

Effects of Potassium Sorbate and Ascorbic Acid Concentrations on Physio-Chemical Properties and Stability of Pumpkin Puree in Chilled Storage

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This study investigated the storage conditions for pumpkin puree at cold temperature to ensure safety and prolong the shelf life. The study aimed to observe the changes in physio-chemical and microbial aspects at different potassium sorbate (0, 0.05, and 0.1 %), and ascorbic acid (0, 0.025, 0.05, and 0.075 %) concentrations during the storage at 10 ± 2 °C. The results showed that puree processed without potassium sorbate had a shelf life of 3 d, while the combination of 0.05 % potassium sorbate and 0.05 % ascorbic acid in puree could extend the shelf life of puree up to 9 d at 10 ± 2 °C. Total carotenoids content (16.72 ± 4.21 mg%), water activity (0.91 ± 0.01), and the total aerobic microorganism (3.64 ± 0.28 log CFU/g), yeast, molds, and *E.coli*/Coliforms were not observed in puree pumpkin over nine d of storage. The results also illustrated that microbial growth was the leading cause of puree quality degradation, spoilage, and short shelf life.

1. Introduction

Pumpkins are a wealth of superior nutritional components, containing carotenoids, K⁺, vitamins B₂, C, and E, and a large amount of dietary fiber (de Escalada Pla et al., 2007). Carotenoids found primarily on pumpkin pulps and seeds (Martins and Ferreira, 2017), help combat chronic diseases like cancer (Men et al., 2021), cardiovascular, and macular degeneration (Obulesu et al., 2011). Pumpkins can be widely converted into common added-value products, especially puree, which are rich in carotenes, dietary fiber, and other substances beneficial to health (Santos et al., 2018). However, pumpkin puree has low acidity (pH 7.17 -7.46), which is favorable for the growth of microorganisms (Guinebretiere et al., 2003). Sorbic acid and potassium sorbates (PS) are common antimicrobial agents used in food products, effective against yeasts and molds (Ihl et al., 2003). There is a wide range of studies associated with the effect of thermal treatments (Provesi et al., 2012) and types of packages on pumpkin puree stability during storage (Gliemmo et al., 2009), but there are few studies involving the effects of additives concentrations on the quality of pumpkin puree, especially processed from Vietnamese pumpkin (*Cucurbita moschata*). This research aimed to investigate the effects of potassium sorbate and ascorbic acid concentrations on the quality of pumpkin puree during chilled storage at 10 ± 2 °C for 15 d.

2. Material and Methods

2.1 Preparation of pumpkin puree

Fresh pumpkins (F1 hybrid pumpkin variety, long fruit, *Cucurbita moschata* D.) contain high amounts of protein, total phenolic content, and total soluble solids (Pham et al., 2023) were purchased at a supermarket. The pumpkin seeds were removed manually, sliced (thickness: 4 - 5 cm), and blanched (90 °C for 7 min). The remaining pulp was crushed and homogenized by blender (Philips HR2223, Viet Nam) with 30 % water, 10 % sucrose, and potassium sorbate at 0, 0.05, and 0.1 % (Weifang JS, China). A concentrator machine then concentrated the puree at 100 °C for 15 min. The obtained puree was then cooled at ambient temperature and aseptically added ascorbic acid at 0, 0.025, 0.05, and 0.075 % (Guangzhou Zio Chemical, China). All samples

were packed into 500 mL plastic bottles (250 g/ plastic bottle) sealed, and stored in chilled condition at 10 ± 2 °C over 15 d of storage.

2.2 Determination of physio-chemical characteristics of pumpkin puree

Color (L, a, b, total color difference of ΔE) using a colorimeter (FRU, China) described by Provesi et al., (2012), total soluble solid (TSS) analyzed by Atago refractometer (NM 2773, Japan) as described by the method of AOAC (2000), pH was measured using a pH-electrode (Vemier, USA), water activity was measured by Rotronic Hygropalm (HP-23A, UK). Total carotenoid content was analyzed using the Rojas et al. (2020) methodology with some modifications at wavelengths at 450 nm using a UV/VIS spectrophotometer (752N, China).

2.3 Microbiological analysis

25 g puree was mixed with 225 mL of Maximum Recovery Diluent (MRD, Merck, Darmstadt, Germany) for the initial dilution, followed by a ten-fold serial dilution. Total aerobic mesophilic counts, total yeast and molds, lactic acid bacteria, and Coliform/*E.coli* count were determined on Plate Count Agar (Merck, Darmstadt, Germany), Yeast Extract Glucose Chloramphenicol Agar (Merck, Darmstadt, Germany), De Man, Rogosa and Sharpe agar (Merck, Darmstadt, Germany), and Coliform Agar ES (Merck, Darmstadt, Germany) by pour plating the decimal dilutions. The Petri dishes were incubated for 48 h at 37 °C for total mesophilic counts, yeast and molds, and lactic acid bacteria, and for 24 h at 37 °C for Coliform/*E.coli*. The analysis results were averaged in logarithmic form with the number of colonies formed as log CFU/g.

2.4 Statistical analysis

Results are reported as mean value \pm standard deviation of these duplicate analyses. The data were compared using analysis of variance (ANOVA) with a significance level of 5 % via Statgraphics Centurion 18.1.12 (Statgraphics Technologies, Inc., The Plains, Virginia).

3. Results and Discussion

3.1 Effect of potassium sorbate and ascorbic acid on the total carotenoids content of pumpkin puree

The changes in total carotenoid content over 15 d had statistically significant differences, as shown in Table 1. The samples added 0.05 % potassium sorbate (PS) showed no significant difference in carotenoid content compared to the control ($p > 0.05$). Adding ascorbic acid (AA) alone or with PS did not prevent carotenoid decline in pumpkin puree despite varying additive concentrations.

Table 1: Change of total carotenoids content (mg/100g dry matter) of pumpkin puree during chilled storage

Samples	Storage intervals (d)							Mean
	0	3	6	9	12	15		
0 ¹ -0 ²	15.75 2.08	\pm 19.81 4.15	\pm 21.44 \pm 4.22	21.01 \pm 3.64	22.41 \pm 3.01	22.14 \pm 2.62	20.70 \pm 1.70 ^a	
0 - 0.025	15.75 2.08	\pm 15.98 0.47	\pm 16.11 \pm 0.91	16.36 \pm 1.52	16.16 \pm 3.04	15.91 \pm 1.52	16.19 \pm 0.25 ^{cde}	
0 - 0.05	15.75 2.08	\pm 16.76 3.15	\pm 18.83 \pm 5.91	18.56 \pm 6.19	18.72 \pm 4.61	19.99 \pm 0.62	18.36 \pm 1.06 ^b	
0 - 0.075	15.75 2.08	\pm 15.38 1.67	\pm 16.95 \pm 5.54	17.13 \pm 4.69	17.15 \pm 3.84	24.23 \pm 8.14	18.17 \pm 2.83 ^{bc}	
0.05 - 0	15.75 2.08	\pm 22.87 13.06	\pm 22.82 \pm 4.27	18.76 10.41	\pm 22.37 10.91	\pm 21.62 10.05	21.23 \pm 1.73 ^a	
0.05 - 0.025	15.75 2.08	\pm 19.71 8.38	\pm 17.97 \pm 7.43	17.95 \pm 7.25	19.01 \pm 5.96	19.46 \pm 8.10	18.14 \pm 1.67 ^{bc}	
0.05 - 0.05	15.75 2.08	\pm 18.21 7.99	\pm 18.95 \pm 6.55	16.72 \pm 4.21	19.88 \pm 8.25	19.85 \pm 8.57	18.04 \pm 1.86 ^{bc}	
0.05 - 0.075	15.75 2.08	\pm 17.02 7.99	\pm 17.99 \pm 5.43	16.33 \pm 5.22	20.02 \pm 9.23	17.64 \pm 7.74	17.73 \pm 1.14 ^{bcd}	
0.1 - 0	15.75 2.08	\pm 16.72 7.70	\pm 15.82 \pm 4.34	17.29 \pm 5.68	16.63 \pm 2.52	15.54 \pm 5.33	15.89 \pm 1.27 ^{de}	
0.1 - 0.025	15.75 2.08	\pm 14.48 4.54	\pm 12.19 \pm 4.49	14.20 \pm 5.89	15.33 \pm 2.41	18.75 \pm 0.05	14.97 \pm 1.95 ^e	
0.1 - 0.05	15.75 2.08	\pm 14.73 4.82	\pm 12.56 \pm 2.16	16.24 \pm 7.52	15.01 \pm 1.53	15.48 \pm 4.68	14.37 \pm 1.48 ^e	
0.1 - 0.075	15.75 2.08	\pm 14.72 5.32	\pm 13.69 \pm 2.31	15.64 \pm 6.39	14.92 \pm 2.46	13.03 \pm 0.23	14.21 \pm 0.94 ^e	
Mean	15.75 2.08 ^b	\pm 17.19 2.44 ^{ab}	\pm 17.11 3.14 ^{ab}	\pm 17.18 1.68 ^{ab}	\pm 18.13 2.57 ^a	\pm 18.63 3.11 ^a		

Note: The a, b, c,... superscript letter(s) in the same column and row are significantly different ($p < 0.05$). ¹PS and ²AA concentration (%).

3.2 Effect of potassium sorbate and ascorbic acid on color parameters of pumpkin puree

The increase in ΔE value of pumpkin purees during chilled storage ($p < 0.05$) is shown in Table 2. The increase of L^* value (black to white), a^* value (green to red), b^* value (blue to yellow) resulted in an increase ΔE of pumpkin purees during chilled storage. In detail, pumpkin puree samples added 0.05 % potassium sorbate and 0.05-0.075 % ascorbic acid had the highest value of ΔE compared to the others ($p < 0.05$). The changes in the color of pumpkin products could be due to the degradation of carotenoids and the behavior of enzymatic browning of phenolic compounds (Dutta et al., 2006).

Table 2: Change of ΔE^* value of pumpkin puree during chilled storage

Samples	Storage interval (d)					Mean			
	3	6	9	12	15				
0 ¹ -0 ²	2.12 ± 0.86	2.97 ± 1.38	2.31 ± 0.13	2.68 0.25	±	3.09 ± 0.11	2.64 0.37 ^{abcd}	±	
0 - 0.025	1.04 ± 0.91	2.29 ± 0.31	1.33 ± 1.79	2.22 1.31	±	2.08 ± 1.68	1.79 ± 0.51 ^{ef}		
0 - 0.05	1.35 ± 0.01	2.69 ± 0.27	2.83 ± 0.56	2.81 0.19	±	3.12 ± 0.05	2.56 0.62 ^{abcd}	±	
0 - 0.075	1.16 ± 0.24	2.70 ± 1.09	2.34 ± 0.13	2.56 0.12	±	2.94 ± 0.03	2.34 0.62 ^{bcde}	±	
0.05 - 0	2.98 ± 1.46	2.60 ± 0.99	3.10 ± 1.99	2.93 1.95	±	2.99 ± 0.98	2.92 ± 0.17 ^{ab}		
0.05 0.025	-	1.30 ± 1.12	2.28 ± 1.33	2.96 ± 3.64	1.86 1.16	±	1.94 ± 1.14	2.07 ± 0.55 ^{def}	
0.05 - 0.05	2.58 ± 2.11	3.01 ± 1.64	3.35 ± 2.73	3.07 2.07	±	3.40 ± 2.02	3.08 ± 0.29 ^a		
0.05 0.075	-	2.13 ± 1.11	3.20 ± 1.97	3.44 ± 2.17	3.12 1.72	±	3.66 ± 2.03	3.11 ± 0.53 ^a	
0.1 - 0	2.37 ± 0.34	1.72 ± 0.85	1.42 ± 0.43	1.35 1.51	±	1.43 ± 1.76	1.66 ± 0.38 ^f		
0.1 - 0.025	1.92 ± 0.88	2.25 ± 0.69	2.63 ± 0.61	2.86 0.69	±	3.23 ± 0.06	2.58 0.46 ^{abcd}	±	
0.1 - 0.05	2.13 ± 0.55	2.60 ± 0.41	2.73 ± 0.41	3.03 0.72	±	3.26 ± 0.28	2.74 ± 0.40 ^{abc}		
0.1 - 0.075	1.42 ± 0.98	2.11 ± 0.63	2.32 ± 0.52	2.57 0.24	±	2.67 ± 0.37	2.22 0.45 ^{cdef}	±	
Mean	1.88 0.59 ^b	± 2.53 0.40 ^a	± 2.56 0.64 ^a	± 2.59 0.51 ^a	±	± 2.82 0.64 ^a			

Note: The a, b, c,... superscript letter(s) in the same column and row are significantly different ($p < 0.05$).

¹PS and ²AA concentration (%).

3.3 Effect of potassium sorbate and ascorbic acid on pH and TSS of pumpkin puree

The pH of pumpkin puree remained unchanged during the surveyed period ($p > 0.05$). The pH value of pumpkin puree samples decreased by adding a higher concentration of ascorbic acid ($p < 0.05$) (data not shown). The average pH value of pumpkin puree was 5.89 ± 0.21 on d 0 which was slightly lower compared to a finding of Kampuse et al. (2019) in which pH ranged from 6.2 to 6.6 at the beginning of storage.

The TSS of pumpkin puree dropped significantly and decreased by 1.35 (from 15.78 ± 0.79 to 14.43 ± 0.53 °Bx) after 15 d ($p < 0.05$) (data not shown). This behavior is likely due to microorganisms consuming sugars and soluble solids for microorganisms' growth (Huang et al., 2015). The same results were also found by Assous et al. (2014) with a slight decrease in total soluble solids of pumpkin cubes packed with sucrose solution, pumpkin cubes packed with orange juice, and pumpkin cubes packed with orange juice after six months of storage of pumpkin cubes packed with orange juice.

3.4 Effect of potassium sorbate and ascorbic acid on water activity of pumpkin puree

Water activity is a vital parameter for predicting the stability and shelf-life of food products (Lianou et al., 2016). The water activity of pumpkin puree is displayed in Table 3. Generally, the water activity of all samples was high with a range of 0.91 to 0.92 and these values kept stable after 15 d. Besides, the water activity value of pumpkin puree changed remarkably with the addition of potassium sorbate and ascorbic acid, reaching 0.1 and 0.075 % ($p < 0.05$). This behavior contrasts with Kampuse et al. (2019), who reported a decrease in water activity in pumpkin puree stored at 4 ± 2 °C during 24 d of storage. This is probably because the length of storage intervals is proportional to the growth of microorganisms, so the free water might have been taken up by the bacteria

from the surroundings through their cell wall resulting in a reduction in water activity. The high water activity of all samples indicated that it was high susceptibility to microbiological spoilage (Devi et al., 2016).

Table 3: Change of water activity value of pumpkin puree during chilled storage

Samples	Storage intervals (d)							Mean
	0	3	6	9	12	15		
0 ¹ -0 ²	0.92 ± 0.01	0.93 ± 0.02	± 0.92 ± 0.01	0.91 ± 0.01	± 0.92 ± 0.01	± 0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01
0 - 0.025	0.92 ± 0.01	0.93 ± 0.01	± 0.92 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01	± 0.91 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01
0 - 0.05	0.92 ± 0.01	0.92 ± 0.01	± 0.91 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01	± 0.91 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01
0 - 0.075	0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.02	0.90 ± 0.01	± 0.90 ± 0.01	± 0.90 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01
0.05 - 0	0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01	0.92 ± 0.01	± 0.91 ± 0.01	± 0.91 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01
0.05 - 0.025	0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.02	0.91 ± 0.01	± 0.91 ± 0.01	± 0.91 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01
0.05 - 0.05	0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01	0.91 ± 0.01	± 0.90 ± 0.01	± 0.90 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01
0.05 - 0.075	0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01	0.91 ± 0.01	± 0.90 ± 0.01	± 0.90 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01
0.1 - 0	0.92 ± 0.01	0.91 ± 0.01	± 0.88 ± 0.06	0.91 ± 0.01	± 0.92 ± 0.01	± 0.92 ± 0.01	0.92 ± 0.01	± 0.92 ± 0.01
0.1 - 0.025	0.92 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01	0.92 ± 0.01	± 0.91 ± 0.01	± 0.91 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01
0.1 - 0.05	0.92 ± 0.01	0.93 ± 0.04	± 0.93 ± 0.02	0.90 ± 0.01	± 0.91 ± 0.01	± 0.91 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01
0.1 - 0.075	0.92 ± 0.01	0.91 ± 0.02	± 0.90 ± 0.03	0.91 ± 0.02	± 0.91 ± 0.01	± 0.91 ± 0.01	0.91 ± 0.01	± 0.91 ± 0.01
Mean	0.92 ± 0.01 ^{ab}	± 0.92 ± 0.01 ^a	± 0.91 ± 0.01 ^{bc}	± 0.91 ± 0.01 ^c	± 0.91 ± 0.01 ^c	± 0.91 ± 0.01 ^c	± 0.92 ± 0.01 ^{abc}	± 0.92 ± 0.01 ^{abc}

Note: The a, b, c,... superscript letter(s) in the same column and row are significantly different ($p < 0.05$).

¹PS and ²AA concentration (%).

3.5 Effect of potassium sorbate and ascorbic acid on microbiology of pumpkin puree

3.5.1 Yeast and molds, lactic acid bacteria, and *E. coli*/Coliforms

After 15 d of storage, the growth of total yeast and molds, lactic acid bacteria, and *E. coli*/Coliforms was not observed in any of the samples during the storage ($< 1.00 \log \text{CFU/g}$). It is due to the effectively selected processing methods that reduce the counts of lactic acid bacteria, yeast, and molds under the detection limit. The current study agreed with the previous research, stating that yeast cell development was delayed, when samples were stored at low temperatures, providing at least six months of shelf-life (Kampuse et al., 2019). When stored at $4 \pm 2 \text{ }^\circ\text{C}$ and frozen at $-20 \pm 2 \text{ }^\circ\text{C}$, yeast cells in pasteurized pumpkin puree are in anabiosis (Kampuse et al., 2019). From a microbial aspect, the pumpkin puree product remained edible throughout the surveyed storage period. This assessment is based on microbial quality indicators, including total yeast and molds and lactic acid bacteria, as well as safety quality indicators, such as *E. coli* and coliforms.

3.5.2 Total aerobic mesophilic counts

Total aerobic mesophilic counts in food products reflects the overview quality and safety of food (Mendonca et al., 2020). An evaluation of microbiological counts of pumpkin puree during storage in different concentrations of potassium sorbate and ascorbic acid is shown in Table 4. Table 4 indicates that the initial total aerobic mesophilic counts of all samples were below $3.00 \log \text{CFU/g}$. These aerobic mesophilic bacteria counts were noticeably lower than pumpkin puree under vacuum-cooked stored at $4 \text{ }^\circ\text{C}$ on d 0 ($4.00 \log \text{CFU/g}$) (Kampuse et al., 2019). The average total aerobic mesophilic counts of pumpkin puree increased significantly ($p < 0.05$) over 15 d of preservation and reached levels of $4.28 \pm 0.28 \log \text{CFU/g}$ on 12 d and remained unchanged until 15 d ($p > 0.05$). However, adding chemical additives at various concentrations could not show an apparent inhibition ability among experiments ($p > 0.05$). Regarding decision 46/2007/QĐ-BYT of the Ministry of Health of Viet Nam, the limit for the number of total aerobic mesophilic counts is less than $4.00 \log \text{CFU/g}$ in terms of processed vegetable products; in turn, all samples of pumpkin puree were acceptable to microbial aspects under 6 d of storage. The same results were found by Gliemmo et al. (2010) that adding potassium sorbate to pumpkin

puree significantly inhibited the aerobic bacterial, fungal, and yeast population while storing at 25 °C compared to puree without potassium sorbate.

Table 4: Change of total aerobic mesophilic counts (log CFU/g) of pumpkin puree during chilled storage

Samples	Storage intervals (d)						Mean	
	0	3	6	9	12	15		
0 ¹ -0 ²	2.84 ± 0.19	3.28 ± 0.24	4.02 ± 0.27	4.33 ± 0.06	4.80 ± 0.04	4.74 ± 0.06	4.00 0.72 ^a	±
0 - 0.025	2.84 ± 0.19	3.52 ± 0.55	4.35 ± 0.25	4.42 ± 0.06	4.78 ± 0.08	4.79 ± 0.02	4.12 0.71 ^a	±
0 - 0.05	2.84 ± 0.19	3.49 ± 0.38	4.08 ± 0.08	4.17 ± 0.08	4.31 ± 0.05	4.43 ± 0.05	3.89 0.55 ^a	±
0 - 0.075	2.84 ± 0.19	3.74 ± 0.25	4.20 ± 0.11	4.47 ± 0.08	4.61 ± 0.03	4.77 ± 0.08	4.11 0.65 ^a	±
0.05 - 0	2.84 ± 0.19	2.87 ± 0.34	3.11 ± 0.34	3.88 ± 0.27	4.08 ± 0.06	4.26 ± 0.02	3.51 0.58 ^a	±
0.05 0.025	2.84 ± 0.19	2.87 ± 0.65	3.26 ± 0.01	3.45 ± 0.44	4.08 ± 0.05	4.27 ± 0.13	3.46 0.55 ^a	±
0.05 - 0.05	2.84 ± 0.19	2.92 ± 0.71	3.16 ± 0.95	3.64 ± 0.28	4.14 ± 0.04	4.45 ± 0.13	3.52 0.61 ^a	±
0.05 0.075	2.84 ± 0.19	2.85 ± 0.57	2.86 ± 0.5	3.22 ± 0.43	4.11 ± 0.09	4.43 ± 0.27	3.38 0.65 ^a	±
0.1 - 0	2.84 ± 0.19	3.29 ± 0.06	3.40 ± 0.13	3.81 ± 0.24	4.25 ± 0.11	4.38 ± 0.04	3.66 0.54 ^a	±
0.1 - 0.025	2.84 ± 0.19	3.13 ± 0.11	3.63 ± 0.03	3.79 ± 0.33	4.31 ± 0.14	4.44 ± 0.33	3.69 0.58 ^a	±
0.1 - 0.05	2.84 ± 0.19	3.18 ± 0.11	3.42 ± 0.03	3.76 ± 0.27	3.92 ± 0.41	4.21 ± 0.11	3.56 0.46 ^a	±
0.1 - 0.075	2.84 ± 0.19	3.10 ± 0.13	3.55 ± 0.12	4.09 ± 0.56	3.99 ± 0.51	4.38 ± 0.05	3.66 0.55 ^a	±
Mean	2.84 0.19 ^e	± 3.19 0.27 ^d	± 3.59 0.46 ^c	± 3.92 0.37 ^b	± 4.28 0.28 ^a	± 4.46 0.19 ^a		

Note: The a, b, c,... superscript letter(s) in the same column and row are significantly different ($p < 0.05$).

¹PS and ²AA concentration.

4. Conclusion

Pumpkin puree with 10 % sucrose, 0.05 % potassium sorbate, and 0.05 % ascorbic acid maintained quality and safety for up to 9 d at chilled storage of 10 ± 2 °C. After 9 d of storage, the pumpkin puree remained acceptable with pH (5.93 ± 0.20), TSS (14.5 ± 2.97 °Bx), water activity (0.91 ± 0.01), the total carotenoids content (16.72 ± 4.21 mg%), the total aerobic mesophilic counts (3.64 ± 0.28 log CFU/g), the total yeast and molds (< 1.00 log CFU/g), the lactic acid bacteria (< 1.00 log CFU/g), and undetectable *E.coli* and Coliforms. These results are valuable for producers using puree pumpkin in added valued products.

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