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BIOSYNTHESIS OF SINGLE CELL PROTEIN (SCP) BY YEASTS FROM CRUDE CHICKEN WASTE

By

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إستخدام مخلف الدجاج لانتاج بروتين وحيد الخلية بواسطة الخمائر

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تم انماء ثلاث سلالات من الخميرة على منابت غذائية تحتوي على مستخلص مخلف الدجاج كمصدر وحيد للكربون والنيتروجين بتركيزات مختلفة . وقد وجد أن المنبت الذي يحتوي على المخلف بنسبة ٢٪ كان أكثر المنابت ملاءمة لنمو كل من فطرة «سكارو مايسيس سيريفيزي» و« سكارو مايسيس كارلسبرجنسيس » وانتاجها للبروتين . أما فطرة «كانديدا يوتيليس » فقد كان المنبت المحتوي على تركيز ٣٪ من المخلف هو أحسن المنابت ملاءمة لنموها وانتاجها للبروتين . وقد أعطت « كانديدا يوتيليس » أعلى ناتج من الكتلة الحيوية (٣٤٥.٨ مجم / ١٠٠ ملليلتر) تليها فطرة « سكارو مايسيس سيريفيزي » (٢٩٦ مجم / ١٠٠ ملليلتر) ثم جاءت في المؤخرة فطرة « سكارو مايسيس كارلسبر جنسيس » التي أعطت أقل ناتج من الوزن الحيوي (٢٧٤ مجم / ١٠٠ ملليلتر) . وبالرغم من أن فطرة « سكارو مايسيس كارلسبر جنسيس » أعطيت أقل ناتج من الوزن الحيوي الا أنها أنتجت أعلى نسبة من البروتين الخلوي (٤٣.٢٪) تليها فطرة « سكارو مايسيس سيريفيزي » (٣٦.٥٪) حيث تعادلت أو كادت تتعادل مع فطرة « كانديدا يوتيليس » (٣٦.٤٪) في كمية البروتين الخلوي الذي أنتجته كلتاهما . وتؤكد النتائج التي تم الحصول عليها من هذا البحث امكانية استخدام مخلف الدجاج بكفاءة كمصدر وحيد لكل من الكربون والنيتروجين لانتاج بروتين وحيد الخلية بواسطة الخمائر .

Key Words: Biosynthesis, Single cell protein, yeasts, Crude chicken waste.

ABSTRACT

Three strains of yeasts have been grown on media consisting of Chicken waste (CW) as a sole source of carbon and nitrogen in different concentrations. The nutrient medium which contains 2% CW extract was found to be the most suitable fermentation medium for growth and SCP production by S. cervisiae and S. carlsbergensis, while the medium containing 3% concentration of the waste was the most suitable for C. utilis. Candida utilis produced the highest yield of biomass (345.8 mg/100 ml) followed by S. cerevisiae (296.0 mg/100 ml) then S. carlsbergensis gave the least biomass yield, yet it produced the highest level of SCP (43.2%), followed by S. cerevisiae (36.5) then C. utilis (36.4%). The results obtained in this work insure that chicken waste could be used perfectly as a sole carbon and N source for SCP production by yeasts.

INTRODUCTION

Because of the SCP importance as a food and feed matter, more attention has been offered to yeasts than any other group of heterotrophic micro-organisms as a source of human food and animal feed (Bunker, 1963; Cooney and Levine, 1975; El-Rafai *et al.,* 1986; Masuda and Yoshikawa, 1972; Reuse *et al.,* 1975; Uene *et al.,* 1974 a,b,c). Increasing the concern about the pollution that occurs form different types of wastes has stimulated the interest of scientest in converting waste materials into valuable products, especially single cell protein (SCP) (Jwanny *et al.,* 1990; Maurizio & Federico, 1986; Moriguchi, 1982: Peitersen, 1975; Silva & Nicoli, 1985). Chicken waste is one of many wastes present in nature and produced in large quantities constituting a source of pollution in the environment. It is the objective of the present communication to evaluate the suitability of this waste for SCP production by yeasts.

MATERIALS AND METHODS

Micro-organisms

1. *Saccharomyces cerevisiae:* was isolated from local commercial baker's yeast.
2. *Saccharomyces carlsbergensis:* was from NRRL (Northern Regional Research Laboratory, Peoria, 111, USA).
3. *Candida utilis:* was from MIRCEN.

Maintenance and preparation of the inocula:

The cultures were maintained on Phaff's medium which used also as control (basal medium) and has the following composition (Phaff *et al.,* 1966) (gm/l): D-glucose, 10.0; $(NH_4)_2$ SO_4, 5.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.1; $CaCl_2$, 0.1; distilled water to liter. Before inoculation, the micro-organisms were serially subcultured at least three times in the same medium, whereby all fermentations received a 5% (V/V) inoculum at age 48 h.

Fermentation Medium:

The dried locally obtained crude chicken waste (CCW) was boiled with distilled water, and filtered. Six sets of media, in triplicates, have been prepared as follows:

1. Control (basal medium).
2. CCW extract, 1% in distilled water.
3. " ", 2% " " ".
4. " ", 3% " " ".
5. " ", 4% " " ".
6. " ", 5% " " ".

Initial pH was adjusted at 5.5, and media were distributed in 50 ml amounts in Erlenmeyer flasks and sterilized at 121° C for 15 minutes.

Cultivation:

The micro-organisms were suspended in sterile distilled water to an optical density of 2.5 at 400 nm. The flasks (3 for each treatment) were inoculated each with 5% (V/V) cell suspension and incubated at 28° C on a rotary shaker at 180 r.p.m. for 6 days. At the end of the fermentation period, the contents of each flask were centrifuged and the necessary analyses were made.

Analyses:

The original, as well as the unassimilated, sugar contents of the fermentation media were determined as glucose, as described by (Dubois *et al.,* 1956).

The growth of yeasts was estimated by separation of the yeast cells by centrifugation, washing and drying at 70° C to constant weight. The dry cells were analysed for their content of crude protein (SCP) using the procedure mentioned by Fawcett and Scott (1960). The fermentation media have been analysed for their content of total nitrogen (Fawcett and Scott, 1960). Total sterols were determined using Libermann-Burchard reaction (Snell and Snell, 1960). The obtained data were statistically analysed by using F test, Duncan's methods and L.S.D. test (Steel and Torrie, 1980).

Each treatment was carried out in triplicates and the results illustrated in this work are the arithmatic mean.

RESULTS AND DISCUSSION

Chemical composition of the crude chicken waste (CCW):

Analytical results of CCW revealed that water constitutes about 26.5% of its fresh weight (Table 1). CCW was found to be rich in total reducing sugars (about 57%). It contained an appreciable amount of total N (about 12%), some of which is present in the form of peptides (6.6%), while part of the rest is in the form of ammonia and amino acids. It also contains a minor amount (about 1.3%) of total sterols.

Table 1
Approximate chemical composition of the crude chicken waste (CCW).

Constituents	Mg/100 mg fresh weight
Moisture	26.46
Total solids	73.54
Total reducing sugars	57.32
Ash	7.69
Ammonia N	1.11
Amino acids N	2.73
Peptides N	6.62
Total soluble N	10.46
Insoluble N	1.28
Total N	11.74
Total sterols	1.27

SCP Production

The results presented in Tables 2, 3 & 4 clearly show that the three tested yeast strains manifested large variations in growth values and in their capacity to SCP production. However, these yeast strains exhibited good growth yields when grown on CCW as a sole carbon and N source comparable to control. It is clear that sugar and N uptake increased form the fermentation media containing the waste more than from control. The increase in sugar and N uptake reached its maximal level at 2% waste concentration in case of *S. cerevisiae* and *S. carlsbergensis* (1047 mg, 978 mg sugar and 90.9, 106.1 mg N uptake, respectively), (Tables 2 & 3). On the other hand, sugar and N uptake by *Candida utilis* were maxima at 3% waste concentration (Table 4). The relationships between cell biomasses and accumulation of SCP in the yeast cells were not consistent. Thus, a high cell biomass was not necessarily associated with a concomitant high level of SCP content. Although a high growth level accompanied with a relatively low SCP content were obtained with *C. utilis,* maximal SCP yield accompanied with a comparatively lower growth level were observed with *Saccharomyces carlsbergensis* (Table 5). These results are consistent with the findings of El-Rafai *et al.*, (1986).

Sterels formation:

Total sterols-formation was found to follow the same sequence which occurred in case of SCP production.

Table 2
Growth features and SCP production by *S. cerevisiae* grown on crude chicken waste media (CCW). (amounts are represented as mg/100 ml medium)

CCW concentration	Final pH	Sugar uptake (mg)	Nitrogen uptake (mg)	Dry weight (mg)	SCP (mg)	Protein percent dry wt.	Total sterols (mg)	EC	PCC
Control	4.1	740	48.2	107.6	34.4	31.9	0.26	14.5	4.6
1%	4.0	786	66.1	154.5	54.7	35.4	0.42	19.7	6.9
2%	3.9	1047	90.9	296.0	108.0	36.5	0.63	28.3	10.3
3%	3.9	910	48.7	220.4	79.7	36.2	0.52	24.2	8.8
4%	3.9	895	78.4	224.0	81.8	36.5	0.53	25.0	9.1
5%	3.9	674	62.0	141.8	51.2	36.1	0.41	21.0	7.6
L.S.D. at 0.01		2.94	3.12	13.52	5.21		0.255		
L.S.D. at 0.05		2.13	2.25	9.78	3.76		0.185		

$$\text{EC (Economic Coefficient)} = \frac{\text{Dry weight of biomass}}{\text{Sugar uptake}} \times 100$$

$$\text{PCC (protein conversion coefficient)} = \frac{\text{Weight of SCP}}{\text{Sugar uptake}} \times 100$$

Table 3
Growth features and SCP production by *S. carlsbergensis* grown on crude chicken waste (CCW). (amounts are represented as mg/100 ml medium).

CCW concentration	Final pH	Sugar uptake (mg)	Nitrogen uptake (mg)	Dry weight (mg)	SCP (mg)	Protein percent dry wt.	Total sterols (mg)	EC	PCC
Control	3.9	684	63.6	97.7	35.6	36.4	0.27	14.3	5.2
1%	4.1	720	81.4	143.6	57.4	39.9	0.44	19.9	7.9
2%	4.0	978	106.1	274.6	118.5	43.2	0.67	28.1	12.1
3%	4.0	870	97.2	208.4	88.2	42.3	0.56	23.9	10.1
4%	3.8	840	82.9	201.6	83.1	41.2	0.54	24.0	9.9
5%	3.8	612	70.4	123.5	52.3	42.3	0.42	20.2	8.5
L.S.D. at 0.01		2.83	3.15	4.06	5.88		0.274		
L.S.D. at 0.05		2.05	2.28	2.94	4.25		0.198		

Table 4
Growth features and SCP production by *C. utilis* grown on crude chicken waste (CCW).
(amounts are represented as mg/100 ml medium).

CCW concentration	Final pH	Sugar uptake (mg)	Nitrogen uptake (mg)	Dry weight (mg)	SCP (mg)	Protein percent dry wt.	Total sterols (mg)	EC	PCC
Control	4.0	820	42.7	125.2	38.2	30.5	0.29	15.3	4.6
1%	4.0	898	61.5	179.2	61.8	34.5	0.46	19.9	6.9
2%	3.9	1014	78.9	237.0	84.5	35.6	0.54	23.4	8.3
3%	4.0	1155	87.6	345.8	125.7	36.4	0.70	29.9	10.9
4%	3.9	966	73.7	247.2	87.5	35.4	0.56	25.6	9.1
5%	3.9	815	57.2	153.6	54.8	35.6	0.44	18.8	6.9
L.S.D. at 001		3.59	3.2	15.7	6.01		0.288		
L.S.D. at 0.05		2.59	2.3	11.3	4.3		0.208		

Table 5
Comparison between the growth features of the tested yeast strains grown on chicken-containing media.

Micro-organism	Final pH	CCW concentra tion	Sugar uptake (mg)	Nitrogen uptake (mg)	Dry Wt. of biomass (mg)	SCP (mg)	Protein percent dry wt.	Total sterols (mg)	EC	PCC
S. cerevisiae	3.9	2%	1047	90.9	296.0	108.0	36.5	0.63	28.3	10.3
S. carlsbergensis	4.0	2%	978	106.1	274.0	118.5	43.2	0.67	28.1	12.1
C. utilis	4.0	3%	1155	87.6	345.8	125.7	36.4	0.70	29.9	10.9

As protein accumulation within the yeast cells increased, formation of total sterols subsequently increased. Maximal amounts of total sterols were attained at 2% level of waste in case of *S. cerevisiae* and *S. carlsbergensis*, and at 3% level of waste in case of *C. utilis*.

Utilization of sugar contents of the media:

The efficiency of conversion the sugar content of CCW into SCP by the tested yeast strains has been affected by the waste concentration in the fermentation media. It was maximum at 2% level of the waste in case of *S. cerevisiae* and *S. carlsbergensis* (10.3% & 12.1%, respectively), while it was maximum at 3% level of waste in case of *C. utilis* (10.9%) - (Table 5). *S. carlsbergensis* converted the sugar's content of CCW into SCP more efficiently (12.1%) than the other tested yeast strains (Table 5). This micro-organism seems to exhibit a lower carbohydrate assimilation activity as shown from the relatively low amount of consumed sugar comparable to the other tested micro-organisms (Table 5). These results agree with the results recorded by Lindquist, 1953 a, b and El-Rafai *et al.*, 1986. All tested yeast strains had the capacity of conversion the sugar content of the CCW into cell biomass efficiently with an economic coefficient (EC) higher than that of the control. The economic coefficient reached maximal values according to the following sequence: *C. utilis* (29.9), *S. cerevisiae* (28.3) and *S. carlsbergensis* (28.1), respectively (Table 5). The present results are in accordance with the observations of Ghanem *et al.*, (1986) and Litchfield (1979).

pH - relationships:

The biosynthesis of SCP and maximum SCP production were accompanied by a drop in the pH - value of the media to 3.9, 4.0 for *S. cerevisiae*, *S. carlsbergensis* and *C. utilis*, respectively. This means that good fermentation yields were achieved at this level of pH value (Table 5). This may be attributed to the more favourable reaction of the media during the fermentation process. The adjustment of the initial pH value of the medium at 5.5 allowed high biomass and SCP yielding. This is consistent with the observations of Anderson *et al.*, (1974) and Ghanem *et al.*, (1986).

All data obtained were statistically analysed and the analysis revealed the presence of significant differences between the data.

Finally, we can conclude, according to the results of this work, that crude chicken waste (CCW) can be utilized efficiently as a sole carbon and N source for the biosynthesis of SCP by yeasts.

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