mL of water and 100 μL of Folin-Ciocalteu reagent (Kemika, Croatia). The mixture was allowed to equilibrate for 5 minutes, and then 300 μL of sodium carbonate solution (20 %) was added. After incubation at room temperature in the dark for 30 minutes, the absorbance of the mixture was read at 725 nm (Camspec, M501, UK). Acidified methanol was used as a blank. Total polyphenols were determined for both raw and extruded samples with 4 replications. Gallic acid (Carlo Erba reagents, Italy) was used as a standard (calibration curve $y = 0.1602x - 0.0008$, $R^2 = 0.9998$), and the results were expressed in mg of gallic acid equivalents per 100 g of sample.

Antioxidant activity (ABTS)

The $ABTS^{\cdot+}$ radical was obtained by mixing 7.4 mmol L^{-1} ABTS (Fluka, Switzerland) solution and 2.6 mmol L^{-1} solution of ammonium persulfate in 1:1 ratio. The solution was left in the dark overnight in order to develop a stable radical, and then the radical solution was diluted with ethanol in a 2:70 ratio to obtain absorbance approximately 1.100 (A_{ABTS}). An aliquot of the extract obtained in the same way as for polyphenols determination, was mixed with 3.2 mL of diluted $ABTS^{\cdot+}$ radical. After incubation at room temperature in the dark for 95 minutes, the absorbance of the mixture was read at 734 nm (A_{EXTR}), and ΔA was calculated $A_{ABTS} - A_{EXTR}$. Trolox (Sigma Aldrich, USA) was used as a standard. Decrease in absorbance caused by trolox was done in the same way as for the samples, and the standard curve ΔA/trolox concentration was created ($y = 496.11x - 18.506$, $R^2 = 0.9962$). The results are expressed in μmol of trolox equivalents per 100 g of sample.

Experimental design and data analysis

Physical analysis data were analysed using *Design expert 6.0.8.* software. The RSM (response surface methodology) was chosen to build up mathematical models, using 3-level factorial design. Tomato powder (variable A) and ascorbic acid levels (variable B) were set as independent variables. The statistical significance of the regression coefficients was determined by analysis of variance (ANOVA), at a 95 % level. Chemical composition data were analysed by Statistica 8 software, using *post hoc LSD* at 95 % level.

Results and discussion

Hardness of the extrudates

The hardness of expanded extrudates is a perception of the human being, it is related to the force applied by the molar teeth to compress the food. It is associated with expansion and cell structure of the product.[15,18] Hardness of the extrudates obtained in this research, (Figure 1), are within the values obtained by Cortazzo Menis *et al.* (2013)[19] during extrusion of flavoured corn grits. Comparing the hardness of the extrudates with the same composition but obtained at different extrusion temperatures, (Figure 1), lower extrusion temperatures (E2) had increased hardness, which has also been observed by Altan *et al.* (2008)[14]. Increasing temperature decreases melt viscosity, but it also increases the vapour pressure of water. This favours the bubble growth, which is the driving force for expansion that produces low density products, thus decreasing hardness of extrudates. The addition of tomato powder caused a decrease in hardness at both extrusion temperatures, contrary to the results obtained by Huang *et al.* (2006)[9] and Potter *et al.* (2013)[20]. It is believed that several factors affect hardness of the extrudates. The amount of fibre is one of the most important factors, since it affects cell wall thickness.[21] Therefore, it is expected that the addition of vegetables rich in fibre will increase the hardness of the extrudates. Nevertheless, Stojceska *et al.* (2008)[22] showed that the addition of cauliflower in extruded products had not significantly affected the hardness. The addition of some other ingredients to starch basis, like proteins, also showed variable influence on the extrudates hardness.[23] The regression equation for the relationship between independent variables of tomato powder and ascorbic acid levels and hardness of the extrudates obtained in terms of coded variables (Table 1) is presented in Table 2. The proposed model for E1 temperatures has relatively good correlation to experimental data (R^2 =

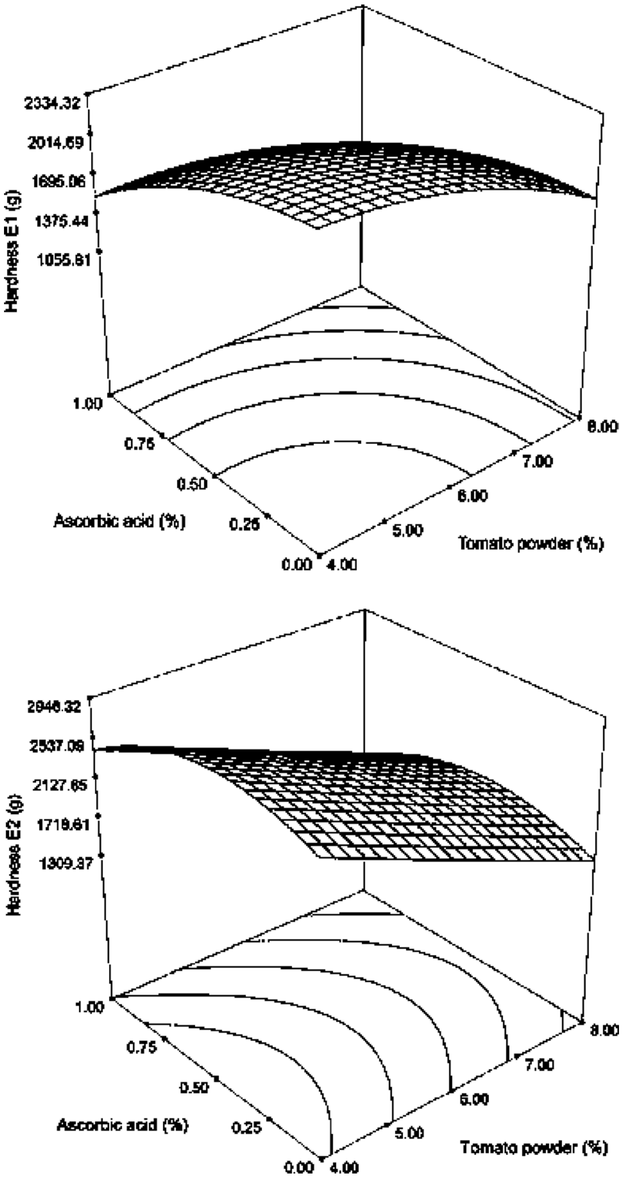

Fig. 1 – *Response surface plots for hardness of extrudates at E1 (135/170/170 °C) and E2 (100/150/150 °C) extrusion temperatures as a function of tomato powder and ascorbic acid levels*

Table 1 – *Coded levels for the independent variables*

	Coded level		
Independent variable	–1	0	1
A: Tomato powder (%)	4	6	8
B: Ascorbic acid (%)	0	0.5	1

Table 2 – *Regression model corresponding to each response*

Response	Model	R^2	R^2_{adj}
Hardness E1	$1977.04 - 282.28 \cdot A - 356.98 \cdot B - 164.10 \cdot A^2 - 170.37 \cdot B^2 + 52.49 \cdot AB$	0.8411	0.7276
Hardness E2	$2336.61 - 585.25 \cdot A - 117.97 \cdot B + 9.23 \cdot A^2 - 364.31 \cdot B^2 + 31.06 \cdot AB$	0.9590	0.9297
Expansion ratio E1	$2.60 - 0.085 \cdot A - 0.053 \cdot B + 0.037 \cdot A^2 + 2.414 \cdot 10^{-3} \cdot B^2 + 0.063 \cdot AB$	0.7920	0.6434
Expansion ratio E2	$2.86 - 0.31 \cdot A - 0.063 \cdot B - 0.061 \cdot A^2 - 0.086 \cdot B^2 + 0.018 \cdot AB$	0.8732	0.7827

E1 – 135/170/170 °C extrusion temperatures, E2 – 100/150/150 °C extrusion temperatures

Table 3 – *Degree of significance (P-values) of the polynomial regression model coefficients corresponding to each response*

Source	Hardness E1	Hardness E2	ER E1	ER E2
A	0.0109	< 0.0001	0.0069	0.0003
B	0.0034	0.0543	0.0494	0.2213
A^2	0.2178	0.9059	0.2960	0.4129
B^2	0.2026	0.0019	0.9440	0.2584
AB	0.6183	0.6349	0.0575	0.7708

A – tomato powder level, B – ascorbic acid level, E1 – 135/170/170 °C extrusion temperatures, E2 – 100/150/150 °C extrusion temperatures
ER – expansion ratio

0.8411), and is statistically significant, but the lack of fit was significant ($P < 0.05$) (Table 4). Analysis of variance (Table 3) showed that the addition of TP and the addition of AA had a statistically significant linear negative influence on hardness of the extrudates at higher temperatures. On the other hand, at E2 extrusion temperatures, the AA level showed a significant quadratic influence on hardness, which can also be seen by the curvature of the surface plot (Figure 1). The regression equation for E2 hardness of the extrudates has much better correlation to experimental data ($R^2 = 0.9590$), than for E1 hardness (Table 2), but the lack of fit is also significant as it was at higher extrusion temperatures (Table 4). A decrease in hardness of the extrudates with the increase in sulphurous acid level was observed by Chang and El-Dash (2003).[24]

Expansion ratio

Texture is a critical sensory attribute that can dominate the quality of a product, as in snacks obtained through thermoplastic extrusion. In extruded snacks, expansion is desired, and puffed products are expected, which is why texture plays an important role regarding the acceptability of snacks among consumers.[18] Figure 2 shows that higher extrusion temperatures decreased expansion of the extrudates, with the exception of the extrudates with the high-

Table 4 – *Analysis of variance results for fitted models of product properties*

Response	Source	df	Sum of squares	Mean squares	F-value	P-value
Hardness E1	Regression	5	$1.503 \cdot 10^6$	$3.007 \cdot 10^5$	7.41	0.0102
	Lack of fit	3	$2.728 \cdot 10^5$	90939.75	32.62	0.0029
	Pure error	4	11149.95	2787.49		
	Residual	7	$2.840 \cdot 10^5$	40567.03		
	Total	12	$1.787 \cdot 10^6$			
Hardness E2	Regression	5	$2.563 \cdot 10^6$	$5.126 \cdot 10^5$	32.73	0.0001
	Lack of fit	3	99623.65	33207.88	13.27	0.0151
	Pure error	4	10010.78	2502.69		
	Residual	7	$1.096 \cdot 10^5$	15662.06		
	Total	12	$2.673 \cdot 10^6$			
ER E1	Regression	5	0.081	0.016	5.33	0.0245
	Lack of fit	3	0.014	$4.769 \cdot 10^{-4}$	2.76	0.1761
	Pure error	4	$6.920 \cdot 10^{-3}$	$1.730 \cdot 10^{-3}$		
	Residual	7	0.021	$3.032 \cdot 10^{-3}$		
	Total	12	0.10			
ER E2	Regression	5	0.64	0.13	9.65	0.0048
	Lack of fit	3	0.066	0.022	3.17	0.1472
	Pure error	4	0.028	$6.920 \cdot 10^{-3}$		
	Residual	7	0.093	0.013		
	Total	12	0.74			

*significant at $P < 0,05$; df – degrees of freedom; ER expansion ratio;
E1 – 135/170/170 °C extrusion temperatures, E2 – 100/150/150 °C extrusion temperatures

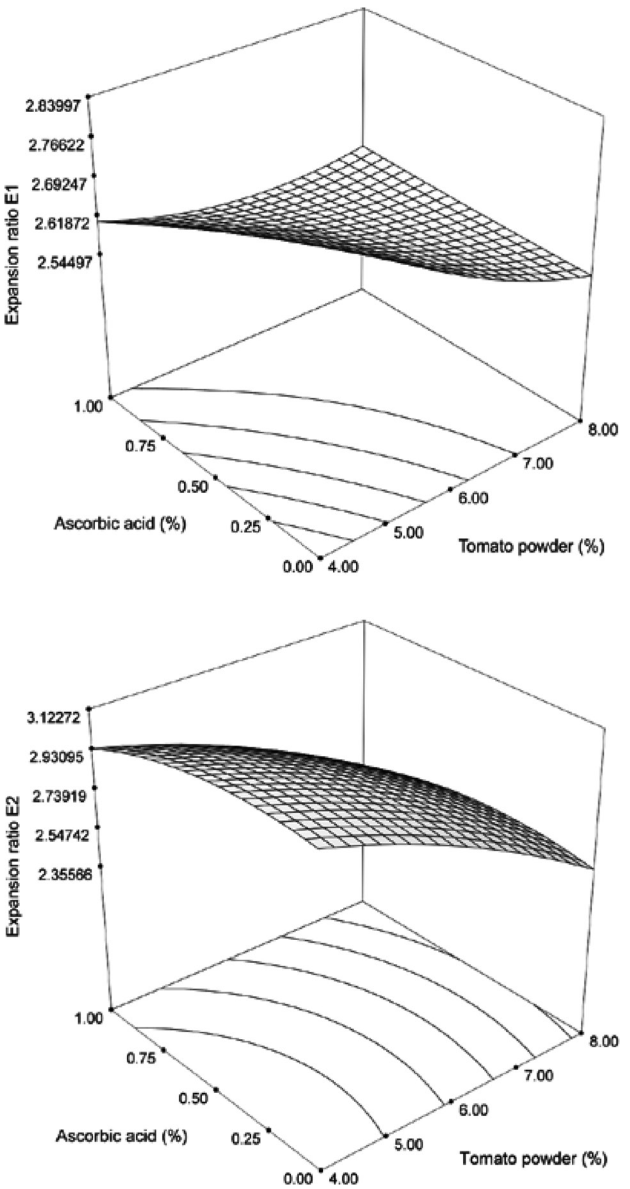

Fig. 2 – *Response surface plots for expansion ratio of extrudates at E1 (135/170/170 °C) and E2 (100/150/150 °C) extrusion temperatures as a function of tomato powder and ascorbic acid levels*

est TP level (8 %). The general trend in literature is that higher extrusion temperatures mostly increase expansion of the extrudates, because at higher temperatures the overheating of water during extrusion increases, resulting in greater expansion. Several studies have reported opposite results,[14,25] which is possibly attributed to the greater degradation of starch during extrusion cooking which prevents the bubble growth and weakens the structure.[25] Besides, the product temperature increases with the extrusion temperature, decreasing the product viscosity. Low viscosity of the extruded cereal melt is important for the expansion, since it allows the matrix cells to collapse under the high vapour pressure. There is a temperature range in which radial expansion of starch reaches a maximum: this optimal temperature range depends on the type of starch and moisture content. Expansion decreases with temperature, most likely due to excessive softening and potential structural degradation of the starch melt, which becomes unable to withstand the high pressure and, therefore, collapses.[26] On the other hand, Dehghan-Shoar *et al.* (2010)[10] showed that the extrusion temperature had no significant influence on expansion during extrusion of corn snacks enriched with tomato lycopene.

TP addition caused a decrease in expansion at both extrusion temperatures (Figure 2). This can be explained by the interaction between fibres and proteins originated from vegetables and starch, and by the reduced elasticity due to the presence of proteins and fibres. The development of the cellular matrix in expanded extrudates depends on the expansion and subsequent collapse of the bubbles in the melt, and is governed by a complex balance between forces driving and resisting deformation, and the extensibility or film forming ability of the melt.[27] Fibres may bind water more strongly than starch, inhibiting water loss at the die and reducing its ability for expansion. Fibres can also cause rupture of cell walls and prevent air bubbles from expanding to maximum level.[25]

The regression equation for the relationship between independent variables of tomato powder and ascorbic acid levels, and E1 expansion ratio of the extrudates obtained in terms of coded variables (Table 1) are presented in Table 2. Although the proposed equation is statistically significant (P = 0.0245), and the lack of fit is not significant (Table 4), the coefficient of determination is not very high (R^2 = 0.7920). Table 3 shows that the TP addition and AA addition had a statistically significant linear negative influence on expansion at E1 extrusion temperatures. Although samples with 8 % of TP and 1 % of AA showed a slight increase in expansion compared to samples with 6 % of TP and 1 % of AA, it is not statistically significant. The addition of ascorbic acid at lower extrusion temperatures (E2) caused a decrease in expansion, but the influence was not statistically significant. TP addition showed a significant linear negative influence on expansion (Table 4).

Polyphenols and antioxidant activity

Recently, consumer interest in the use of natural antioxidants has increased due to the belief that they will offer more health benefits than synthetic antioxidants. In particular, phenolic compounds isolated from plants are recognised as the most promising group of molecules that help to prevent oxidation and maintain product quality.[28] Since such natural antioxidants are very susceptible to oxidation/degradation during thermomechanical conditions during extrusion,[7] different antioxidants have been studied in order to preserve them.[29] Synthetic antioxidants, such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate) and TBHQ (tert-butylhydroquinone), have potential health hazards.[28]

The addition of 4 % TP to corn grits increased total polyphenols content from 72.95 mg_{GAE}/100 g to 201.21 mg_{GAE}/100 g (Table 5). This was expected, since agricultural products, especially fruits and vegetables are considered good sources of natural antioxidants.[30] The increase in TP level had increased the polyphenols content in the raw samples. Polyphenols in the raw samples containing ascorbic acid were not determined since Folin-Ciocalteu reagent reacts not only with polyphenols, but also with other compounds with high antioxidative activity, especially ascorbic acid.[31] In samples without ascorbic acid, extrusion cooking caused a decrease in polyphenol content, at E1 and E2 extrusion temperatures. Similar observations have been reported in many studies.[8,32] During extrusion, the phenolic compounds may undergo decarboxylation due to higher melt temperature and moisture content, which may promote polymerisation of phenols, leading to reduced extractability and antioxidant activity.[33] Nevertheless, in this research, application of a lower extrusion temperature (E1) increased degradation of polyphenols, compared to higher temperatures (E2), except in samples with 6 and 8 % of TP and 1 % of AA, which is in contradiction with the results shown by Sharma *et al.* (2012)[17]. The obtained results can be explained by Maillard reactions favoured by high temperatures, whose products show antioxidant activity.[20] On the other hand, some studies observed an increase in phenolic content after extrusion,[34,35] which is explained by liberation of bound phenolics from cell wall structural components.[36] E1 extrusion temperatures, in this study, obviously induced such liberation. Extrudates containing ascorbic acid showed higher polyphenolic values than extrudates without acid obtained at same temperature, but this cannot be explained only by ascorbic acid retention, since ascorbic acid values decreased more at higher temperatures (results not shown), contrary to total phenolics.

Antioxidant activity in the raw samples ranged from 121.25 µmol TE/100 g in corn grits to 438.59 µmol TE/100 g in the sample with 8 % of TP (Table 6). The raw and extruded samples containing AA showed much higher antioxidant activity compared to samples without the addition of AA. The extrusion process reduced antioxidant activity at both ap-

Table 5 – *Polyphenols in raw and extruded samples*[a,b]

TP (%)	AA (%)	Polyphenols (mg_{GAE}/100 g) before extrusion	after extrusion E1	after extrusion E2
0	0	72.95^{c}±1.64	51.27^{b}±2.79	41.23^{a}±0.98
0	0.5	*	141.14^{b}±1.00	84.59^{a}±0.45
0	1	*	239.34^{b}±1.36	181.35^{a}±2.27
4	0	201.21^{c}±6.80	71.45^{b}±1.98	39.84^{a}±0.98
4	0.5	*	132.17^{b}±0.68	79.94^{a}±2.04
4	1	*	205.54^{b}±0.68	123.84^{a}±0.68
6	0	216.91^{c}±2.36	80.58^{b}±1.13	73.77^{a}±2.96
6	0.5	*	176.62^{b}±3.95	135.45^{a}±2.15
6	1	*	168.85^{a}±1.95	202.66^{b}±4.76
8	0	236.94^{b}±4.30	96.60^{a}±3.40	85.39^{a}±2.95
8	0.5	*	166.93^{b}±3.61	145.25^{a}±4.35
8	1	*	184.07^{a}±0.68	196.09^{b}±3.62

[a]Results expressed as the mean of four repetitions ± standard deviation.

[b]Means followed by the same letter in the lines are not statistically different at 5 % probability.

*not determined

TP – tomato powder level, AA – ascorbic acid level, E1 – 135/170/170 °C extrusion temperatures, E2 – 100/150/150 °C extrusion temperatures

Table 6 – *Antioxidant activity in raw and extruded samples*[a,b]

TP (%)	AA (%)	Antioxidant activity (µmol TE/100 g) before extrusion	after extrusion E1	after extrusion E2
0	0	121.25^{b}±0.70	88.01^{a}±1.98	88.50^{a}±2.86
0	0.5	3093.19^{c}±35.08	406.84^{b}±4.99	255.20^{a}±0.70
0	1	4469.90^{c}±87.70	765.69^{b}±7.02	543.44^{a}±4.21
4	0	391.63^{c}±7.02	180.62^{b}±5.00	103.38^{a}±0.57
4	0.5	5978.90^{b}±71.61	419.90^{a}±7.71	284.97^{a}±0.70
4	1	6181.48^{c}±17.54	597.02^{b}±7.01	394.61^{a}±2.81
6	0	402.54^{c}±5.61	259.17^{b}±3.51	230.14^{a}±1.70
6	0.5	5449.72^{c}±35.08	599.66^{b}±7.45	471.01^{a}±4.05
6	1	6553.56^{c}±52.62	593.05^{b}±8.42	715.59^{a}±10.52
8	0	438.59^{c}±9.79	307.29^{b}±5.25	228.91^{a}±6.19
8	0.5	4581.51^{c}±0.00	587.59^{b}±4.91	489.86^{a}±4.21
8	1	7831.04^{b}±105.24	663.00^{a}±0.71	644.14^{a}±0.70

[a]Results expressed as the mean of four repetitions ± standard deviation.

[b]Means followed by the same letter in the lines are not statistically different at 5 % probability.

*not determined

TP – tomato powder level, AA – ascorbic acid level, E1 – 135/170/170 °C extrusion temperatures, E2 – 100/150/150 °C extrusion temperatures plied temperatures, and E2 temperatures degraded antioxidant activity more than E1 temperatures (except in the sample containing 6 % of TP and 1 % AA), as observed in polyphenols. The high correlation between polyphenols and antioxidant activity during extrusion has also been reported in many studies.[8,32,37] On the other hand, some studies reported opposite results,[38, 39] but it is necessary to note that the obtained results for antioxidant activity depend to a large degree on the analysing method.

Conclusions

An experimental design to investigate the effect of tomato powder addition and ascorbic acid addition on the hardness and expansion of extrudates was employed. TP and AA addition decreased hardness at both applied temperature regimes, but higher extrusion temperatures acted favourably on the hardness of the extrudates. Higher extrusion temperatures decreased hardness owing to excessive starch degradation. TP and AA addition caused a decrease in expansion, but still the extrudates showed good expansion for this type of product. Polyphenols content and antioxidative activity of raw samples increased with the addition of TP and AA. Extrusion degraded the polyphenols, yet the obtained values were much higher than for the pure corn extrudates. The results indicate that products with satisfying physical properties and improved functional properties can be produced with the addition of TP and AA, however, more research in the field of consumer acceptance and other antioxidants like carotenoids are needed.

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