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EFFECT OF THE ADDITION OF SELECTED HERBS ON THE TECHNOLOGICAL AND SENSORY QUALITY OF BEER

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ABSTRACT

The aim of this work was to enhanced functionality of beer with natural materials such as selected herbs from *Lamiaceae* family (salvia, oregano, wild thyme, lemon balm) and consequently to determine the impact of the addition of herbs on the technological parameters and sensory profile of beer. For purposes of this study a top-fermented beer was produced and herbs: salvia (*Salvia officinalis* L), oregano (*Origanum vulgare* L.), wild thyme (*Thymus serpyllum* L.) and lemon balm (*Melissa officinalis* L.) were used for beer fortification. The basic qualitative parameters of beer as real extract, alcohol content, pH, colour, total polyphenol content and antioxidant activity were determined. Results showed that in the analysed samples, the values of the actual extract ranged from 3.64 wt.% to 4.02 wt.%. Alcohol content ranged from 3.99 vol.% to 4.46 vol.%. Total polyphenol content was significantly increased by the herbs addition to beer. The content of phenolic substances increased the most in beer with oregano 801.63 mg GAE.g^{-1}. Moreover, the antioxidant activity in beer samples enriched with herbs was significantly increased; it ranged from 50.30 mg TEAC.g^{-1} (control beer) to 78.17 mg TEAC.g^{-1} (beer with oregano). Values of antioxidant activity were positively correlated with the content of total polyphenols. Sensory evaluation of beer was conducted using a nine-level hedonic scale. Sensory analyses revealed that the most intense, pleasurable herbal aroma and taste was evaluated in beer with salvia. From the overall impression point of view beer enriched with wild thyme manifested the best performance.

Keywords: beer, herbs, antioxidant activity, sensory quality

INTRODUCTION

Beer belongs to the world's most popular beverages. Beer, as a completely natural product, contains numerous beneficial ingredients such as carbohydrates, amino acids, organic acids, vitamins, bitter substances of hops, phenolic compounds, but also specific ingredients with potentially beneficial effects on a human body (**Olšovská *et al.*, 2014; Djordjevic *et al.*, 2016**). From a nutritional point of view, the interest in beer is very high. This is due to the rich content of antioxidant compounds. Beer contains a wide range of phenolic compounds, most of which come from barley malt (80%) and hops (20%). Antioxidants with the structure of polyphenols are also contained in barley and malt. The phenolic compounds of barley have antioxidant and antiradical properties. They also play an important role in determining the stability of beer and its sensory properties (**Běláková *et al.*, 2010; Dvořáková *et al.*, 2010**). **Mikyška *et al.* (2011)** state that polyphenolic substances are the basic structural components of the cellular structures of barley grain. The content and composition of polyphenols depends on the genetic disposition of barley, but also on the climatic conditions during its vegetation. The most widespread group of polyphenols are flavonoids. In brewing raw materials, they are present in both monomeric and polymeric form. The second most represented group of polyphenols are phenolic acids, which are divided into a group of cinnamic acid derivatives (ferulic acid is the best known of them in the brewing industry) and ρ-hydroxybenzoic acid derivatives (**Leskošek-Čukalović *et al.*, 2010; Pluháčková *et al.*, 2020**). The antioxidant activity of beer depends on both the amount and composition of polyphenols. Polyphenolic antioxidants are important not only from a sensory point of view, but also from a health point of view (**Mikyška *et al.*, 2011; Sohrabvandi *et al.*, 2012**). It is reported that drinking beer to a reasonable extent increases the antioxidant and anticoagulant activities of blood plasma, positively affects the level of lipids in the blood, and has protective effects on the human cardiovascular system (**Nardini and Foddai, 2020**).
The effort to improve beer sensory and qualitative properties has always been an attraction for many brewers. Adding herbs to beer is a known practice since the Middle Ages, used to enhance taste and aroma (**Habschied *et al.*, 2020**). Aromatic plants are considered very interesting for their taste and medicinal properties along with human consumption as well as animal food. A large number of these aromatic species belong to the *Lamiaceae* family (**De Falco *et al.*, 2013**). Ancient brewers affected beer sensory properties, its stability and health safety by adding herbs. The herbs used differed mainly according to the territory where the beer was brewed (**Hornsey, 2003**). Herbal ingredients can interestingly change the taste, aroma, colour, and sharpness of beer (**Pluháčková *et al.*, 2020**). Similarly, according to **Djordjevic *et al.* (2016)** extracts of aromatic herbs can be a suitable solution for refining beer. Authors state that herbal extracts can be used in the food and beverage industry for the production of a wide range of products with defined functional properties.
Lamiaceae is a family of species, also known as the mint family widely cultivated as culinary herbs, such as basil, oregano, rosemary, thyme, mint and sage (**Marchioni *et al.*, 2020**). Wild thyme (*Thymus serpyllum*) comes from the Mediterranean region. *Thymus serpyllum* is an aromatic plant that contains a high amount of essential oils. Essential oils are biologically active substances that show a high radical scavenging potential and anti-inflammatory activity. These properties are used in the pharmaceutical industry to prepare antihypertensive, anti-inflammatory, anti-proliferative and anti-cancer drugs (**Galovičová *et al.*, 2021**). Also, experimental results show that commercially produced beer enriched with *Thymus serpyllum* tinctures shows improved antimicrobial and antioxidant properties (**Leskošek-Čukalović *et al.*, 2010**). Oregano (*Origanum vulgare*) comes from the Mediterranean region and western Eurasia. It contains a large number of active components, including phenolic glycosides, flavonoids, tannins, sterols and a high amount of terpenoids. Monoterpene hydrocarbons (ρ-cymene and γ-terpinene), oxygenated monoterpenes (thymol, 4-terpineol, carvacrol, trans-sabinene hydrate), sesquiterpene hydrocarbons (γ-terpinene, caryophyllene oxide) are considered to be the main components of *Origanum vulgare* essential oils (**Pezzani *et al.*, 2017**). Several studies indicate a connection between the antioxidant potential of medicinal plants and the concentration of their phenolic compounds, which include phenolic acids, flavonoids, anthocyanins and tannins (**Khiya *et al.*, 2021**). Authors also claim that phenolic compounds such as carnosol, rosmarinic and carnosic acids, caffeic acid, rosmanol, rosmadial, and cirsimaritin, which have the highest antioxidant activity among them, can be isolated from salvia (*Salvia officinalis* L) extract. The commercial importance of the plant *Salvia officinalis* is due to the richness of phenolic and volatile compounds such as essential oil. *S. officinalis* has a power antioxidant activity. In addition, rosmarinic acid and flavonoids as quercetin and rutin also have strong antioxidant activity. Chemical studies on the composition of lemon balm *Melissa officinalis* have shown that it mainly contains flavonoids, terpenoids, phenolic acids and tannins. The main active ingredients are volatile compounds (citral, neral, citronellal and geraniol), triterpenes (ursolic acid and oleanolic acid), phenolic compounds (rosmarinic acid, caffeic acid and protocatechuic acid) and flavonoids (quercetin, rhamnocitrin, luteolin) (**Petrisor *et al.*, 2022**). Experimental results indicate that commercially

produced bottled beer enriched with *Thymus vulgaris* and *Melissa officinalis* tinctures exhibits improved antimicrobial and antioxidant properties (**Leskošek-Čukalović *et al.*, 2010**). Thanks to its light citrus aroma, lemon balm is suitable as an addition to Belgian wheat beers, strong light beers (Tripel) and blonde ales (**Boulton, 2013**).
The aim of this study was to enhanced functionality of beer with natural materials such as selected herbs from *Lamiaceae* family (salvia, oregano, wild thyme, lemon balm) and consequently to determine the impact of the addition of herbs on the technological parameters and sensory profile of beer.

MATERIAL AND METHODS

Herbs

Herbal plants as salvia (*Salvia officinalis* L), oregano (*Origanum vulgare* L.), wild thyme (*Thymus serpyllum* L.) and lemon balm (*Melissa officinalis* L.) were collected in Slovak Republic and after drying were added to the beer during fermentation. Herbal plants were before application to beer homogenized to powder.

Beer

Control beer and beer for fortification with herbs (pale unfiltered top-fermented) was prepared in microbrewery of AgroBiotech Research Centre of Slovak University of Agriculture in Nitra. For beer production the Pilsner malt and hops from the Hallertau Blanc Company (α-acids content 10.1% by weight) and lyophilized yeast Safale US-05 were used. Crystal sugar was added to support the yeast fermentation.

Fortification of beer with herbs

To prepare the beer, 10 kg of Pilsner malt was used. The wort in the volume of 37.5 L was obtained by the process of decoction mashing into three mashes. In laboratory conditions, the wort was brought to a boil and then the hopping was run for 60 minutes. Hallertau Blanc hop granulate in the amount of 50 g was used. The resulting bitterness reached 20 IBU. The wort so prepared was cooled to the fermentation temperature and inoculated with top-fermentation yeast *Saccharomyces cerevisiae* var. *cerevisiae* (Safale US-05). Subsequently, 50 g of a specific type of herbs was added. Primary fermentation process was running for five days at room temperature 20 °C. After the main fermentation process, the young beer was filled into 500 mL glass bottles, where the secondary fermentation was carried out. 4.09 g of granulated sugar was added to each bottle to promote yeast activity. Secondary fermentation was carried out for six days at 2 °C in order to saturate with carbon dioxide and ensure the desired sensory properties of the beer.

Determination of beer basic technological parameters

All analyses of beer technological parameters were carried out in accordance with recognized methods within European Brewery Convention **(EBC Analytic, 2010)**. Qualitative parameters such as real extract, alcohol content, pH, colour, total polyphenol content and antioxidant activity were evaluated in the samples.
Alcohol content was determined in beer samples degassed (freed of CO_2) based on the methods of EBC 9.43.2 Analytic, using Alcolyzer Beer with DMA 4500M Densitometric Module (Anton Paar GmbH, Austria). The real wort extract was calculated based on the determined alcohol content of the beer according to the specific calculations given in the EBC Method 9.4 (**EBC Analytic, 2010**). Determination of beer pH was conducted according to EBC Method 9.35. Determination of colour was performed spectrophotometrically according to the method of EBC Method 9.6. This method can be applied to all types of beers. Beer absorbance was measured at a wavelength of 430 nm in a 10 mm cuvette.

Total polyphenol content

Total polyphenol content (TPC) of samples was measured spectrophotometrically, using the Folin-Ciocalteu reagent as described by **Singleton and Rossi (1965**). 0.1 mL of sample was mixed with 0.1 mL of the Folin-Ciocalteau reagent, 1 mL of 20% sodium carbonate, and 8.8 mL of distilled water. The samples were then mixed. After 30 minutes, the absorbance was measured at a wavelength of 700 nm. The total content of polyphenols was expressed based on the gallic acid calibration curve in mg GAE.g^{-1} (R^2=0.9978). All analyses were performed in triplicate.

Antioxidant activity DPPH method

Free radical scavenging activity of samples was measured using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) according to the procedures described by **Sánchés-Moreno *et al.* (1998**). 0.9 mL of the DPPH radical (Sigma Aldrich, GE; 0.025 g was dissolved in 100 mL of ethanol and subsequently diluted as necessary) and 0.1 mL of the sample was pipetted into the cuvette. The mixture was mixed and placed in the dark for 10 minutes. Subsequently, the decrease in absorbance was monitored on a spectrophotometer at 515 nm. The antioxidant activity was expressed on the basis of the calibration curve of Trolox (TEAC) in mg TEAC.g^{-1} of the sample (R^2=0.9881). All analyses were performed in triplicate.

Sensory analysis of beers

Beer samples were assessed by subjective evaluation of a sensory panel of 9 trained evaluators (2 men + 7 women) by means of an evaluation sheet using a nine-level hedonic scale. For the sensory evaluation of beer, 5 samples were prepared in triplicate, 4 of which contained the addition of herbs and one was a control sample, without the addition of herbs. Intensity scale from 1 - 9 points was used to rate each descriptor, with 1 being dislike extremely and 9 being like extremely. The evaluators compared individual samples based on their aroma, taste, fullness, sharpness and overall impression.

Statistical analysis

The experimental results were analysed with the statistical software Statsoft Statistica 12.5 (Statsoft Inc., Tulsa, USA). The experiment was performed in three replicates. To assess statistically significant differences among samples, the LSD multiple comparison test at $P<0.05$ was used and correlation between measured values was calculated by Pearson´s coefficient.

RESULTS AND DISCUSSION

The term extract refers to all soluble components present in raw materials for beer production (**Boulton, 2013**). For maltsters and brewers, the extract is one of the most important quality parameters. According to **Li *et al.* (2008)** the amount of extract will always be of fundamental economic importance as it affects the amount of beer that can be produced. Authors state that extract consists mainly of carbohydrates, along with a number of nitrogenous substances, polyphenols, salts and many other substances. Fermentable sugars represent a large part of the extract produced during mashing, which make up 61 to 65% of the total extract. The malt extract is mainly influenced by the characteristics of the barley grain, such as the composition of the husk, the thickness of the cell walls and the modification of the endosperm. It is also influenced by cultural and environmental factors. **Esslinger (2009**) states that the actual extract is an indicator of the amount of sugars, dextrin and proteins found in the wort, hopped wort which remains in the beer during and after fermentation. Results of this study showed that in the analysed samples, values of the actual extract ranged from 3.64 wt.% up to 4.02 wt.% (Table 1). The addition of herbs slightly reduced the values of the actual beer extract. Statistically significant ($p<0.05$) the highest content of extract was confirmed in control beer and the lowest in beer with oregano (Table 1).
Basařová *et al.* (2015) state that pH of beer is the result of the organic acids which were formed during fermentation. From the pH value point of view beer is considered to be a slightly acid drink. According to **Chládek (2007)**, the pH of beer should range from 4.0 to 4.9. **Anderson *et al.* (2019)** reports that lager beers have a pH in the range from 4.0 to 5.0. The level of acidity is largely influenced by the presence of organic acids. The results showed that the addition of herbs had almost no effect on the pH values (Table 1) and the beer could still retain its acid character.
In their study, **Anderson *et al.* (2019)** state, that the presence of alcohol is noticeable only during fermentation. Its amount does not depend only on the original level of the wort, but also on the composition of the malt, the mashing process, the yeast strain used, the course of fermentation, or artificial interventions by the brewer. The production of beers with higher sugar content and lower alcohol content is ensured by rapidly flocculating yeast with a low degree of fermentation. On the other hand, good quality beers with higher alcohol content and lower sugar concentrations are obtained by adding slowly flocculating yeast with a high degree of fermentation **(Viejo *et al.*, 2018).** Ethanol, together with carbonyl compounds and esters, is involved in creating the aroma and taste profile of beer **(Anderson *et al.*, 2019)**. Alcohol content ranged from 3.99 vol.% (control beer) to 4.46 vol.% (beer with lemon balm). The alcohol content of beers with herbs (salvia, oregano, lemon balm) was significantly different ($p<0.05$) from a control sample. According to **Esslinger (2009)**, the alcohol content in pale lagers usually range from 3.5-4.5 vol.%, which was also confirmed by the results of our study.
Colour measurement is very important in the brewing industry. The colour of beer is a parameter that depends primarily on the raw material used for its preparation and also on the processes that the grain goes through during the technological production process. The coloured components are partly caused by the Maillard reaction, but also by the oxidation processes of polyphenols released from barley husk (**Leskošek-Čukalović *et al.*, 2010**). The colour of beer is an important sensory attribute that the consumer notices first. **Koren *et al.* (2020)** claim that low colour values in EBC units are rather typical for pale beers, and higher colour values in EBC units belong to the darker types of beers. An important role in its stability plays the alcohol content in beer, which tends to react with free radicals, thereby protecting the stability of the beer itself, as well as the colour. Table 1 shows the colour values among the investigated beers which ranged from 12 to 18 EBC units. The lowest colour value was achieved by the control sample (6.82 EBC units) and the highest increase in colour was observed in the sample containing

lemon balm (18.59 EBC units). Following the basics of the beer colour values (12-18 EBC units) a pale beers were prepared according to the valid **Decree no. 30/2014 (2014).**

Table 1 Determination of basic parameters in herbal beers and control sample

Sample	Real extract [wt.%]	Alcohol [vol.%]	pH	Total polyphenols [GAE.l^{-1}]	Antioxidant activity [mg TEAC.g^{-1}]	Colour [EBC units]
Control	4.02 c	3.99 a	4.22 a	253.62 a	50.30 a	6.82 a
Salvia	3.88 bc	4.36 b	4.22 a	589.07 b	76.60 c	17.16 c
Oregano	3.64 a	4.36 b	4.22 a	801.63 c	78.17 d	18.20 cd
Lemon balm	3.84 bc	4.46 b	4.22 a	763.00 c	76.70 cd	18.59 d
Wild thyme	3.76 ab	4.02 a	4.22 a	553.82 b	72.90 b	12.50 b

Notes: a-d = homogeneous groups, different letters at mean represent statistically significant differences among samples ($p<0.05$)

From a nutritional point of view, the interest in beer is very high. This is due to the rich content of antioxidant compounds. The main sources of antioxidants in beer are malt and hops. The antioxidant activity of beer therefore depends on various factors associated with the malting process, temperature and pH during mashing and brewing, and on different types of hops and yeast. According to **Ramos da Silva *et al.* (2021),** aromatic and medicinal plants are also considered natural sources of antioxidants, because their secondary metabolites inhibit the formation of free radicals. Specifically, plants of the *Lamiaceae* family are known for their biological activity and especially their antioxidant properties **(Skendi *et al.*, 2017).** Antioxidant activity in samples was determined using the DPPH method. Beers fortification with herbs of the *Lamiaceae* family had a beneficial effect on increase in the antioxidant activity. Based on Table 1, it can be concluded that the addition of herbs increased the antioxidant activity by approximately 34% compared to the control sample. Differences among variants were confirmed by LSD test. Significantly the highest antioxidant activity was achieved in beer with oregano (78.2 mg TEAC.g^{-1}). High values were also measured in beer with lemon balm (76.7 mg TEAC.g^{-1}) and salvia (76.6 mg TEAC.g^{-1}). The lowest antioxidant activity was demonstrated by the control sample of 50.3 mg TEAC.g^{-1}. Based on the antioxidant activity, the following descending order of the individual samples was determined: > oregano > lemon balm > salvia > wild thyme > control. In their study, **Djordjevic *et al.* (2015)** have shown that the results of the determination of the antioxidant activity analysed by the DPPH test of standard beer samples and beer in combination with selected herbs was in correlation with the level of total polyphenols. Based on author´s measurements, the greatest increase in antioxidant activity was recorded in beer samples containing herbs form genus *Thymus* and *Melissa*. These results are in accordance with our study that the values of antioxidant activity are positively correlated with the level of total polyphenols (Table 2) with a positive correlation coefficient (r=0.91). According to **Tzima *et al.* (2018),** plants from the *Lamiaceae* family are potential sources of natural antioxidants due to their high content of polyphenols. Eating foods rich in polyphenols is associated with health benefits, such as reducing the risk of cardiovascular disease. Phenolic compounds such as rosmarinic acid, carvacrol and thymol are found in several genus of the *Lamiaceae* family **(Skendi *et al.*, 2017).** Our results confirmed that fortification by herbs had a beneficial effect on increasing the content of total polyphenols (TPC) in beer. TPC ranged from 253.62 mg GAE.g^{-1} (control beer) to 801.63 mg GAE.g^{-1} (beer with oregano). By multiple comparisons of means by LSD test, the varieties were divided into 3 homogeneous groups. Statistically significant ($p<0.05$) the highest TPC was detected in beer with oregano (801.63 mg GAE.g^{-1}). The lowest content of total polyphenols was measured in the control sample (253.62 mg GAE.g^{-1}). Based on the total polyphenol content expressed as mg GAE.g^{-1}, the following descending order of individual samples was determined: > oregano > lemon balm > salvia > wild thyme > control. Positive correlation coefficient (r=0.94) was also found between TPC and beer colour (Table 2). **Djordjevic *et al.* (2015)** demonstrated a significant increase in the content of polyphenols in beer with a minimal dose (0.05%) of herbal extract. The content of polyphenols in beer samples enriched with extracts from plants belonging to the *Lamiaceae* family was 30-37% higher compared to standard beer. Similarly, **Pluháčková *et al.* (2020)** observed, up to a four times higher increase in the content of polyphenols and phenolic acids in beer due to the addition of herbal extracts.

Table 2 Correlation matrix between monitored parameters of beer quality based on the Pearson correlation coefficient

Parameter	Alcohol [vol.%]	pH	TPC [GAE.l^{-1}]	AA [mg TEAC.g^{-1}]	Colour [EBC units]
Extract (wt.%)	-0.4130	-0.0453	-0.6448**	-0.6455**	-0.5267*
Alcohol [vol.%]		0.3499	0.7151**	0.6410**	0.8192**
pH			0.1021	0.2165	0.1765
TPC [GAE.l^{-1}]				0.9130**	0.9415**
AA [mg TEAC.g^{-1}]					0.9312**

Notes: AA – total antioxidant capacity, TPC – total phenolic content expressed as gallic acid equivalent, *statistically significant at $p<0.05$, **statistically significant at $p<0.01$

Results of sensory analysis

The sensory characteristics of beers play an important role in consumer´s acceptance or rejection. For consumer acceptance, significant factors are its taste, flavour and colour **(Díaz *et al.*, 2022).** Results of sensory analysis of beer are demonstrated in Figure 1. Addition of herbs to beer had a beneficial effect on increasing the intensity of the beer's taste. The most intense, pleasurable herbal aroma and taste was evaluated in beer with salvia. The lowest values of aroma intensity among the samples with the addition of herbs were assigned to the sample containing wild thyme. Fullness and sharpness intensity was rated best in beer with oregano and salvia. On the contrary, weak fullness was assigned to the sample with the addition of lemon balm. From the overall point of view the wild thyme in the combination with beer manifested the best performance (Figure 1).

From the consumer's point of view, herbal beers can be attractive not only for their antioxidant properties, but also from a sensory point of view. Brewing such beers could be undertaken by craft breweries in order to expand the offer and subsequently increase the demand for an unusual product. Beers with the addition of herbs can provide the consumer with an unconventional way to refresh or diversify consumer gastronomic habits.

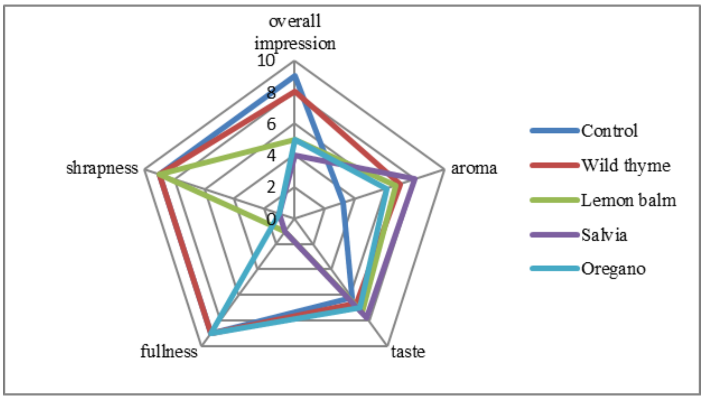

Figure 1 Sensory analysis of beers enriched with herbs

CONCLUSION

The purpose of this study was to determine the impact of the addition of selected herbs such as lemon balm, salvia, oregano, wild thyme on the technological parameters and sensory profile of beer with the aim to enhanced functionality of beer. The effort to improve beer sensory and qualitative properties has always been an attraction for many brewers. Data presented in this study demonstrate that fortification of beer with herbs lead to obtaining a beer with increased functional and new sensory attributes. Antioxidant capacity of the beer enriched with plant herbs was improved and it reached values from 72.9 mg $TEAC.g^{-1}$ to 78.2 mg $TEAC.g^{-1}$, which is 34% more than in the case of the control sample. The high antioxidant activity suggests that beers with the addition of herbs may have beneficial effects on human health and may be attractive to craft breweries. Moreover, the amount of total polyphenol content was obviously improved as well. In the case of beer with oregano, TPC was several times higher (801.63 mg $GAE.g^{-1}$) as in control beer (253.62 mg $GAE.g^{-1}$). Positive correlation was found between total polyphenol content and antioxidant activity. Sensory analysis revealed that the most intense, pleasurable herbal aroma and taste was evaluated in beer with salvia. From the overall impression point of view the wild thyme in the combination with beer manifested the best performance. The obtained results indicate that selected herbs can be a very interesting raw material for the brewing industry. It is possible to produce different types of beers with defined functional properties, created with a special purpose and for different target groups. Such products could bring innovation in beer production and in the development of products with health-promoting properties that meet market needs while attracting new consumers.

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