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Research Article:

Solid state fermentation of tomato pomace waste by different lactic acid bacteria and yeast strains for quality and nutritional improvement

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SUMMARY: The study on solid state fermentation of industrial processed tomato pomace by different

lactic acid bacteria viz., Lactobacillus plantarum, L. acidophilus, isolate Lactobacillus spp. and yeast

strains viz., Saccharomyces cereviciae, S. boulardii, isolate Saccharomyces spp. were evaluated for

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the nutritional improvement of tomato pomace. The results revealed that the tomato pomace fermented by lactic acid bacteria strain (*L. plantarum*) and yeast strain (*S. boulardii*) were found to be more efficient in reduction of pH(4.54 and 4.31 %), TSS (1.37 and 1.50 %) and enhancement in protein (16.15 and 17.89 %), fat (9.54 and 9.77 %), titrable acidity (1.16 and 1.78 %), energy (223.31 and 230.16 k.cal) and minerals of Ca (413.33 mg and 422.33 mg/100 g)), Mg (342.67 mg and 342.33 mg/100 g), P (95.33 mg and 91.67 mg/100g) and Fe (15.00 mg/100 g and 13.67 mg/100 g) of the fermented tomato pomace, respectively. The results clearly indicated that the solid state fermentation of tomato pomace bylactic acid bacteria and yeast helps to enhance the quality and nutritional improvement with reduction in fibre of tomato pomace and which could be a good source of animal feed supplement.

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BACKGROUND AND OBJECTIVES

Tomato (*Solanum lycopersicum* Mill.) belongs to family Solanaceae, is the most important warm season fruit vegetable grown throughout the world. The main tomato producers are China, USA, India, Turkey, Egypt, Italy, Iran, Spain, Brazil and Mexico (FAO, 2011). More than a 33 per cent of this production is grown for the processing industry, which makes tomatoes the world's leading vegetable for processing. In 2007, tomato

wastes were estimated as 11 million tons, including a little more than 4 million tons of tomato pomace (FAO, 2011 and Anonymous, 2010). The tomato fruits are eaten raw as salad or cooked and are used in the preparation of products like sauces, pickles, puree, paste, syrup, juice, ketchup etc. After the juice is extracted, a residue, tomato pomace, which primarily consists of water, tomato seeds and peels, is left. The high water content of this by-product limits its length of storage. Hence, tomato pomace is often dried and is fed to dairy cows and sheep (Weiss *et al.*, 1997). Tomato seeds have a high protein (25-28 %), fibre (54 % ADF) and fat (20-24 %) content on dry matter basis (Persia *et al.*, 2003).

The role of these wastes as a cheap source of nutrients capable of supplying adequate calories to livestock is very significant. Fermentation technology has been identified as one of the less expensive means of increasing/ enhancing the nutrients of fruits and vegetable wastes. The use of micro-organisms to convert carbohydrates, celluloses and other industrial wastes into food stuffs rich in protein is possible due to the different characters of micro-organisms viz., very fast growth rate, easy genetic modification for growth on a particular substrate, their protein content is very high ranging from 35-60 per cent, their nutritional values are as good as other conventional foods rich in protein. Apple pomace after fermentation with different species of yeast followed by drying, makes the feed enriched with proteins, vitamins, minerals and fats; which could be used for feeding animals (Joshi et al., 1996). Solid state fermentation of Apple pomace with Candida utilis significantly increased 2.5 fold in protein content, 3.4 fold in niacin, 1.5 fold in thiamine (Joshi and Sandhu, 1996). Apple pomace fermented with C. utilis and Aspergillus niger results significant increase in protein content (Bhalla and Joshi, 1994).

The limitation in utilization of fresh tomato pomace as animal feed is because of its high moisture and fibre contents. Presently, huge quantity of this valuable byproduct waste is either composted or dumped in landfills, roadsides or rivers leading to environmental hazards. An alternative to such disposal methods could be recycling of this waste through solid state fermentation technology for developing animal feed supplements. Hence, the study was under taken to improve the nutritional quality of industrial processed tomato waste by different yeast and lactic acid bacteria under solid state fermentation technology. This approach would also help in proper utilization of tomato fruit wastes which is otherwise discarded.

RESOURCES AND METHODS

Collection of tomato pomace waste:

Industrial processed raw tomato pomace wastes was collected from the Mother Dairy processing plant (SAFAL, unit of Mother Dairy (National Dairy Development Board), near Whitefield, Bengaluru) and stored in a deep freezer for the experimental studies (Plate A). The experiment was conducted at All India Coordinated Research Project of Post-Harvest Engineering and Technology Scheme, University of Agricultural Sciences, GKVK, Bengaluru. The experiment was conducted to evaluate the efficiency of different strains of yeast and lactic acid bacteria for the nutritional improvement of tomato pomace under solid state fermentation. The experiment was conducted with a Completely Randomized Design (CRD) with seven



Plate A : SAFAL fruit and vegetable industrially processed tomato pomace waste generated processing unit@white field

treatments and 3 replications.

Treatments:

LAB/yeast	Treatment details		
	T_1 = Tomato pomace (Control)		
LAB fermentation	$T_2 = TP + Lactobacillus acidophilus (RSL-1)$		
	T ₃ = TP + Lactobacillus plantarum (RSL-2)		
	$T_4 = TP + Isolated Pomace LAB (ITPL-2)$		
	$T_5 = TP + Saccharomyces boulardii (RSY-1)$		
Yeast fermentation	$T_6 = TP + Saccharomyces cerevisiae (RSY-2)$		
	$T_7 = TP + Isolated Pomace Yeast (ITPY-1)$		

Preparation of tomato pomace waste for solid state fermentation

The industrially processed tomato pomace of 1kg weight with a moisture content about 80 - 85 per cent was placed in autoclavable polythene bags of 400 gauge and subjected for pasteurization (heat treatment at 115°C) using autoclave for removal / reduction of anti-nutritional factors. After heat processing, the bags were inoculated with microbial broth cultures containing 10^7 cfu / ml at 5 per cent inoculums and completely air tightened by sealing the bags. The sealed bags were kept for incubation at 37°C for 6 days under solid state fermentation (Plate B). The biochemical analysis was carried out as per standard procedures.



Plate B : SSF of tomato pomace by different LAB and yeast strains

Microbial cultures used :

Authenticated microbial cultures of lactic acid bacteria viz., Lactic acid bacteria L. plantarum (RSL2)

MTCC 6161, L. acidophilus MTCC 10307 (RSL1) and yeast S. cereviceae MTCC 170 (RSY2) were obtained from Microbial Type Culture Collection Centre, Chandigarh, India in the form of lyophilized cultures and the same were revived in the form of agar based slant cultures. Probiotic yeast, S. boulardii (RSY1) was isolated from the commercially available sachets from local medical stores using Sabourauds culture media. These cultures were purified and characterized based on morphological and biochemical tests and maintained in respective agar culture media in the form of agar slant culture. Similarly, one each yeast and lactic acid bacteria from tomato pomace was isolated and characterized based on morphological and biochemical characters and identified as yeast Saccharomyces spp. (ITPY1) and lactic acid bacteria Lactobacillus spp. (ITPL1) were also used in the study.

Preparation of different yeast starter cultures :

Purified and authenticated loop full of inoculums of different yeast cultures are transferred to conical flask containing Yeast Extract Peptone Dextrose broth (YEPDA). The probiotic yeast *S. boulardii* was inoculated in Sabourouds Hi-media broth. The inoculated flasks were kept for 2 days incubation at 28° C. These broth cultures of yeast were inoculated at 5 per cent containing 10^{7} cfu/ml to 400g of tomato pomace contained in polythene bags under solid state fermentation.

Preparation of different LAB starter culture :

Loop full inoculums of purified and authenticated different lactic acid bacteria were transferred to conical flasks containing 100 ml of MRS broth. The inoculated flasks were incubated for 3 days at 37°C. These broth cultures were inoculated at 3 per cent containing 107 cfu /ml to 400 g of tomato pomace contained in polythene bags. The experiment treatment details were as follows:

Tray drying and grinding:

After 6 days of fermentation, the samples were subjected to drying in tray drier at 55°C for 48 hours. Samples were spread uniformly in the tray for effective drying (Plate C). The tray dried samples were grinded in mixer to get powder and subjected to biochemical and microbiological analysis by following standard procedures.



Biochemical and microbiological analysis :

The fermented selected fruit waste flours were subjected to proximate analysis for different nutrients such as moisture, protein, fat, fibre, ash and carbohydrate content and mineral contents of calcium, phosphorus, potassium, zinc, magnesium, iron. The biochemical analysis such as pH, TSS, titrable acidity were done by employing standard methods (AOAC, 2005) and similarly, the proximate analysis of the samples were determined by standard procedures.

Determination of phosphorus and magnesium :

The phosphorus and magnesium contents of the samples were estimated using a Flame Photometer. Ash obtained from the ignition of mango peel waste was digested in 6N HCl, evaporated over a water bath and dissolved using a little quantity of 6N HCl and the volume was made upto 100 ml. This ash solution was used for estimating above minerals content.

Determination of calcium, iron :

The iron, calcium and zinc contents of fruit waste samples were estimated using Atomic Absorption Spectrometer (AAS). Ash obtained from the ignition of fruit waste sample was digested in 6N HCl evaporated over water bath and dissolved using little quantity of 6N HCl and the volume was made upto 100 ml. This ash solution was used for estimating ions of above three elements with help of AAS.

Statistical analysis :

Statistical interpretation of data was done by following the Fischer's Analysis of Variance (ANOVA) technique (Persia *et al.*, 2003). The results were computed at five per cent level of significance. Critical differences were worked out whenever F-test was significant using Duncan's multiple range test.

OBSERVATIONS AND ANALYSIS

The raw processed tomato pomace was fermented by different lactic acid bacteria and yeast by solid state fermentation. The changes in pH, TSS and titrable acidity of the fermented tomato pomace as influenced by different lactic acid bacteria and yeast is presented in Table 1.

The results revealed that the initial pH of the tomato pomace was 4.70 (control). After 6 days of fermentation by different LAB and yeast strains, the change in pH of the fermented tomato pomace varied from 4.31 to 4.63 between the strains. The tomato pomace fermented by reference LAB strain *L. plantarum* (RSL-2) and reference yeast *S. boulardii* (RSY-1) showed more reduction in pH (4.54) and (4.31), respectively compared

lactic acid ba	cteria and yeast strains			
LAB and yeast fermentation	Treatments	pH	TSS(° brix)	Titrable acidity (%)
	T_1 = Tomato pomace (Control)	4.70 ^a	2.43 ^a	1.04 ^e
	T ₂ = Tomato pomace + Lactobacillus acidophilus	4.59 ^a	1.60 ^b	1.28 ^c
LAB fermentation	T ₃ = Tomato pomace + Lactobacillus plantarum	4.54 ^a	1.37 ^b	1.16^{d}
	T_4 = Tomato pomace + Isolated Pomace LAB (IPB)	4.63 ^a	2.27 ^a	1.09 ^e
	T ₅ =Tomato pomace + Saccaromyces boulardii	4.31 ^b	1.50 ^b	1.78^{a}
Yeast fermentation	T_6 = Tomato pomace + Saccaromycescerevesiae	4.55 ^a	1.33 ^b	1.28 ^c
	T_7 = Tomato pomace + Isolated Pomace Yeast (IPY)	4.61 ^a	2.37 ^a	1.45 ^b
S.E.±		0.08	0.15	0.03
C.D. (P=0.05)		0.23	0.44	0.08

Table 1: Changes in pH, total soluble solids (TSS) and titrable acidity of the fermented dried tomato pomace waste as influenced by different lactic acid bacteria and veast strains

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to other strains indicating that both the strains of yeast and LAB were more efficient in fermentation of tomato pomace thereby maintaining quality of the product. The decrease in pH is the indication of fermentation of the substrate and carbohydrate, an organic compound, is expected to yield organic acids such as lactic acid. The production of acid will increase the acidic content and thus reduction in pH value. Similar results were reported by (Abalaka et al., 2011) in spent sorghum waste, where in the decrease in pH from 5.1 to 3.2 after fermentation by yeast. Similarly, rapid decrease in the pH during silage was observed due to fermentation of lactic acid bacteria by (Carpintero et al., 1979). Zheng et al. (2015) reported that the pH of acidification system containing fruit and vegetable wastes could automatically decrease from 3.0 to 4.0. The tomato pomace fermented with LAB strain Lactobacillus plantarum (RSL-2) recorded lowest TSS (1.37 brix) and the tomato pomace fermented by yeast strain S. cerevisiae (RSY-2) recorded less TSS (1.33 brix) may be due to the utilization of more sugars during fermentation and others utilized less sugar during fermentation time. Upon the fermentation of tomato pomace by lactic acid bacteria and yeast, the TSS content was found to decrease. Similar results were reported by (Joshi and Sandhu, 1996) in apple pomace by Saccharomyces spp. and Candida spp. reduction of total sugar during solid state fermentation.

The tomato pomace fermented with LAB strain *L. acidophilus* (RSL-1) and yeast *S. boulardii* (RSY-1) recorded highest titrable acidity (1.28 %) and (1.78 %), respectively. The increase in titrable acidity is due to the fermentation efficiency of the organisms. Fermentation has been reported to cause decrease in pH with a

simultaneous increase in titrable acidity in several fermented products. These results partially related with the investigations of (Ogunsua, 1980) in fermentation of cassava tuber (Murekatete *et al.*, 2012) reported that the titrable acidity increased progressively with fermentation time.

The changes in crude protein, crude fat, fibre and ash of the fermented tomato pomace as influenced by different yeast and bacterial culturesis presented in Table 2 and Fig 1.



- $T_1 = Tomato pomace (Control)$
- T_2^{1} = Tomato pomace + Lactobacillus acidophilus
- $T_3 = Tomato pomace + Lactobacillus plantarum$
- $T_4 =$ Tomato pomace + Isolated Pomace LAB (IPB)
- T_{5} = Tomato pomace + Saccaromycesboulardii
- $T_6 = Tomato pomace + Saccaromycescerevesiae$
- T_{7} = Tomato pomace + Isolated Pomace Yeast (IPY)
- Fig. 1 : Influence of different yeast and lactic acid bacterial strains on crude fibre, crude fat and crude of the fermenteddried tomato pomace waste

Crude protein :

The tomato pomace fermented with *L. acidophilus* (RSL-1) was more efficient in utilization of carbohydrates

LAB and yeast fermentation	Treatments	Protein (%)	Fat (%)	Fibre (%)	Ash (%)
	T_1 = Tomato pomace (Control)	15.15 ^d	3.25 ^d	37.89 ^a	2.29 ^d
	T ₂ = Tomato pomace + Lactobacillus acidophilus	16.55°	8.31 ^{bc}	37.30 ^b	3.13 ^{abc}
LAB fermentation	T ₃ = Tomato pomace + Lactobacillus plantarum	16.15 ^c	9.54 ^a	36.23c	3.22 ^{ab}
	T ₄ = Tomato pomace + Isolated Pomace LAB (IPB)	15.26 ^d	7.97°	37.10 ^b	2.95 ^c
	T ₅ = Tomato pomace + Saccaromycesboulardii	17.89 ^a	9.77 ^a	36.29 ^c	3.25 ^a
Yeast fermentation	$T_6 = Tomato pomace + Saccaromycescerevesiae$	17.78 ^{ab}	8.34 ^{bc}	36.14 ^c	3.05 ^{bc}
	$T_7 = Tomato pomace + Isolated Pomace Yeast (IPY)$	16.96 ^{bc}	8.52 ^b	37.31 ^b	3.10 ^{abc}
S.E.±		0.17	0.08	0.09	0.04
C.D. (P=0.05)		0.51	3.04	0.28	0.13

Table 2: Changes in protein, fat, fibre and ash content of the fermented dried tomato pomace waste as influenced by different lactic acid

and sugars from the substrate which indicates the enhancement of protein (16.55%). Similarly, the yeast strain S. boulardii (RSY-1) was more efficient in enhancement of crude protein (17.89%) compared to other strains. The ability of carbohydrate degradation is more by yeast strains than LAB strains. Similar results have been reported by (Joshi and Sandhu, 1996) in apple pomace fermented by Saccharomyces and Candida under solid-state fermentation (SSF). This has resulted in the increase in the crude protein 3 times more than the control. Similar results were reported by (Odetokum, 2000) in mango peel waste where, increase in protein and fat content of the fermented mango peel and authors attributed to the fact that the micro-organisms degrade the sample as well as microbial biomass. Similarly, Iyayi and Losel (2001) reported that cassava peels waste fermented by S. cerevisiae increased the protein content from 5.60 to 16.74 per cent. The results of the present study are in agreement with the findings of (Ojokoh et al., 2007) who found that the fermented mango peel showed an increase in protein content and decrease in pH, fibre, tannin and phytate contents.

Crude fat and crude fibre :

In the present study, among LAB and yeast strains, the highest crude fat was obtained in the tomato pomace fermented by L. plantarum (RSL-2: 9.54%) and S. boulardii (RSY-1: 9.77%). Both yeast and LAB strains showed significant difference in crude fat content of tomato pomace. Similarly, the increase in fat content of the fermented mango peel sample may be attributed to the fact that the micro-organisms degrade the sample as well as microbial biomass (Odetokum, 2000). The crude fibre of fermented tomato pomace showed less significant

with the reduction of crude fibre by yeast and LAB strains. However, crude fibre reduction was more in the yeast fermentation compared to LAB fermentation. This may also be attributed to the fact that during fermentation, carbohydrate including cellulose pectin, lignocellulose and starch are broken down by fermenting micro-organisms, thereby reducing the fiber content (Raimbault and Tewe, 2001). These results also support the work of (Oboh and Akindahunsi, 2003) who reported that fermentation of cassava waste by S. cerevisiae resulted in nonsignificant changes in the crude fibre contents of the cassava fermented products. (Onifade et al., 2004) reported that the crude fibre, carbohydrate, sugars (reducing, non-reducing and total) and starch contents of both flours and tubers of sweet potato reduced after fermentation.

Ash :

Tomato pomace fermented by both LAB Lactobacillus plantarum (3.22%) and yeast S. boulardii (3.25%) showed significant difference in ash content compared to uninoculated. The improvement in ash content may be due to increase in the mineral content of the waste due to fermentation activity. Similar results were earlier reported by (Joshi and Sandhu, 1996) that solid-state fermentation (SSF) of dried apple pomace by Saccharomyces and Candida were found to be increases the ash content of the product. Onifade et al., 2004 also reported that fermentation increases the ash content of the samples.

Changes in moisture, carbohydrates and energy of the fermented tomato pomace as influenced by different lactic acid bacteria and yeast strains is showed in Table 3.

yeast strain	ls		·	
LAB and yeast fermentation	Treatments	Moisture (%)	Carbohydrates (%)	Energy (k cal)
	T_1 = Tomato pomace (Control)	4.33 ^{cd}	36.86 ^a	185.28 ^e
	T ₂ = Tomato pomace + Lactobacillus acidophilus	4.87 ^b	29.84 ^c	213.97 ^d
LAB fermentation	T ₃ = Tomato pomace + Lactobacillus plantarum	5.20 ^a	29.66 ^c	223.31 ^b
	T ₄ = Tomato pomace + Isolated Pomace LAB (IPB)	4.37 ^{cd}	32.36 ^b	214.86 ^{cd}
	T ₅ = Tomato pomace + Saccaromycesboulardii	4.45 ^c	28.35 ^d	230.16 ^a
Yeast fermentation	T ₆ = Tomato pomace + Saccaromycescerevesiae	4.18^{d}	30.51°	219.87 ^{bc}
	T ₇ = Tomato pomace + Isolated Pomace Yeast (IPY)	4.46 ^c	29.64 ^c	216.51 ^{cd}
S.E.±		0.05	0.20	0.94
C.D. (P=0.05)		0.16	0.61	2.84

Table 3: Changes in moisture, carbohydrates and energy of the fermented dried tomato waste as influencedby different lactic acid bacteria and

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Moisture :

The moisture content in the fermented tomato pomace by different LAB and yeast strains was analyzed and the moisture content in the fermented tomato pomace did not varied too much. However, the highest moisture content was obtained in the tomato pomace fermented by *L. plantarum* (5.20) and the yeast strain Isolated Pomace Yeast (4.46). The moisture content variation in fermented tomato pomace may be attributed to drying process and depends upon the moisture content present in substrate. The increase in moisture content could be due to the addition of water to the sample prior to fermentation as observed by Ojokoh *et al.* (2007).

Carbohydrates :

The carbohydrate content varied from 28.35 to 32.36 per cent between LAB and yeast fermentation of tomato pomace. Among LAB strains, the highest carbohydrate content (32.36 %) was observed in the tomato pomace fermented by isolated tomato pomace LAB (ITPY-1) and among yeast strains, Saccharomyces cerevesiae (RSY-2) showed higher carbohydrate content (30.51%). Both LAB and yeast strains showed non-significant difference in carbohydrate content of tomato pomace. This could be attributed to the soluble carbohydrates which are essential substrates for the growth of yeast and lactic acid bacteria for proper fermentation (Mc Donald et al., 1991). Odetokum (2000) and Ogunsua (1980) also reported that increase in carbohydrate content during fermentation may be due to a reduction in the fibre content and increase in both reducing sugars and total soluble sugars.

Energy :

The energy content increased in the tomato pomace

after fermentation by allthe yeast and LAB strains. Among LAB and yeast strains, the tomato pomace fermented with *L. plantarum* (223.31 k cal) and *S. boulardii* (230.16k cal) recorded the highest energy value (Table 3).

Changes in mineral contents of the fermented tomato pomace as influence by different lactic acid and yeast strains is showed in Table 4.

Minerals :

The result of mineral composition revealed that tomato pomace fermented by L. plantarum (RSL-2) showed highest Ca (413.33 mg/100g), Mg (342.67 mg/ 100g), P (95.33 mg/100g) and Fe (15.00 mg/100g) content. Among yeast strains, the highest Ca (422.33 mg/100g), P (91.67mg/100g) and Fe (13.67mg/100g) and Mg (342.33 mg/100g) content was recorded in the tomato pomace fermented by S. boulardii (RSY-2). This might be due to the fact that the combined fermentation sample was rich in some essential minerals which perform various functions in the body (Abalaka et al., 2011). The result concluded that the tomato pomace fermented by L. plantarumand S. cerevesiae significantly enhanced minerals over un-inoculated control and other treatments. Onifade et al., (2004) similarly reported that the mineral elements such as Zn, Fe and Na contents increased after fermentation (Table 4)

Hence, the study indicated thatthe yeast strain Saccharomyces boulardii and LAB strain Lactobacillus plantarum performed better for solid state fermentation of tomato pomace waste with respect to enhancement of nutrients. Tomato pomace waste fermentation helps to increase the protein and mineral contents, reduction in fibre, thus, improving its nutritional quality. Hence, it could be concluded that fermented

Table 4: Changes in mineral contents of the fermented tomato pomace as influence by different lactic acid and yeast strains						
LAB and yeast	Treatments	Minerals (mg/100g)				
fermentation		Ca	Mg	Р	Fe	
	T ₁ = Tomato pomace (Control)	399.00 ^c	320.00 ^b	90.33 ^{bc}	12.33 ^b	
	T ₂ = Tomato pomace +Lactobacillus acidophilus	400.00°	333.67ª	86.33°	13.00 ^b	
LAB fermentation	T ₃ = Tomato pomace + <i>Lactobacillus plantarum</i>	413.33 ^b	342.67 ^a	95.33ª	15.00 ^a	
	T_4 = Tomato pomace + Isolated Pomace LAB (IPB)	402.67 ^c	330.67 ^{ab}	89.00 ^{bc}	12.33 ^b	
Yeast fermentation	T ₅ = Tomato pomace + Saccaromyces boulardii	421.67 ^a	342.33 ^a	90.00 ^{bc}	13.33 ^b	
	T_6 = Tomato pomace + Saccaromycescerevesiae	422.33ª	340.33 ^a	91.67 ^{ab}	13.67 ^b	
	$T_7 = Tomato pomace + Isolated Pomace Yeast (IPY)$	403.67 ^c	331.67 ^{ab}	89.33 ^{bc}	12.67 ^b	
S.E.±		1.63	4.15	1.32	0.42	
C.D.(P=0.05)		4.94	12.57	4.01	1.27	

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tomato pomace could serve as a good source of animal feed supplement.

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