IMPACT OF THE FERMENTATION PROCESS WITH IMMOBILIZED YEAST CELLS ON THE AROMA PROFILE AND SENSORY QUALITY OF DISTILLATES PRODUCED FROM CAROB PODS (*CERATONIA SILIQUA L.*)

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT: The aim of this research was to investigate the influence of immobilized cell fermentation on aroma and sensory characteristics of distillates produced from carob pods (*Ceratonia siliqua L.*) commonly grown in coastal Croatia. Distillate samples were produced both by classical and immobilized yeast technology. The aroma profile was determined using GC/FID and a sensory analysis was conducted according to the German DLG model. Results showed that a immobilized cell technique gave distillates with lower ester contents, nevertheless satisfying sensory quality.

KEYWORDS: immobilized yeast cells, carob distillate, aroma, sensory quality

INTRODUCTION

Carob (*Ceratonia siliqua L.* - derived from the Greek word *keras* - horn and Latin *siliqua* - hard) is a wild tree or a shrub of the legume family (*Leguminosae*). Tree has a broad canopy and up to 15 m high, and the fruit is 20 cm long pods, at first green and maturing exceeds that in dark brown. The pods ripen in late summer. A mature follicle is of a sweet taste [1].

Endosperm in the mature follicle is processed into farina [2]. Carob grows wild or is cultivated in the coastal regions of the Mediterranean region countries: Croatia, Israel, Turkey, Algeria, Morocco, Tunisia, Spain, Portugal, Greece and Italy, under various names: Hebrew *kharuv*, Arab *Kharrūbah*, in Spain it is called, *Garrofer*, *Carrubo* in Italy*, Caroubier* in France, *Karubenbaum* in Germany, *Alfarrobeira* in Portugal, *Charaoupi* in Greece and *charnup* in Turkey. Carob has been used in the human diet for more than 4,000 years, it has been mentioned as food from Biblical times, which fed John the Baptist, and hence derives an English synonym for carob - *St.John's bread* [3].

The husk of the carob contains app. 13% of simple sugars (fructose, glucose and maltose), 20% sucrose, 2-3% pectin, 4% protein and 35% starch. It is rich in minerals: 36% calcium, 24% potassium, 29% copper. Carob pulp has a high content of phenolic compounds of which the most important are tannins (16-20%), while the proportion of phenolic compounds in the husk of the carob varies and depends on the climate, carob varieties, husk maturity degree and manner of processing [4]-[6].

Carob is offered to the market in fresh and dried state. In the food industry carob is used as thickener, stabilizer, cocoa replacement and medical dietetics use it as low-energy food. Carob extract is used as a flavour in the production of wide range of products (alcoholic and non-alcoholic beverages, frozen dairy products, candies, baked goods, puddings, meat products, spice mixes, fruit products, etc. [2], [7].

For distillate and brandy production, a completely dry carob pod with the high sugar content is used, as well as various essential oils and resinous substances, which give the distillate and brandy with a characteristic smell and taste [3].

The aim of this study was to investigate the influence of fermentation with immobilized yeast cells on the flavour and sensory properties of distillate produced from farina of the dried carob pod.

MATERIAL AND METHODS

SHUCK

The samples of shuck produced from flour of the dried carob pod (harvest 2015) were taken from the technological process of carob distillate production at “Zvečevo” JSC.

SHUCK FERMENTATION

The samples in two parallels were produced using the classical technological procedure and immobilized yeast fermentation as described previously [8].

All samples were taken at the end of fermentation before sedimentation, therefore the samples were insufficiently clear and slightly dull, which is appropriate for the selected procedure for the distillate production.

DISTILLATION

The selected samples were distilled in a copper clip distillation device, according to the procedure described previously [8].

The samples containing approximately 70% vol. alcohol were taken from the middle fraction, or with recommended alcohol concentration in distillates, while the first (head) and the last (tail) fraction were not used.

All selected samples were distilled according to the same distillation protocol.

ANALYSIS OF DISTILLATES

Instrumental analytical techniques were applied on the basis of the European Community Reference Methods for the analysis of spirits using gas chromatography [9], [10], [11], using a Hewlett Packard 5890 gas chromatograph with a split /splitless injector and a FID detector, as described previously [8].

SENSORY ANALYSES

The sensory analysis of samples was performed according to the method of positive ranking according to the German DLG model [12]. This model was based on 4 sensorial experiences, which were marked with grades from 0 to 5, including 0, while the average grade was multiplied by the significance factor.

The sensory assessment was conducted in two repetitive cycles; each group had ten qualified professional testers, from the alcoholic beverages industry, selected by selection procedure [13], with extensive experience in the sensory assessment of distillates.

RESULTS AND DISCUSSION

The results of the chemical analyses of distillate samples are shown in Table 1. The results show that the fermentation process with immobilised yeast cells resulted in increased content of ethanol in the immobilized yeast sample (69.87% vol. compared to 69.22% vol). A similar trend was recorded in our previous research [14].

Table 1. Selected chemical characteristics of carob distillate samples (mean±standard error).

Assessment characteristics	**Classical fermentation**	**Immobilized yeast-fermentation**	**p**
Total extract (g/L)	0.048±0.021	0.014±0.004	0.16575
Total SO_2 (mg/L)	4.255±0.007	6.16±0.070	**0.00069**
Total acidity (mg/L)	470.85±18.73	344.60±1.97	**0.01095**
Benzaldehyde (mg/L)	2.02±0.08	2.38±0.14	**0.09086**
Ethanol (% vol.)	69.22±0.04	69.87±0.04	**0.00423**
Methanol (mg/L)	0.135±0.02	0.04±0.05	0.15610
Propan-1-ol (mg/L)	2.67±0.04	1.80±0.14	**0.01434**
Butan-1-ol (mg/L)	0.175±0.049	0.090±0.014	0.14460
Isobutyl alcohol (mg/L)	3.71±0.14	3.41±0.014	0.09628
Isoamyl alcohol (mg/L)	8.79±0.02	6.18±0.28	**0.00583**
2-phenyl ethanol (mg/L)	4.13±0.388	2.59±0.212	**0.03873**
Linalool (mg/L)	0.73±0.18	0.76±0.16	0.88094
α-Terpineol (mg/L)	2.01±0.21	2.64±0.28	0.12790
Benzoic acid(mg/L)	21.74±0.87	60.48±7.16	**0.01690**
Quercetin (mg/L)	5.74±0.87	25.48±0.09	**0.00099**
Gallic acid (mg/L)	31.27±0.12	35.68±0.37	**0.00010**
Ethylgallate (mg/L)	20.20±0.21	35.68±0.37	**0.00039**
Ellagic acid (mg/L)	31.11±0.07	65.98±0.79	**0.00026**
Furfural (mg/L)	0.06±0.007	tr.	**0.00586**
5-Methylfurfural (mg/L)	0.025±0.035	n. d.	0.42226
5-Methyl -2-furancarboxaldehyde (mg/L)	0.100±0.014	0.055±0.007	0.05650
Pyranone (mg/L)	3.49±0.22	1.12±0.04	**0.00468**
2-Acetylpyrrole (mg/L)	1.56±0.01	1.06±0.01	**0.00079**
5,6-Dihydro-2-pyranone	0.88±0.01	0.36±0.01	**0.00073**
Ethyl lactate (mg/L)	0.09±0.01	n. d.	**0.01212**
Ethyl octanoate (mg/L)	2.74±0.09	2.40±0.31	0.27150
Ethyl decanoate (mg/L)	0.92±0.15	n. d.	**0.01399**
Ethyl acetate (mg/L)	6.33±0,18	4.45±0.04	**0.00049**
Isoamyl acetate (mg/L)	8.50±0.14	8.03±0.01	**0.04593**
Acetaldehyde(mg/L)	4.44±0.45	3.81±0.94	0.48552
Ethyl hexanoate (mg/L)	2.48±0.52	2.33±0.45	0.79617
Methyl octanoate (mg/L)	0.40±0.06	0.39±0.09	0.91091

Assessment characteristics	**Classical fermentation**	**Immobilized yeast-fermentation**	**p**
2-Phenylethyl acetate (mg/L)	0.71±0.14	0.58±0.007	0.34171
Methyl decanoate (mg/L)	0.31±0.02	0.23±0.01	**0.04215**
Benzyl acetate (mg/L)	0.22±0.007	n. d.	**0.00049**
Ethyl benzoate (mg/L)	0.15±0.03	n. d.	**0.02504**
Ethyl decanoate (mg/L)	28.43±0.007	27.89±0.07	**0.00839**
Isoamyloctanoate (mg/L)	0.41±0.02	0.35±0.04	0.23799
Ethylundecanoate (mg/L)	2.11±0.02	1.49±0.48	0.21443
3-Methylbutyl dodecanoate (mg/L)	0.35±0.02	0.24±0.02	0.05219
Ethyl dodecanoate (mg/L)	1.74±0.16	1.22±0.04	0.05219
3-Methylphenyl butanoate (mg/L)	0.25±0.007	0.21±0.01	0.05654

n. d. – not detected; tr. – traces; results with bolded p values are statistically different

The content of methanol, an important safety factor when producing alcoholic drinks [15], was in acceptable range in both samples [16], [17], with markedly lower content in the immobilized-yeast sample (0.04 mg/mL compared to 0.135 mg/mL).

On the other hand, total SO_2 was significantly higher in the immobilized-yeast sample (6.16 mg/L compared to 4.255 mg/mL). This potentially increases the risk of bonding SO_2 on acetaldehyde and results in an intense heavy smell [18], [19].

The total extract and total acidity were reduced by the fermentation process with the immobilised yeast cells method, which shows the higher degree of fermentation [14].

The content of main components that affect the aroma profile (Table 1) was reduced when using the fermentation process with immobilised yeast cells, which can result in reduced distillate sensory characteristics [17], [20].

Furthermore, the total share of typical phenolic substances, described by Rakib *et al.* [21], dominated by gallic acid was significantly higher in distillates produced by immobilized yeast cells, the share of quercetin increased nearly by 5-fold (25.48 mg/L compared to 5.74 mg/L), ellagic acid nearly 2-fold and ethylgallate by 1.5-fold.

Also, volatile substances typical for roasted carob beans [22] were notably more predominant in the samples produced by a classic method of fermentation. Furfural was not detected in the immobilized-cell sample, while in the classically produced sample the amount of 0.06 mg/L was measured. The content of pyranone was 3 times higher in the classically produced sample, acetopyrole 1.5 times and 5,6-dihydro-2-pyranone 2.4 times higher than in the immobilized-yeast sample (Table 1). Although not statistically significant, the content of 5-methylfurfural and 5-methyl-2-furancarboxaldehyde was also slightly higher in the classically produced sample.

Overall, the level of all identified esters in this study was reduced in the samples produced by the fermentation process with immobilized yeast cells, indicating that distillates produced by the classic fermentation process have a deeper aroma [23], [24].

This was confirmed by the sensory analysis where the samples produced by the fermentation process with immobilised yeast cells were given a significantly lower score for taste and odour in comparison to samples that were produced by the classical fermentation process (Figure 1), and this is in accordance with the research of Vila *et al.* [25] and Yajima and Yokotsuka [26]. Although the overall score of the sensory analysis was higher for the classically produced sample, the sample produced with immobilized yeast cells had over 90 points, which places it in a category of highly acceptable to consumers.

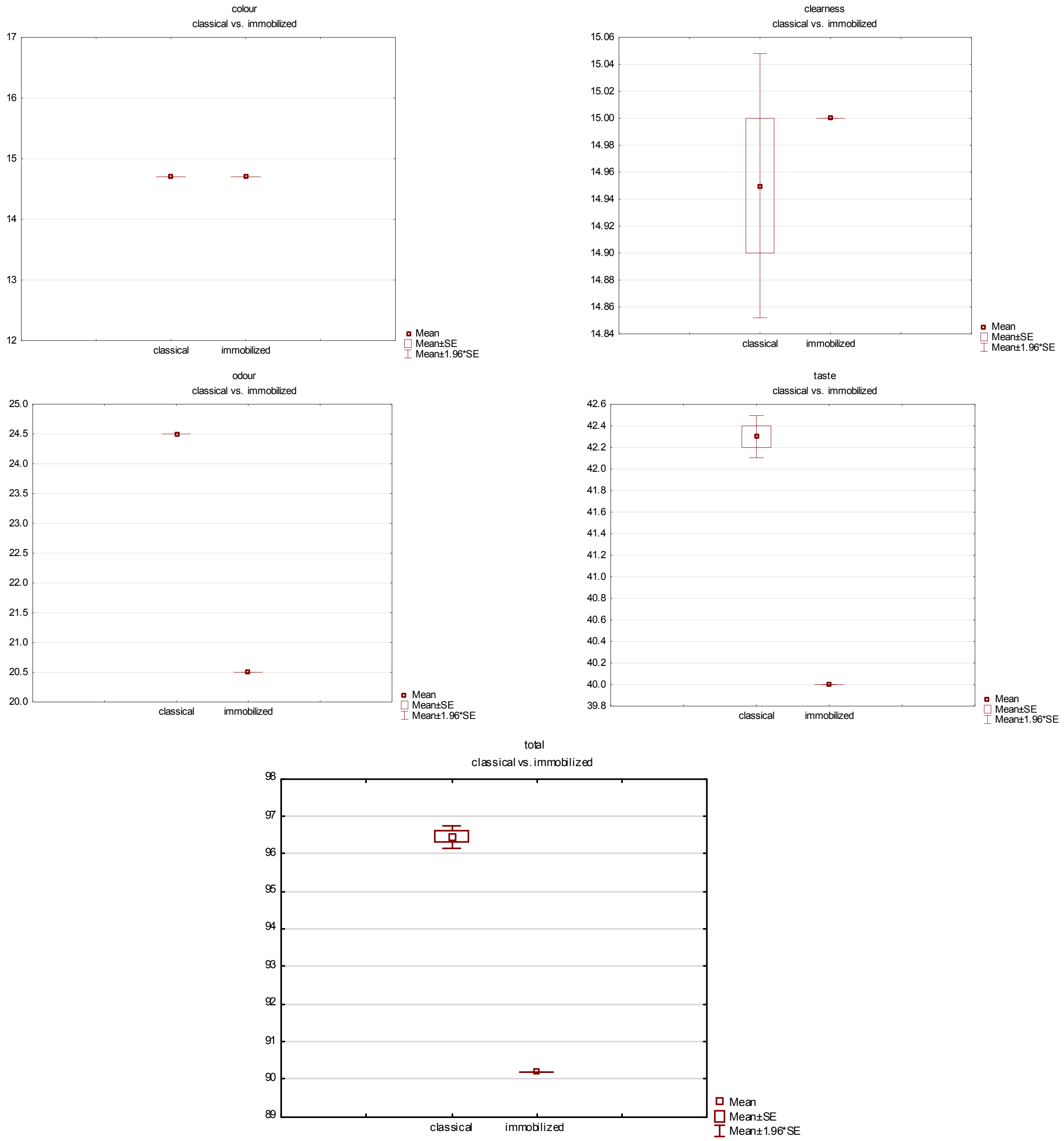

Figure 1. Results of sensory analysis of carob distillates produced by classical fermentation and fermentation with immobilized yeast cells - *German DLG model* (Koch, 1986.).

CONCLUSION

Although the present research shows that the original carob aroma was partly lost by immobilized yeast cell fermentation, as well as ester content, which gave less pronounced odour and taste of distillate, the overall sensory score shows that carob distillate acceptable to the consumers may be produced by immobilized yeast fermentation.

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