

VALORIZATION OF AGRI-FOOD WASTES IN YEAST BIOTECHNOLOGIES

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Abstract: The article presents the research results the purpose of which was to evaluate the possibility of utilization of nutrient media obtained from carrot and celery agri-food wastes as a single source of nutrition for the cultivation of pigmented yeast strains of the genus *Rhodotorula*. It was established that nutrient media based on carrot and mixture of carrot and celery present an effective substrate for the cultivation of pigmented yeasts *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-03, which allow stimulation of the biomass productivity of yeasts, significantly increase the concentration of carotenoids in biomass and increase the total antioxidant activity of the strain biomass. The medium based on celery wastes inhibits the growth and development of the strains, affects the yeast productivity, significantly modifies the biochemical composition of the biomass and significant increases its total antioxidant activity.

Keywords: *biochemical composition, carrot wastes, celery wastes, nutrient media, pigmented yeasts, total antioxidant activity*

INTRODUCTION

Agri-food wastes are becoming an increasingly acute global problem, which generate approximately one billion tons of wastes annually [1, 2]. In addition to the high costs of managing these wastes, it also creates environmental problems. Various scientific events are organized worldwide, designed to draw the attention of researchers and civil society to this problem and possible solutions [3]. At the same time, agri-food wastes represent an endless source of various substances with biological activity and nutritional value, which after processing can serve as a source of nutrients in animal feed or as a substrate for the generation of value-added products [4].

A large part of agri-food wastes is the by-products of fruit and vegetable processing. These by-products include solid residues of peels, seeds, pits and pulp [5].

Carrot processing industries produce 25 - 30 % of wastes in the form of carrot residues, peels and pomace, which contain a large amount of high-value bioactive components [6]. Carrot is an important root vegetable, rich in bioactive compounds such as carotenoids and dietary fiber, with appreciable level of other biologically active compounds. It is a good source of carbohydrates and minerals such as Ca, P, Fe and Mg. Depending on the variety, carrot contains up to 10 % proteins, 60 % carbohydrates, mainly sucrose and up to 1.4 % ethereal extract from dry biomass, vitamin C (300 - 700 mg·kg⁻¹ d.w.) and carotenoids (200 - 1000 mg·kg⁻¹ d.w.) [5].

Celery is also an intensively processed vegetable and a reputed medicinal plant with low caloric value, rich in fiber, minerals such as Ca, P, Fe, K and Mg, vitamins such as B₁, B₂, B₃, A, K, C and flavonoids [7]. Celery hypocotyl contains 84 – 85 % water and 15 – 16 % dry matter, of which 77.5 % carbohydrates, 18.0 % proteins and up to 4.4 % fats [8]. Celery owes its characteristic aroma to the presence of the volatile essential oils, compounds of terpenes, sesquiterpenes and phthalides [9].

Currently, the research of interest is to evaluate the possibility of using agri-food wastes in various microbial biotechnologies, as a substrate for cultivating microorganisms and obtaining metabolites of interest, contributing to the reduction of environmental risks [10, 11].

Microbiological biosynthesis is the basis of many biotechnological processes, with bacteria, fungi, microscopic algae and yeasts being widely used as producers of microbial biologically active substances [12].

From an economic and ecological point of view, the best producers of biologically active substances are yeasts, because they have a number of advantages compared to other biotechnological objects. Yeasts are characterized by the high rate of biomass accumulation, metabolize a wide range of carbon sources, are resistant to contamination with foreign microflora, do not pollute the environment with spores. Yeasts can be easily separated from the culture media liquid and have the ability to grow on relatively cheap nutrient media, including agricultural and food industry by-products, which significantly reduce the production cost of biologically active substances [13]. In addition to these advantages, yeasts, including those of the genus *Rhodotorula*, are considered an excellent source of proteins, carotenoids, polysaccharides, vitamins, enzymes, antioxidants, etc. with high biological activity, safe for human and animal consumption [14, 15].

In order to maximum exploit the potential of yeasts to produce biologically active substances, various synthetic and natural nutrient media are being investigated, which would stimulate the productive performance of the strains. In this context, the purpose of

this research was to evaluate the possibility of utilization of nutrient media, obtained from carrot and celery agri-food wastes, as a single source of nutrition for the cultivation of 2 pigmented yeast strains of the genus *Rhodotorula*, by estimating their productive performance and the antioxidant activity of the biomass, compared to the potential of the strains cultivated on YPD medium.

MATERIALS AND METHODS

Object of the study

As the object of study the red pigmented yeast strains *Rhodotorula mucilaginosa* CNMN-Ys-10 and *Rhodotorula gracilis* CNMN-Ys-03 from the Fungal Biotechnology Laboratory of the Institute of Microbiology and Biotechnology of the Technical University of Moldova, stored in the National Collection of Nonpathogenic Microorganisms, were served.

Media and fermentation conditions

The inoculum was obtained by cultivation of the yeast strains in YPD liquid nutrient medium, for 48 hours, on the stirrer (200 rpm), at the temperature of 27 – 28 °C. The inoculum (2×10^6 cells·mL⁻¹) constituted 5 % of the volume of the nutrient medium.

In the experiment, the YPD medium (1 % yeast extract, 2 % glucose, 2 % peptone, 1 L distilled water, pH 5.5) [16] as the control medium (CM) was used. In the experimental nutrient media were used carrot peel and tip wastes of *Daucus carota* (CarE), celery hypocotyl peel wastes of *Apium graveolens* (CelE) and the mixture of CarE and CelE in the ratio of 1:1 v/v.

The CarE is obtained as follows: carrot peels from the factory are washed repeatedly with water in order to remove soil particles and other impurities; they are ground to the particle size of 0.5 - 1.0 cm; then 1 volume of peels (750 g wet weight) is mixed with 1 volume of distilled water (750 mL) and autoclaved for 30 min. at the temperature of 115 °C, after cooling the liquid supernatant (CarE) is separated from the sediment (carrot peels) by centrifugation; the CarE is sterilized in an autoclave for 30 min. at the 115 °C. The CarE has the sugar content of 5.0 °Bx, pH 5.58 and is used as the nutrient medium, as the single source of nutrition for submerged cultivation of pigmented yeasts.

The CelE is obtained as follows: celery peels from the factory are washed repeatedly with water in order to remove soil particles and other impurities; they are ground to the particle size of 0.5 - 1.5 cm; then 1 volume of peels (750 g wet weight) is mixed with 1 volume of distilled water (750 mL) and autoclaved for 30 min. at the temperature of 115 °C, after cooling the liquid supernatant (CelE) is separated from the sediment (celery peels) by centrifugation; the CelE is sterilized in an autoclave for 30 min. at the 115 °C. The CelE has the sugar content of 4.5 °Bx, pH 5.48 and is used as the nutrient medium, as the single source of nutrition for submerged cultivation of pigmented yeasts.

The third type of medium - CarE:CelE 1:1 is obtained by mixing of the extracts CarE and CelE in the 1:1 ratio and sterilizing in an autoclave for 30 min. at 115 °C. The CarE:CelE 1:1 has the sugar content of 4.8 °Bx, pH 5.52 and is used as the nutrient medium as the single source of nutrition for the submerged cultivation of pigmented yeast strains.

Depth cultivation was carried out in Erlenmeyer flasks containing 0.2 L nutrient medium, on the stirrer with the rotation speed of 200 rpm, at the temperature of 27 – 28 °C, for 120 hours.

Methods of achieving research

The yeast biomass productivity was determined gravimetrically prepared by centrifugation at 4000 rpm for 15 min., dry biomass was determined by drying the known weight of the yeast biomass at 105 °C to the constant weight.

The protein content was determined spectrophotometrically according to the Lowry method, with crystalline albumin from bovine serum as the standard [17].

The total carbohydrate content was determined spectrophotometrically with the Antron reagent and D-glucose as standard. The absorption was recorded at 620 nm [18].

The carotenoid content was determined spectrophotometrically by extracting carotenoids using 96 % ethanol [19].

Total antioxidant activity (TAA) was determined spectrophotometrically with the use of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS^{•+}) method [20]. Spectrophotometric assays were performed on the UV-VIS Shimadzu spectrophotometer UV-1280.

Statistical analysis

Statistical processing of the results was performed using the MO Excel and Statistics 9.0 software suite. The results were expressed by calculating the mean, standard deviation and confidence interval for an average of three repetitions of the entire process. All differences were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

The strains *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-03 were cultivated on the nutrient media CarE, CelE and CarE:CelE 1:1, in order to establish the possibility of using them as a single source of nutrition for submerged yeast cultivation, by monitoring the biomass productivity, biochemical composition (proteins, carbohydrates, carotenoids) and the biomass total antioxidant activity. It was determined that of the three tested media, CarE medium ensures a significant increase in the biomass productivity of the *R. mucilaginosa* CNMN-Ys-10 strain up to $12.07 \pm 0.6 \text{ g} \cdot \text{L}^{-1}$, compared to $7.20 \pm 0.33 \text{ g} \cdot \text{L}^{-1}$ in the control medium (CM), which constitutes 67.7 %. On the CarE:CelE medium the biomass productivity was $7.71 \pm 0.29 \text{ g} \cdot \text{L}^{-1}$ or of 7.1 % more compared to the control. The lowest productivity was established on the CelE medium of $6.39 \pm 0.35 \text{ g} \cdot \text{L}^{-1}$ (Table 1).

The CarE and CelE media stimulate protein synthesis and allow the *R. mucilaginosa* CNMN-Ys-10 strain to accumulate in the biomass up to $61.67 \pm 5.54 - 74.70 \pm 0.93 \text{ % d.w. proteins}$, which are 12.59 - 36.38 % higher than the control. Cultivated on the CarE:CelE medium the protein content in the strain's biomass was minimal of $48.34 \pm 1.44 \text{ % d.w.}$ (Table 1).

The carbohydrate content in the biomass of the *R. mucilaginosa* CNMN-Ys-10 strain was maximal of 30.93 ± 0.29 % d.w. cultivated on the CarE medium, but did not differ significantly from the values recorded on the control medium of 29.54 ± 2.34 % d.w. On the CelE and CarE:CelE media the carbohydrate content in the biomasses was lower compared to the control and constituted 16.52 ± 0.90 and 24.69 ± 0.1 2% d.w. respectively (Table 1).

Table 1. Productivity and biochemical composition of the *R. mucilaginosa* CNMN-Ys-10 biomass on the carrot and celery peel media

Parameters	Nutrient media			
	CM (YPD)	CarE:CelE 1:1	CarE	CelE
Biomass [$\text{g} \cdot \text{L}^{-1}$]	7.20 ± 0.33	7.71 ± 0.29	$12.07 \pm 0.6^*$	6.39 ± 0.35
Biomass [% of control]	100	107.1	167.7	88.7
Proteins [% d.w.]	54.77 ± 4.16	48.34 ± 1.44	61.67 ± 5.54	$74.70 \pm 0.93^*$
Protein [% of control]	100	88.26	112.59	136.38
Carbohydrates [% d.w.]	29.54 ± 2.34	24.69 ± 0.12	30.93 ± 0.29	16.52 ± 0.90
Carbohydrates [% of control]	100	83.58	104.71	55.93

* $P \leq 0.05$

The tested media had a significant impact on the carotenogenesis of the *R. mucilaginosa* CNMN-Ys-10 strain. Thus, on the control medium the strain synthesizes the content of 86.2 ± 9.4 $\mu\text{g} \cdot \text{g}^{-1}$ carotenoids, but on the CarE:CelE and CarE media of 211.8 ± 11.2 and 281.8 ± 11.7 $\mu\text{g} \cdot \text{g}^{-1}$, respectively, which is by 145.8 and 227.1 % more, respectively. The productivity of carotenoids per liter of medium, when cultivated on CM was 0.62 $\text{mg} \cdot \text{L}^{-1}$, and on the CarE:CelE and CarE of 1.63 and 3.41 $\text{mg} \cdot \text{L}^{-1}$, respectively, which is 2.6 and 5.5 times more, respectively (Figure 1).

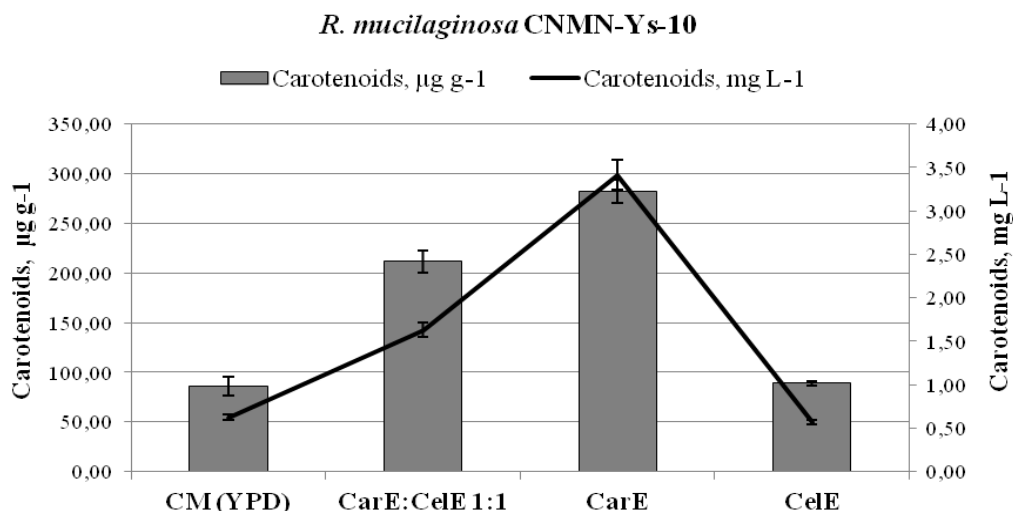


Figure 1. Carotenoid concentration and productivity of the *R. mucilaginosa* CNMN-Ys-10 yeast strain

The *R. gracilis* CNMN-Ys-03 strain also manifested significantly ($P \leq 0.05$) higher biomass productivity on the CarE:CelE and CarE media compared to the control medium. Thus, on CM medium the strain accumulates of 6.57 ± 0.35 $\text{g} \cdot \text{L}^{-1}$ dry biomass, and on the CarE:CelE of 7.55 ± 0.49 $\text{g} \cdot \text{L}^{-1}$ and on the CarE of 9.67 ± 0.17 $\text{g} \cdot \text{L}^{-1}$, which are by

14.9 and 47.2 % more, respectively. The biomass productivity of the strain on the CelE medium was significantly lower compared to the control and the other tested media and amounted to only $3.55 \pm 0.06 \text{ g} \cdot \text{L}^{-1}$ (Table 2), which indicates that the components of this nutrient medium significantly influence the growth of the tested yeast.

At the same time, similar to the *R. mucilaginosa* CNMN-Ys-10 strain, the *R. gracilis* CNMN-Ys-03 biomass, obtained on the CelE medium, accumulates a maximum amount of proteins of $79.42 \pm 2.28 \text{ % d.w.}$, which is significantly ($P \leq 0.05$) by 20.9 % more compared to the control. On the CarE:CelE medium the strain managed to accumulate amount of proteins, comparable to the control of $60.51 \pm 3.19 \text{ % d.w.}$ The CarE medium was the weakest from this point of view and had of $41.90 \pm 1.56 \text{ % d.w.}$ proteins in the biomass (Table 2).

The carbohydrate content in the biomass of the *R. gracilis* CNMN-Ys-03 did not differ significantly on the CM, CarE:CelE and CarE media, recording values of 20.66 ± 0.93 , 21.53 ± 0.89 and $22.03 \pm 1.53 \text{ % d.w.}$ respectively. The CelE medium, as in the case of the *R. mucilaginosa* CNMN-Ys-10 strain, allowed the accumulation of the minimum amount of carbohydrates of $15.38 \pm 1.34 \text{ % d.w.}$ in the strain biomass (Table 2).

Table 2. Productivity and biochemical composition of the *R. gracilis* CNMN-Ys-03 biomass on carrot and celery peel media

Parameters	Nutrient media			
	CM (YPD)	CarE:CelE 1:1	CarE	CelE
Biomass [$\text{g} \cdot \text{L}^{-1}$]	6.57 ± 0.35	$7.55 \pm 0.49^*$	$9.67 \pm 0.17^*$	3.55 ± 0.06
Biomass [% of control]	100	114.9	147.2	54.1
Proteins [% d.w.]	65.67 ± 3.06	60.51 ± 3.19	41.90 ± 1.56	79.42 ± 2.28
Proteins [% of control]	100	92.14	63.8	120.9
Carbohydrates [% d.w.]	20.66 ± 0.93	21.53 ± 0.89	22.03 ± 1.53	15.38 ± 1.34
Carbohydrates [% of control]	100	104.18	106.64	74.43

* $P \leq 0.05$

For the synthesis and accumulation of carotenoids in the biomass of the *R. gracilis* CNMN-Ys-03, the CarE:CelE medium was effective, on which the strain accumulated of $347.9 \pm 6.8 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ carotenoids, which is significantly more of 83 % compared to the control whose value was $190.10 \pm 19.13 \text{ } \mu\text{g} \cdot \text{g}^{-1}$. In contrast to the *R. mucilaginosa* CNMN-Ys-10 strain, the *R. gracilis* CNMN-Ys-03 strain on the CarE medium synthesized significantly fewer carotenoids compared to the control. On the CelE medium, the carotenoid content was comparable to the value recorded in the control and constituted to $192.4 \pm 9.2 \text{ } \mu\text{g} \cdot \text{g}^{-1}$. Regarding the carotenoid productivity of the *R. gracilis* CNMN-Ys-03 per liter of medium, we can mention that on the control medium it was $1.25 \text{ mg} \cdot \text{L}^{-1}$, and on the CarE:CelE medium was of $2.63 \text{ mg} \cdot \text{L}^{-1}$ which is 2.1 times more. On CarE medium this parameter was $1.46 \text{ mg} \cdot \text{L}^{-1}$ or 1.17 times higher compared to the control (Figure 2).

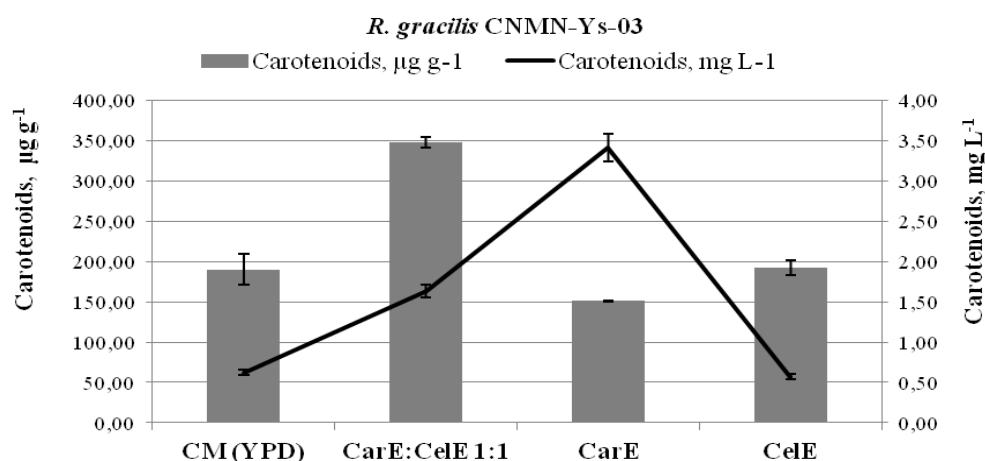


Figure 2. Carotenoid concentration and productivity of the *R. gracilis* CNMN-Ys-03 yeast strain

Similar to these results, some studies have demonstrated the potential of artichoke and asparagus agro-industrial residues for the production of proteins and carotenoids using *R. mucilaginosa* strain [21].

Natural carotenoids, produced by microorganisms on low-cost substrates, present a cost-effective and environmentally friendly alternative to synthetic colorants. Wastes from vegetable and fruit processing are rich sources of carbohydrates, starch, cellulose, soluble sugars, minerals and organic acids.

It is known that nutrient media obtained from various fruit processing wastes with optimized process parameters, reduce the production time of *R. mucilaginosa* YM carotenoids, allow to recycling and/or reuse of wastes for the production of natural pigments [22].

Olive mill waste “Alperujo” from the olive oil industry has the potential to be used as cheap and easily obtainable nutrient media for the cultivation of yeasts and the carotenoid production with industrial applications. The *R. mucilaginosa* strain cultivated on nutrient media based on “Alperujo” wastes produces from 4.9 ± 0.5 to $7.3 \pm 0.6 \text{ mg}\cdot\text{L}^{-1}$ carotenoids [23].

The biomass of yeast *Rhodotorula mucilaginosa* URM 7409 submerged cultivated for 144 hours at temperature of $+25^\circ\text{C}$ with 130 rpm agitation, on optimized nutrient medium YM (yeast and malt extract), with pH 6, composed of $2 \text{ g}\cdot\text{L}^{-1}$ yeast extract, $3 \text{ g}\cdot\text{L}^{-1}$ peptone, $10 \text{ g}\cdot\text{L}^{-1}$ malt extract, $30 \text{ g}\cdot\text{L}^{-1}$ glucose, accumulates up to $252.99 \mu\text{g}\cdot\text{g}^{-1}$ carotenoids, demonstrating the potential of this strain as a source of pigments and its potential for practical use [24].

The optimal conditions for the production of carotenoids by *Rhodotorula* sp. RY1801 were established at temperature $+28^\circ\text{C}$, pH 5.0, carbon source ($10 \text{ g}\cdot\text{L}^{-1}$ glucose), nitrogen source ($10 \text{ g}\cdot\text{L}^{-1}$ yeast extract), to which the strain produced up to $0.99 \text{ mg}\cdot\text{L}^{-1}$ total carotenoids [25].

The maximum concentration of carotenoids in the biomass of *R. mucilaginosa* MTCC-1403 of $717.35 \mu\text{g}\cdot\text{g}^{-1}$ was established on nutrient media from onion peels and mung bean peels cultivated in a bioreactor at pH 6.1, temperature of $+25.8^\circ\text{C}$ with agitation of 119.6 rpm, for 84 hours [26].

Cultivation of the yeast strain *R. mucilaginosa* CCT 3892 on a nutrient medium based on sisal bagasse hydrolysate of *Agave sisalana* supplemented with yeast extract and various

mineral salts, allows a significant increase, by about 50 %, of the strain's biomass productivity from 8.42 to 12.17 - 12.87 g·L⁻¹ dry biomass, but with a low carotenoid concentration of 92.44 μg·g⁻¹, which allows the obtaining of 1.13 mg·L⁻¹ carotenoids. At the same time, in the variant with the maximum carotenoid concentration of 179.66 μg·g⁻¹, the biomass productivity decreases significantly to 4.69 g·L⁻¹ d. w, which allows the obtaining of only 0.84 mg·L⁻¹ carotenoids [27].

The carotenoid biosynthesis capacity of *R. glutinis* BIM Y-253 strain on natural beer wort medium was 150.9 ± 78.4 μg·g⁻¹ biomass, but its supplementation with malic acid or sunflower seed oil significantly increased the carotenoid concentration in the strain biomass by 2.4 - 2.6 times to 362.01 ± 24.3 and 397.22 ± 73.72 μg·g⁻¹, respectively. Cultivation of the strain for 96 hours in the beer wort medium supplemented with 0.05 % malic acid, at the agitation speed of 180 rpm, with artificial lighting, increases the carotenoid yield to 3.5 mg·L⁻¹, or 2.2 times more than the control value [28].

Comparing the results presented by us with those from other scientific sources, we can conclude that the CarE and CarE:CeE tested media present an efficient substrates for the cultivation of the tested pigmented yeasts, stimulate the biomass productivity of the strains, the concentration of carotenoids in it and the production of carotenoids per liter of medium, compared to the control medium and allow the obtaining of biologically active products in concentrations and quantities comparable and/or superior to those reported in the specialized literature [29].

The low biomass productivity of the *R. mucilaginosa* CNMN-Ys-10 strain and the significantly lower one of the *R. gracilis* CNMN-Ys-03 on the CeE medium, accompanied by the significant increase in protein concentration and the decrease in carbohydrate concentration in the biomass, indicate that the medium is not favorable for yeast cultivation, and the biologically active substances from celery manifest antagonism towards the tested yeasts, inhibiting their growth and development. These results are consistent with those obtained by Rožek *et al.* [30], who demonstrated that the phthalide components of celery present antioxidant, larvicidal, nematicidal and fungicidal activities. Probably, substances with antimicrobial and antioxidant characters inhibit the growth of cells, which in response to stress, begin to synthesize significant amounts of proteins.

Further it was established that the biomass of both strains manifested comparable total antioxidant activity (TAA) or significantly ($P \leq 0.05$) higher than the control on all tested media. In the case of cultivation of the *R. mucilaginosa* CNMN-Ys-10 on the CarE medium, AAT did not differ significantly from the reference value, but on the CarE:CeE and CeE media this parameter was 89.87 ± 1.71 and 157.20 ± 1.24 % inhibition respectively, which is significant by 37.0 and 139.7 % respectively (Figure 3). Total antioxidant activity of the *R. gracilis* CNMN-Ys-03 biomass on all three tested media was significantly with 44.7 - 210.9 % higher compared to the control and ranged among 96.86 ± 3.46 and 208.13 ± 3.10 % inhibition (Figure 3).

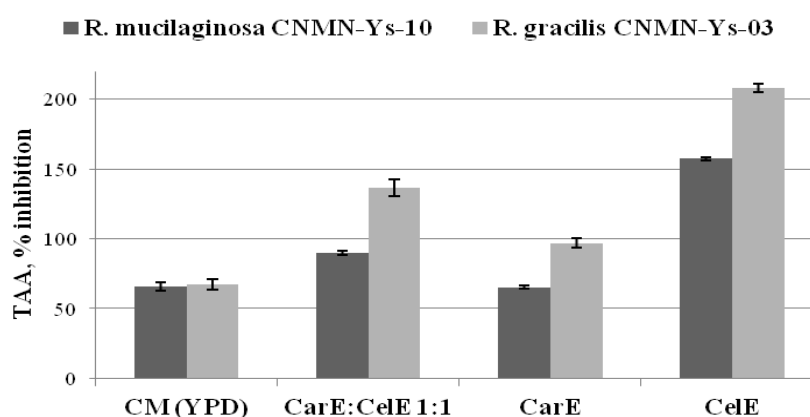


Figure 3. Total Antioxidant Activity (TAA) in biomasses of the *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-03 cultivated on carrot and celery peel media

The significant increase of total antioxidant activity in the biomass of the strains based on the tested carrot and celery nutrient media is probably due to the valuable biochemical composition of these vegetables, especially compounds with antioxidant activity. The aroma and taste of celery, for example, are largely determined by two compounds butylphthalide and sedanolide, with high antioxidant activity [31, 32], and carrots in turn are rich in carotenoids, amino acids and phenols [33]. It is known that yeast cells have the property of absorption of substances from the nutrient medium onto the membrane surface, which explains the significant increase in TAA in the biomass of cultures.

For example, the increase in antioxidant activity of *Saccharomyces cerevisiae* products has been correlated with the high content of phenols in the nutrient media based on cornmeal [34].

In this context, we can affirm that the tested nutrient media, based on carrot and celery, allow the obtaining of the *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-03 biomass with increased antioxidant activity compared to the control medium and have potential for practical use in various biotechnologies based on the yeasts.

CONCLUSIONS

In conclusion, we can mention that the obtained CarE and CarE:CeE nutrient media, based on carrot and celery wastes, have proven effective on a laboratory scale as a substrate for the cultivation of pigmented yeasts *R. mucilaginosa* CNMN-Ys-10 and *R. gracilis* CNMN-Ys-03. These media stimulated the biomass productivity, the accumulation of carotenoids and the increase of the total antioxidant activity of the strain biomasses. Instead, the medium CeE inhibited the growth of the strains and modified the biomass composition, although it also determined higher total antioxidant activity. The obtained results refer to laboratory conditions, and their application on a larger scale requires further studies.

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