



Evaluation of Antioxidant Capacity and Bioactive Compounds in *Rosa damascena*: A Comparative Study of Drying Methods and Growth Phases

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Abstract

Rosa damascena, renowned for its fragrant flowers and therapeutic properties, is highly valued. Given the economic and therapeutic value of *Rosa damascena* essential oils, optimizing various factors is crucial for enhancing yield and quality. This study investigates the impact of drying temperatures and harvest times on the morphological traits and phytochemical compounds of *Rosa damascena* cultivated in Kafshi-Mahalleh Village, Golestan Province, Iran. Flowers were harvested in three stages: buds, half-bloomed, and fully bloomed, and subjected to drying at 25°C, 30°C, 40°C, and 50°C. The measured parameters include morphological and yield traits (fresh weight, dry weight, receptacle diameter, petiole length, petal length, petiole diameter, and petal length) and phytochemical traits (essential oil percentage, geraniol and citronellol content, total phenol, total flavonoid, and antioxidant activity by DPPH method). The results indicate significant variations in the chemical composition and quality of the essential oils based on the drying temperature and harvest stage. Higher drying temperatures generally reduced essential oil percentages, while optimal harvest timing improved key aromatic compounds. The highest levels of geraniol were observed in dried petals at 30 °C harvested in the first stage. However, the highest level of citronellol was recorded in petals dried at 25 °C in the third harvest (full bloom). Additionally, antioxidant activity was highest in flowers dried at 25°C and 30°C. This finding aligns with the higher phenolic and flavonoid content observed at these temperatures. The results provide valuable insights for improving production practices and ensuring high-quality essential oils.

Keywords: citronellol, essential oil, geraniol, petal, phenol

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Introduction

Rosa damascena Mill L. is a shrub of the Rosaceae family. This plant is a hybrid of *Rosa gallica* L. and

Rosa moschata Herrm. It is one of the wild rose species grown in Iran, Syria, Morocco, and Australia. This dense shrub is world-famous for its pink to red, plump, and fragrant flowers (Moein et al., 2010; Yaghoobi et al., 2022). *Rosa* plant products are used in traditional medicine. They were also used as medications until the first

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decades of the twentieth century. The extract obtained from the distillation of petals was used in the Middle Ages and the Renaissance to treat depression (Akram et al., 2020).

Furthermore, given Iran's arid and semi-arid climate and the lack of water resources, the cultivation of *Rosa damascena* in areas facing water constraints can contribute to increasing water productivity and the sustainable development of the agricultural (Ahmadi et al., 2019). *Rosa damascena* is highly resistant to adverse environmental conditions and has high economic value. Thus, it is grown in many areas across the country, especially in the foothills, where the possibility of growing many crops is limited. For this reason, it is considered a plant with significant strategic and economic value (Loghmani-Khouzani, 2007).

Rosa damascena is the best species of this plant due to its high-quality essential oil. However, it has a low share in production and export due to the need for more processing and post-harvest facilities and the non-compliance of its products with the latest export standards. These problems have permanently restricted the export of plant products, especially medicinal products derived from plants in Iran (Ebrahimi and Sharif Zadegan, 2016). The quality and yield of essential oils from *Rosa damascena* are influenced by various agronomic and post-harvest factors, notably the drying temperature and harvest timing.

The drying process is a critical step in producing essential oils as it affects the preservation of the volatile compounds responsible for the aroma and therapeutic properties of the oil (Chua et al., 2019). Different drying temperatures can lead to significant variations in the essential oils' chemical composition and overall quality. Usually, different parts of harvested plants contain large percentages of moisture (between 60-80%) (Sařata et al., 2020). High moisture levels make the product susceptible to microbial attack and spoilage. To prevent these problems, the moisture content should be reduced to 10-14% for a better product with more stable storage capability (Lira-Ricárdez and Cabello, 2024; Prusinowska and Smigielski, 2015). The drying temperature varies

depending on the type of active ingredients. Drying the desired organs of medicinal plants at high temperatures decreases the population of fungi and bacteria. However, it should be noted that excessive temperature reduces the percentage of essential oil (Rocha et al., 2011). Rapid and complete drying of plants containing essential oils helps preserve their color and secondary metabolites.

Harvest time is another pivotal factor that determines the quality of essential oils (Ostadi et al., 2020). The timing of the harvest influences the flowers' physiological and biochemical state, impacting the essential oils' concentration and composition. For instance, the linalool content decreased in the final harvest time, whereas linalyl acetate, another significant component, slightly increased from the initial to the final harvest date. Notable changes were observed in other individual components as well. Specifically, the concentration of eucalyptol decreased, while terpineol increased up to the last harvest (Khaiper et al., 2024).

Given the importance of these factors, it is essential to optimize both drying temperature and harvest time to enhance the quality and yield of essential oils from *Rosa damascena*. This study aims to systematically investigate the effect of varying drying temperatures (25, 30, 40, and 50 °C) and three harvest times (buds to fully bloomed flowers) on some morphological traits and the phytochemical compounds extracted from *Rosa damascena*. Understanding these effects can provide valuable insights for improving production practices and ensuring the high quality of essential oils, thereby benefiting both producers and end-users in the essential oil industry.

Materials and Methods

Plant Source

This study was conducted on *Rosa damascena* flowers cultivated in Kafshi-Mahalleh Village, located at 366506 E 4102276 N, 1255 m above sea level, in Minoodasht County, Golestan Province, Iran.

Harvest Operations

The sampling phase in this experiment began with the formation of flower buds in early May, in three stages. In the first stage, buds were harvested and transferred to the university in paper bags for drying. The second-stage harvest was performed ten days later, by harvesting samples with half-bloomed flowers. Finally, in the third stage, fully bloomed flowers were picked and transferred to the laboratory. The samples were dried at different temperatures, and physical and biochemical analyses were performed on the flowers and essential oil components.

Drying Condition

The collected *Rosa damascena* flowers were transferred to the laboratory immediately after harvest. After measuring the fresh weight and morphological traits, petals were dried at ambient temperatures of 25 °C, and in an oven at 30, 40, and 50 °C.

Morphological Traits

In this experiment, various physical variables affecting the quality of the product, such as fresh weight, dry weight, receptacle diameter, petiole length, petal length, petiole diameter, and petal length, were measured.

Biochemical Traits

To measure total phenol, flavonoids, and antioxidants, 1 g of each dried sample was mixed in a solution of water and methanol in a shaker for 24 hours. After filtering, the desired factors were analyzed with a spectrophotometer. Furthermore, to evaluate the percentage of essential oil, geraniol, and citronellol, the essential oils of the flowers were extracted with a Clevenger apparatus, and the compounds were measured using a GC/Mass device.

Total Phenol (TP) Determination

Total phenol was measured using the method proposed by Slinkard and Singleton (1977). 20 µl of methanolic extract was diluted with 1.16 ml of

deionized water. Afterward, 100 µl of Folin-Ciocalteu reagent was added to the solution and placed in a dark place for 6 minutes to rest and activate the Folin-Ciocalteu reagent. After the necessary time, 300 µl of sodium carbonate was added to the solution. The solution was then placed in a hot water bath at 40°C for 30 minutes. The control sample was used to calibrate the spectrophotometer at 765 nm. Using the gallic acid calibration curve, total phenol was calculated in terms of gallic acid equivalents in one gram of dry plant.

Total Flavonoid (TF) Determination

The aluminum chloride colorimetric method developed by Chang et al. (2002) was used to measure total flavonoids. First, 0.5 ml of the methanolic extract with 1.5 ml of 80% methanol, 0.1 ml of 10% aluminum chloride in ethanol, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water were mixed in a tube. The resulting mixture was kept in the dark for 30 minutes. Afterward, a spectrophotometer at 415 nm immediately measured the absorbance rate. The total flavonoid content was measured using quercetin equivalents in one gram of dry plant.

Antioxidant Activity (DPPH Assay)

Bartoz (2003) method was used to calculate the total antioxidant capacity (TAC). First, 1 ml of the methanolic extract and 1 ml of the 0.1 mM DPPH reagent were added to the 20 ml tube. The test tubes containing the solution were then kept in the dark for 30 minutes to activate radical inhibition by the DPPH. After the required time, the absorbance rate was measured by a spectrophotometer at 517 nm. For measurement, the device was first calibrated with 80% methanol, and then the DPPH solution was read, followed by the rest of the samples. The readings were calculated using the final antioxidant calculation formula:

$$\text{DPPH free radical scavenging percentage} = \frac{A_c - A_s}{A_c} \times 100$$

Table 1
The effect of harvest time on morphological traits of *Rosa damascene*

Harvest Times	Fresh Weight	Dry Weight	Receptacle Diameter	Petiole Length	Petiole Diameter	Petal Length
First Harvest	27.73 ^b	12.35 ^a	2.96 ^b	1.73 ^c	0.67 ^b	2.71 ^b
Second Harvest	31.58 ^a	12.81 ^a	3.93 ^b	3.65 ^b	0.7 ^b	2.96 ^b
Third Harvest	26.99 ^b	11.93 ^a	4.76 ^a	5.45 ^a	1.5 ^a	4.93 ^a

Where A_c is the control sample's absorbance rate, and A_s is the absorbance rate of the other samples.

Essential Oil Extraction and GC/MS Analysis

The petals from the Kafsh-Mahalleh area were subjected to steam distillation for 4 hours using a Clevenger apparatus, following the procedure outlined in the European Pharmacopoeia to isolate the volatile fraction. GC/MS analysis was performed on an Agilent-5975C mass selective detector coupled with a 7890A gas chromatograph, equipped with a cross-linked 5% pH ME siloxane HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The MS operating parameters were as follows: Ionization potential, 70 eV; ionization current, 2A; resolution, 1000. The initial temperature of the column was 60 °C with a temperature gradient of 5 °/min; after reaching 250 °C, it remained at this temperature for 2 minutes.

Data Analysis

This study used a factorial arrangement within a completely randomized design with three replications. The collected data were analyzed using SAS software. The mean values were compared with LSD, and the curves were plotted using Excel.

Results

The Effect of Harvest Time on Morphological Traits of *Rosa damascene*

The results of the analysis of variance (ANOVA) show that the harvest time significantly affects fresh weight, receptacle diameter, petiole length and diameter ($p < 0.01$), and petal length ($p < 0.05$).

The highest fresh weight (31.58 g) was observed in the flowers harvested in the second stage, in a half-bloomed state. Furthermore, the flowers' highest dry weight was found in the first (12.35 g) and second harvests (12.81 g). However, there was no significant difference in dry weight between the flowers at all three harvest stages. The lowest dry weight (11.93 g) was found in fully bloomed flowers harvested in the third stage (Table 1).

Table 1 also shows the effects of harvest time on petal length, receptacle diameter, and petiole length and diameter. As can be seen, these physical traits have the highest values in fully bloomed flowers compared to the initial stages of plant growth. In medicinal and aromatic plants such as damask rose flowers, the desired economic product is assessed in terms of flower yield and essential oils per unit area, and harvest phases should be managed so that the maximum flower yield is achieved while maintaining the quality and quantity of essential oils.

The Impact of the Interaction of Harvest Time and Drying Temperature on Phytochemical Traits

The analysis of variance and the curves for the interaction between harvest time and oven temperature showed that these two treatments significantly affected the content of the secondary compounds and the essential oil of *Rosa damascena* flowers.

As shown in Fig. 1, the essential oil (EO) content of *Rosa damascena* petals, harvested at three different stages and dried at four different temperatures, ranged from 0.002% to 0.281% based on dry weight (% v/w DW). The results revealed significant differences in EO content ($p \leq 0.05$). Fully bloomed flowers had a notably lower

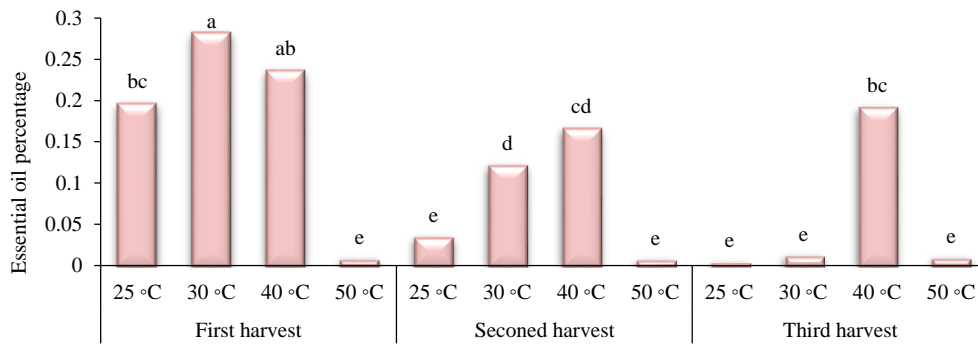


Fig. I. The effect of harvest time and drying temperature on the essential oil percentage of *Rosa damascena*.

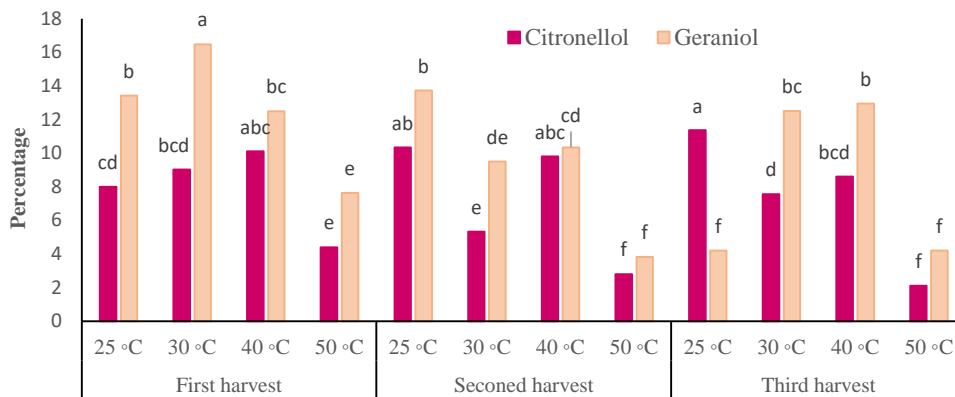


Fig. II. The effect of harvest time and drying temperature on Geraniol and Citronellol of *Rosa damascena*

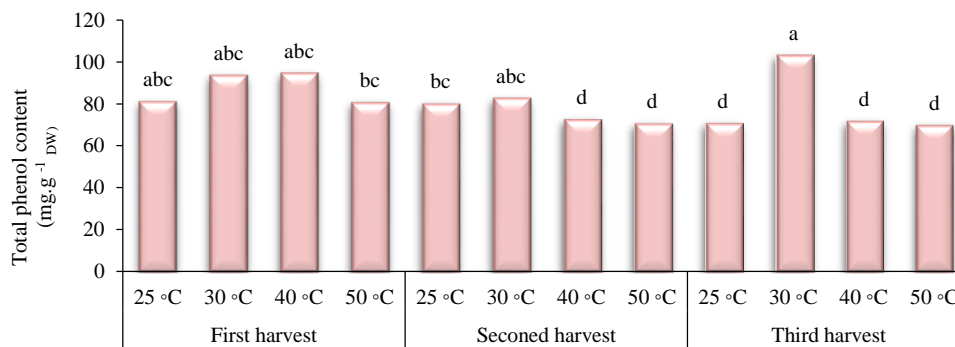


Fig. III. The effect of harvest time and drying temperature on the total phenol of *Rosa damascena*

percentage of essential oil. The highest EO content was observed in petals harvested at the first stage and dried at 30 °C (0.281%), surpassing other treatments. However, no significant differences were found at 50 °C across all three harvest times. Moreover, the highest levels of geranium (16.48%) were observed in dried petals at 30 °C harvested in the first stage (Fig. II). The geranium percentage in the first harvest was at its highest level and then decreased with increasing flower growth.

However, the highest level of citronellol (11.36%) was recorded in petals dried at 25 °C in the third harvest (full bloom). The total phenolic content of *Rosa damascena* populations was measured using the Folin-Ciocalteu colorimetric method. A standard curve of gallic acid ($R^2 = 0.98$, $y = 0.0096x - 0.2077$) was employed to determine the TP content, expressed in mg Gallic Acid Equivalent (GAE) per gram of dry weight. The data in this study revealed that the highest phenol content

(102.61 mg GAE/g DW) was observed in the third harvest stage, where a temperature of 30 °C was very effective in maintaining this compound (Fig. III). The phenol content was reduced at temperatures of 40 and 50 °C. However, the reduction rate was not significant between the second and third harvests. The low content of phenolic compounds at a temperature of 25 °C in the third harvest stage is most likely due to moisture in the *Rosa damascena* flowers and the lack of dry matter in one gram of dry weight.

The flavonoid content in the methanolic extracts was measured using the aluminium chloride colorimetric assay and reported as mg quercetin equivalent (QE) per gram of dry weight (g^{-1} DW), based on the quercetin standard curve ($y = 0.0015x - 0.0217$, $R^2 = 0.99$). The highest TF content (17.51 mg QE/g DW) was obtained from fully bloomed flowers harvested in the third

harvest, which dried at 30 °C (Fig. IV). The lowest content was also observed at 40 and 50 °C in the second and third harvest times. However, this decrease in TF content was also noted during the first harvest, but this decrease is less than the other two harvest times. Furthermore, the mean comparisons (Fig. V) indicated that at the 25 to 50 °C temperature range, the highest percentage of free radical scavenging was obtained from dried flowers at 25 and 30 °C. However, the lowest percentage was observed at 50 °C. This decrease in the percentage of free radical scavenging indicates a decrease in *Rosa damascena* petals' antioxidant properties with a gradual temperature increase. However, previous studies have shown that at very high temperatures (e.g., above 70 °C), antioxidant compounds are more preserved as there is less chance of the degradation of antioxidant compounds and inhibitory and destructive agents. Nevertheless, at low

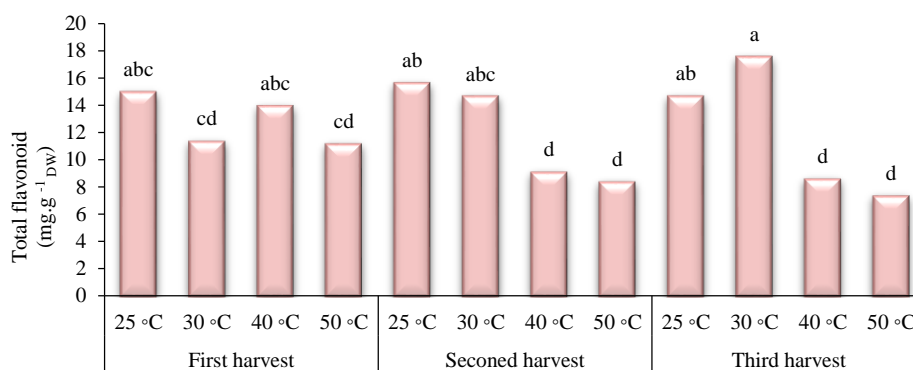


Fig. IV. The effect of harvest time and drying temperature on the total flavonoid of *Rosa damascena*.

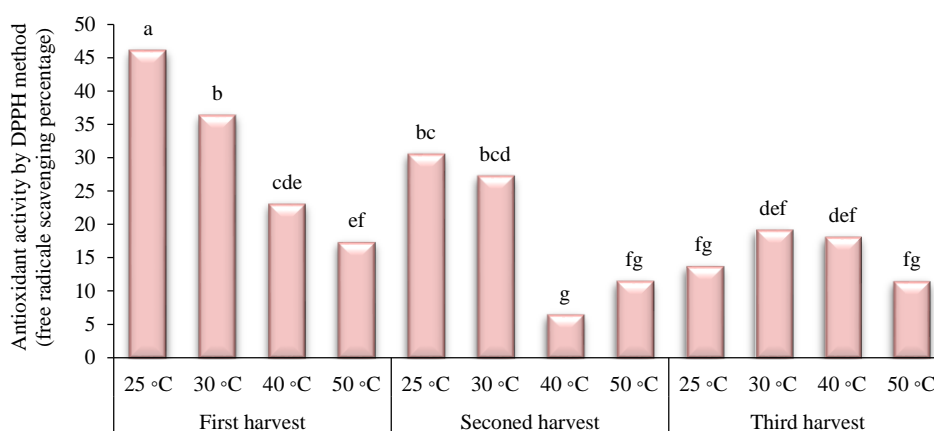


Fig. V. The effect of harvest time and drying temperature on the antioxidant activity of *Rosa damascena*

temperatures of 20 to 50 °C, due to the low adverse effects of heat, antioxidant compounds are affected, and the percentage of these compounds decreases with increasing temperature because, at low temperatures, the temperature erosion stress reduces the resistance of cells.

Discussion

The results of this study demonstrate the significant influence of harvest time and drying conditions on various physical and chemical properties of damask rose (*Rosa damascena*) flowers. The morphological parameters such as petal length, receptacle diameter, and petiole length and diameter reached their maximum values in fully bloomed flowers. This suggests that while physical growth is more pronounced in later stages, biomass accumulation might be more efficient in earlier stages of bloom. These observations are crucial for optimizing flower yield, particularly in medicinal and aromatic plants like *Rosa damascena*, whose economic value is tied to flower yield and essential oil content. Kanani et al. (2021) demonstrated that the fresh weight of the plant increased from the flower bud stage to the fully bloomed flower, and with the beginning of the flower aging process, their amount decreased. The increase in the fresh weight of the flower during the development of the petals until the flower fully bloomed can be due to the increase in water absorption to enhance turgor pressure and ensure the freshness of the flower (Schmitzer et al., 2013). The increase in fresh flower weight in the rose flower during the development of the petals until the full bloom stage was also reported by Sood et al. (2006).

Essential oil (EO) content analysis indicated significant variations depending on the harvest stage and drying temperature. Notably, fully bloomed flowers had a significantly lower EO percentage. The study also evaluated essential secondary compounds such as geraniol and citronellol concentrations. The highest geraniol levels were recorded in petals dried at 30 °C from the first harvest stage, while the highest citronellol content was found in petals dried at 25 °C from the third harvest stage. This differential response

underscores the complex interaction between the harvest stage and drying temperature in determining the profile of secondary metabolites in *Rosa damascena*. Izgi (2022) investigated the harvest date of *Rosa damascena* and reported that the total of major components of essential oil (geraniol, citronellol, and nerol) decreased from the first to the last harvest, which was thought to be attributable to rising temperatures and decreasing relative humidity. Kanani et al. (2021) concluded that the highest ratio of citronellol/geraniol was produced at the full bloom stage.

Flavonoid and phenolic content, another critical quality attribute, was highest in the third harvest stage at 30 °C, emphasizing that later stages of bloom, combined with moderate drying temperatures, yield beneficial compounds. This suggests that while fully bloomed flowers may have lower EO content, they may still hold significant value for their phenolic compounds, which have numerous health benefits. Antioxidant activity was highest in flowers dried at 25 °C and 30 °C. This finding aligns with the higher phenolic and flavonoid content observed at these temperatures, highlighting the importance of moderate drying conditions in preserving the antioxidant properties of rose petals. However, at higher temperatures (e.g., 50 °C), antioxidant activity significantly decreased, reflecting the degradation of heat-sensitive antioxidant compounds. Antony and Farid (2022) state that a significant temperature rise deactivates polyphenol oxidases and damages the structure of polyphenolic compounds.

Additionally, high temperatures lead to the breakdown of phenolic chains and cell walls, causing phenolic compounds to be released and their percentage to diminish (Sim et al., 2017; Smirnoff, 2005; Yang et al., 2017). The findings were consistent with certain studies on mushrooms, fruits, and seeds, challenging the reduction that TP decreases with high-temperature drying (Al Juhaimi et al., 2018; Gąsecka et al., 2020). Mutukwa et al. (2019) reported that oven drying at 43 °C did not impact the TP or TF content in *Pleurotus ostreatus*. In another study, it was clear that oven drying

significantly reduced the DPPH radical scavenging activity of *Grifola frondosa* compared to fresh mushrooms (Sim et al., 2017).

Conclusion

In summary, the optimal harvest stage and drying temperature for *R. damascena* depend on the desired quality attributes. Early-stage harvesting and moderate drying temperatures (around 30°C) maximize essential oil content, while later stages

and similar drying conditions favor the retention of phenolics, flavonoids, and antioxidant properties. These insights are pivotal for the commercial production and processing of *R. damascena*, ensuring maximum yield and quality of essential oils and secondary metabolites. Future studies should explore the mechanistic basis of these observations and consider the potential trade-offs between different quality attributes to optimize overall product value.

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