Regulation of the Amino Acid and Fatty Acid Contents of Baker's Yeast to Improve its Nutritional Value for the Population Growth of the Rotifer *Brachionus plicatilis*

Cyril Glenn SATUITO* and Kazutsugu HIRAYAMA

We reared baker's yeast in growth media of varying compositions and tested the nutritional values of the produced yeasts for the population growth of the rotifer *Brachionus plicatilis*. Amino acid and fatty acid contents of these yeasts were also analyzed.

The type of growth medium influenced the amino acid contents of the yeast cells. Increase in amino acid contents of the yeast resulted in improved growth of the rotifer. The fatty acid composition of yeast was also influenced by the growth medium. Growth of the rotifer fed with the yeast reared in squid liver oil enriched growth medium was improved in the same way as when the lipid was supplemented directly in the food suspension.

Key words: baker's yeast, rotifer, nutritional improvement, yeast growth medium, amino acid, fatty acid

Introduction

Baker's yeast is currently the most practical food for mass culturing the rotifer *Brachionus plicatilis*. It's price is cheap and is readily available. However, baker's yeast is deficient in nutrients essential for the growth of the rotifer[1,2] and hence, unstable rotifer production is often experienced when baker's yeast is used as food[3].

Imada et al.[4] successfully incorporated ω3 highly unsaturated fatty acids (ω3 HUFA) in the yeast cells by culturing baker's yeast in growth medium containing fish oil or cuttlefish liver oil. The so-called "ω-yeast", now commercially available in Japan, was developed to solve the problem of ω3 HUFA deficiency in food organisms fed baker's yeast. If baker's yeast can absorb nutrients added to the growth medium within its cells, this might be an effective method of improving the nutritional value of baker's yeast for the population growth of the rotifer.

The present study was focused to the improvement of the nutritional value of baker's yeast for the growth of the rotifer by artificially regulating the amino acid and fatty acid contents of baker's yeast cells. The effect of adding various enrichments to the yeast growth medium on the nutritional value of baker's yeast for the population growth of the rotifer was investigated, to find out whether enrichments added to the growth medium will be reflected in the yeast cells.

Materials and Methods

First laid eggs, collected from an actively growing group of rotifer, were axenically cultured in food suspensions containing differently reared baker's yeasts. The nutritional values of the differently reared yeasts were

* Graduate School of Marine Science and Engineering.

evaluated by comparing the population growth indices obtained from the control yeast suspension to those obtained from suspensions containing yeasts reared in enriched growth media.

The rotifers used for investigations were amictic females derived from an L-type strain employed in a previous study[2]. During investigations, temperature was maintained at 23 °C and no mictic females or males was observed. The population growth indices used for evaluation were determined from two culture methods ; the batch culture and individual culture methods. In batch culture method, the increase in number of individuals was determined as the index, after culturing rotifers in food suspensions for 6 days from inoculation of 20 individuals in each experimental group. In individual culture method, the intrinsic rate of population increase (r) and the net reproduction rate (R_0) were calculated from survival rates and fecundities obtained by daily observation with renewal of food suspensions during the whole lifespan. The two culture methods are described in details elsewhere[5].

Seven differently prepared yeasts were used as food for the rotifer. These yeasts were suspended in the rotifer culture water strengthened with vitamins B_{12} and C at 1.4 and 4.0 μg/ml and with emulsified fat-soluble vitamins A, D and E at 2.0, 0.2 and 1.0 μg/ml respectively. Baker's yeast reared in 1/2 diluted Mayer's medium was used as the control yeast (yeast M1). Five types of yeast (yeasts M2, M3, M4, M5 and M6, Table 1) were prepared by rearing baker's yeast in five types of growth media containing varying amounts of amino acids. The five types of growth media were prepared by adding varying combinations of amino acids as nitrogen sources to 1/2 diluted Mayer's medium (Table 1), which was used as the basic growth medium. Another type of yeast (yeast W1) was prepared by rearing yeast in Wickerham's medium[6]. The nutritional value of yeast W1 was compared to that of yeast M3 in individual culture method.

Table 1. Composition of 1/2 diluted Mayer's medium (basic medium) and amino acid enrichments added to the different yeast growth media

Formula of 1/2 diluted Mayer's medium (yeast M1)	
Saccharose	75 g
K_3PO_4	2.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaHPO_4 \cdot 2H_2O$	0.4 g
Ammonium tartarate	5 g
Distilled water	1 liter
pH	5.4

Yeasts	Amino acids added to 1/2 diluted Mayer's medium (mg/liter)					
	Trp	Phe	Leu	Met	Val	Lys
M1						
M2		20	20		10	10
M3		20	20	20	10	10
M4	40		20	20	10	10
M5	25	20	20	20	10	10
M6	40	20	20	20	10	10
W1	(Wickerham's medium)					

In another experiment, the nutritional value of squid liver oil enriched yeast (yeast EnW1) on the growth of the rotifer was evaluated in comparison to non-enriched yeast (yeast W1). The enriched yeast was reared in growth medium containing squid liver oil in emulsified state. These yeasts were suspended in the rotifer culture water strengthened with 1.4 μg/ml of vitamin B_{12}. Non-enriched yeast was also suspended in rotifer culture water strengthened with 1.4 μg/ml of vitamin B_{12} and 4.0 μg/ml of squid liver oil.

The yeasts used as food for investigations were all reared with continuous aeration and were washed by centrifugation before resuspending in the rotifer culture water. Cells employed were only those under the exponential stage of growth. The density of yeast suspended in the rotifer culture water was 200 μg/ml. Nutrients were supplemented in all food suspensions to enhance growth of the rotifer. The concentrations of nutrients added

were those which showed the highest supplementary effects in previous investigations[1,2,7]. Food suspensions were shaked with a Circle Shaker (Taiyo Co. Ltd.) set at 130 rotations/min. for 15 minutes 6 times a day to keep the cells in a suspended state throughout the investigations[8].

A small portion of the prepared yeasts were also collected, dried under reduced pressure and sent to the Central Research Laboratory of Nihon Suisan Co. Ltd. for analysis of amino acid and fatty acid contents. At the Laboratory, the samples were again dried prior to analysis. The amino acid contents of the samples were analyzed with a Hitachi 835 amino acid analyzer according to the methods employed by Yagi et al[9]. Tryptophan was measured by the spectrophotometric method after hydrolysis with 4.2 N NaOH at 105°C for 22 hours.

Fatty acid contents of the samples were measured by gas chromatography. Fatty acid methyl esters were prepared by treating lipids, extracted from the samples according to the method employed by Bligh and Dyer[10], with boron triflouride-methanol. Gas chromatography analysis of the fatty acid methyl esters was carried out on a Hewlett Packerd HP5890A Model gas chromatograph fitted with a Supelcowax-10 fused silica capillary column (30m × 0.32mm).

Squid liver oil employed in the study was a product of Riken Vitamin Co. Ltd. Procedures for the collection of first laid eggs, sterilization using antibiotic mixtures, preparation of food suspensions including emulsification of fat-soluble nutrients and sterility tests using STP medium are described elsewhere[1,2].

Results

Effect of Yeast Growth Medium on the Nutritional Value of Baker's Yeast.

Table 2 shows the amino acid profiles of the differently prepared yeasts. No difference was observed in the amino acid profiles and total amino acid contents of yeasts reared in variously modified Mayer's media (yeasts M1, M2, M3, M4, M5 & M6). However, yeast W1 had higher amino acid contents than the other yeasts. Total amino acid contents of the differently prepared yeasts (in % of dry weight) were observed to be constant within the range of 27.2–30.2%, with the exception of yeast W1 which was 40.6%.

Table 3 shows the r and Ro values obtained from culturing rotifers with the different yeasts. The nutritional value of these yeasts for the rotifer were evaluated in comparison to their respective control groups. Yeasts M2, M3, M4, M5 and M6 were compared with yeast M1, while yeast W1 was compared with yeast M3 (Table 3). The relative values are shown in Fig. 1. Out of the different types of yeasts tested, yeasts M3 and W1 showed Ro indices which were 1.52 and 1.95 times higher than their respective controls. Rotifers cultured with yeasts M2, M4, M5 and M6 did not show much difference in growth as compared to those cultured with yeast M1 (control yeast). The same tendency was observed when rotifers were batch cultured with these yeasts. Fig. 2 shows the average increase in number of rotifers obtained from two batch cultures. After 6 days of batch culture from inoculation of 20 individuals, only yeasts M3 and W1 showed an increase which was twice that of the control group.

Nutritional Value of Squid Liver Oil Enriched Yeast.

The total amino acid and fatty acid contents of yeast W1 (control yeast) and yeast EnW1 (squid liver oil enriched yeast) are shown in Table 4. The fatty acid compositions of the two yeasts are shown in Table 5. Data of Imada[11] and Dendrinos and Thorpe[12] are also shown in the Tables for comparison. There was no marked difference observed in the total amino and fatty acid contents between yeast W1 and the enriched yeast EnW1 (Table 4). However, while ω3 HUFA was detected in small amounts

Table 2. Amino acid compositions of differently prepared yeasts

Amino acids (in %)	Yeasts M1	M2	M3	M4	M5	M6	W1
Aspartic acid	2.4	2.6	2.6	2.4	2.5	2.7	4.1
Threonine	1.3	1.6	1.6	1.5	1.4	1.7	2.1
Serine	1.3	1.5	1.6	1.4	1.4	1.5	2.0
Glutamic acid	3.3	3.6	3.6	3.8	4.0	3.6	5.2
Proline	1.2	1.2	1.2	1.4	1.4	1.2	1.4
Glycine	1.1	1.3	1.3	1.4	1.4	1.4	2.0
Alanine	2.3	2.0	2.2	2.5	2.0	2.2	2.4
Cystine	0.6	0.5	0.4	0.4	0.6	0.4	0.6
Valine	1.4	1.5	1.5	1.6	1.4	1.8	2.7
Methionine	0.3	0.4	0.4	0.4	0.3	0.3	0.7
iso Leucine	1.2	1.3	1.4	1.3	1.2	1.5	2.2
Leucine	1.6	1.9	2.1	1.9	1.7	2.3	3.1
Tyrosine	1.0	1.1	1.1	1.0	1.1	1.2	1.6
Phenylalanine	0.8	1.1	1.2	1.0	1.0	1.3	2.1
Histidine	0.9	0.6	0.7	0.8	0.8	0.8	1.0
Lysine	2.1	2.2	2.3	2.2	2.5	2.5	3.6
Arginine	3.1	2.8	2.0	2.9	3.9	2.4	2.7
Tryptophan	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cytrulline	0.8	1.0	1.0	0.4	0.6	0.8	0.6
Ornithine	0.2	0.1	0.1	0.2	0.2	0.1	0.1
Ammonia	0.3	0.4	0.3	0.3	0.3	0.4	0.4
Total (%)	27.2	28.9	28.6	29.1	29.8	30.2	40.6

* n.d.-not detected

Table 3. Indices r and Ro of rotifer cultured with the differently reared yeasts

Yeasts*	Total amino acid contents (%)	Experiment 1 r	Experiment 1 R_0	Experiment 2 r	Experiment 2 R_0	Experiment 3 r	Experiment 3 R_0
M1	27.2	0.29	3.33	0.33	4.77		
M2	28.9			0.35	3.83		
M3	28.6	0.42	6.00	0.39	5.96	0.43	5.42
M4	29.1	0.32	3.24				
M5	29.8			0.32	3.97		
M6	30.2	0.34	3.66				
W1	40.3					0.62	10.57

*Yeasts were each suspended at a density of 200 μg/ml in food suspensions containing vitamins B_{12}, C, A, D & E at 1.4, 4.0, 2.0, 0.2 & 1.0 μg/ml respectively.

(0.4% of the total lipid) from the enriched yeast EnW1, such fatty acid was not detected from the control yeast W1 (Table 5).

Table 6 shows the growth indices of rotifers fed with squid liver oil enriched yeast. Rotifers cultured with enriched yeast showed growth indices which were 1.3 and 1.4 times higher than the respective r and Ro values obtained from those cultured with the control yeast (yeast W1). On the other hand, rotifers cultured in food suspensions containing 4.0 μg/ml of squid liver oil showed growth indices which were 1.4 and

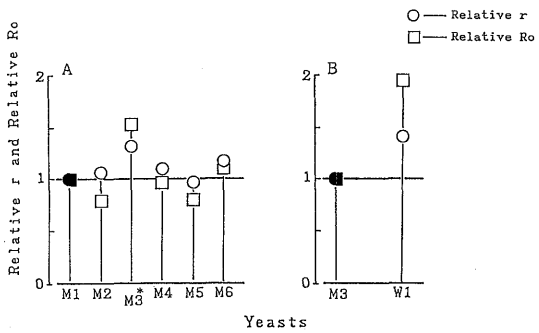

Fig. 1. Relative r and relative R_0 for the differently prepared yeasts.
A shows relative values for the yeasts as compared with values for yeast M1. B shows relative values for the yeast as compared with yeast M3. Shade marks represent the control yeasts with relative values expressed as 1.
* Values shown are averages from two replicate experiments.

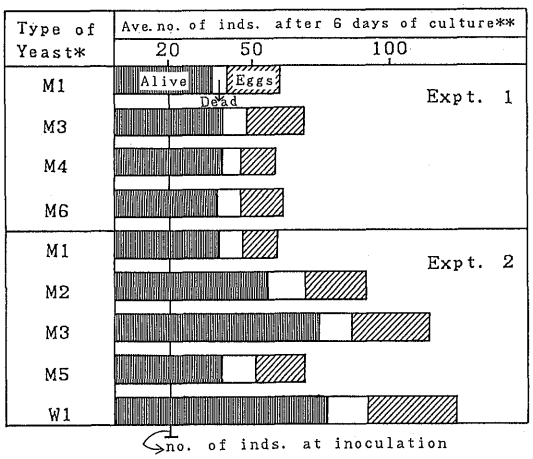

Fig. 2. Average increase in population of the rotifer cultured with the differenly prepared yeasts using batch culture method.
* Yeasts were suspended at μg/ml in the culture water containing vitamins B_{12}, C, A, D and E at 1.4, 4.0, 2.0, 0.2 and 1.0 μg/ml respectively.
** Average values of two replicates.

Table 4. Total amino acid and fatty acid contents of non-enriched and enriched yeasts (in % dry weight)

Type of Yeast	Total Amino Acid	Total Fatty Acid	Source of Data
Yeast W1	47.5	2.4	present experiment
Yeast EnW1	49.3	2.1	〃
Yeast	50.9	1.1	Imada[11]
ω-yeast	38.5	12.7	〃
Sc	30.7		Dendrinos and Thorpe[12]
ScL4	42.9	9.4	〃
ScL5	40.5	8.1	〃

1.6 times higher than the respective r and Ro values obtained from the control yeast suspension.

Discussion

The nutritional values of the differently prepared yeasts were assessed by investigation of the population growth of the rotifer fed with the yeasts and by analysis of the amino acid and fatty acid contents of these yeasts.

Addition of amino acids as nitrogen sources to the yeast growth media did not result in change in the amino acid profiles and total amino acid contents of the yeasts (Table 2). However, the type of growth medium was somehow found to influence amino acid contents in the yeast cells. Yeast W1, which was reared in a medium rich in organic nutrients, showed higher amino acid contents than the other yeasts. Yeast W1 also supported higher population growth for the rotifer than the other yeasts. Rotifers fed with the yeasts reared in modified Mayer's media showed no marked difference in population growth, except for those fed with yeast M3. Although no

Table 5. Fatty acid composition of non-enriched and enriched yeasts

Fatty acid	present expt.		Imada[11]		Dendrinos and Thorpe[12]	
	W1	EnW1	Yeast	ω-Yeast	ScL4	ScL5
14 : 0	1.7	5.4	0.3	4.1	3.4	2.1
14 : 1	0.3	0.2				
15 : 0	–	0.6				
16 : 0	10.6	19.1	8.3	13.4	27.4	21.4
16 : 1	40.8	25.5	38.2	6.6	24.7	26.4
17 : 0	0.2	0.5				
18 : 0	6.2	4.5	4.1	2.4	tr	1.4
18 : 1	37.9	31.5	43.9	16.4	9.3	5.2
18 : 2	–	0.4	2.8	1.1	1.7	5.3
18 : 3			0.5	0.8		
20 : 1	0.2	4.7	0.2	9.1	0.9	tr
20 : 2					} 1.5	} 2.1
18 : 4	0.2	–				
20 : 4 ω3#				1.1		
20 : 3 ω3#				} 3.0		
20 : 4 ω3					2.9	1.6
20 : 5 ω3#				17.7	7.7	4.7
22 : 1				2.1	tr	tr
22 : 5 ω3#				1.0	3.2	4.7
22 : 6 ω3#	–	0.4		12.8	2.5	3.1
24 : 1	1.0	0.5		1.3	2.9	2.9
Unknown	0.9	6.7				
HUFA Σ ω3 (#)	–	0.4	–	35.6	13.4	12.5
Total	2.4	2.1	1.1	12.7	9.4	8.1

tr-trace

Table 6. Indices r and R_0 obtained from non-enriched and enriched yeast suspensions by individual culture method

Type of yeast	Type of Food Suspension*		r	R_0
	Food Density (μg/ml)	Squid liver oil (μg/ml)		
Non-enriched yeast (W1)	200	–	0.35	4.70
W1	200	4.0	0.48	7.57
Enriched yeast (EnW1)	200	–	0.45	6.71

* Food suspensions were supplemented with 1.4 μg/ml of vitamin B_{12}.

apparent difference could be observed in amino acid contents between yeast M3 and the other yeasts, it consistently showed higher nutritional value for the rotifer than the control when evaluated using both culture methods.

Yeast EnW1 (yeast reared in squid liver oil enriched medium) showed no marked difference in total amino acid contents as compared to yeast W1 (Table 4). In contrast, level of protein (% of dry weight) obtained by Imada[11] and Dendrinos and Thorpe[12] varied within about 10% for the different yeasts. Total fatty acid contents of squid liver oil enriched yeast (yeast EnW1) was found at the same level of the

control yeast (yeast W1) (Table 4). Enriched yeasts prepared by Imada[11] and Dendrinos and Thorpe[12] contained total fatty acids (in % dry weight) ranging from 8.1 to 12.7. The total fatty acid contents (in % dry weight) of yeast EnW1 prepared in this investigation was 2.1. However, a small amount of ω3 HUFA (0.4% of the total lipid) was found in yeast EnW1. Since yeast W1 did not contain such fatty acids, ω3 HUFA detected from yeast EnW1 may had been incorporated from the squid liver oil added to the yeast growth medium. Failure to incorporate large amounts of ω3 HUFA into the yeast cells as compared to results of Imada[11] and Dendrinos and Thorpe[12] may be explained by the difference in preparation of the yeast growth medium.

Rotifers fed with yeast EnW1 showed higher growth than those fed with the control yeast W1. Also, rotifers cultured with yeast EnW1 showed growth comparable to those cultured in yeast W1 suspension supplemented with squid liver oil, even though only small amount of fatty acids were incorporated into the yeast cells by enrichment of squid liver oil to the yeast growth medium. With regards to the results of the present experiment, however, fatty acid enriched yeast can improve the growth of the rotifer in the same way as when fatty acids are supplemented directly into the food suspension[7].

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** In Japanese.
*** In Japanese with English summary.

培養液組成がパン酵母のシオミズツボワムシに対する栄養価値に及ぼす影響

サトイトC. G.・平山　和次

パン酵母のシオミズツボワムシ（以下ワムシと略記）に対する栄養価値をその培養組成を改良することによって改善できるかどうか調べた。パン酵母を各種培養液で培養し，生産された酵母をそれぞれワムシに与え，ワムシの増殖を比較した。また，生産された酵母のアミノ酸組成及び脂肪酸組成の面からも培養液の影響を検討した。

窒素源としてMayer氏培養液にアミノ酸を加えパン酵母を培養しても，生産された酵母の全アミノ酸含量を増加させることができなかった。しかし，有機栄養源を多量に含む培養液でパン酵母を培養した場合，全アミノ酸含量が他の培養液で培養した場合に比べて10%程度高い酵母を作ることができた。それをワムシに与えたところ，ワムシの増殖は他の酵母に比べて2倍もよかった。一方，イカ肝油を加えた培養液でパン酵母を培養した場合，$\omega 3$高度不飽和脂肪酸を含む酵母が生産され，そのパン酵母でワムシを飼育したところ，イカ肝油をワムシ飼育水に直接添加したときとほぼ同様な添加効果を示し，その増殖は対照と比べ改善された。