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THE EFFECT OF PREPARATION AND DRYING CONDITIONS OF BACTERIAL AND YEAST-BACTERIAL BIOMASSES ON THE BIOLOGICAL VALUE OF PROTEIN-VITAMIN CONCENTRATES

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Key words: propionic acid bacteria, yeast-bacterial biomasse, spray drying.

Comparative studies involved bacterial and yeast-bacterial biomasses which have been subjected to freeze-drying and spray-drying either with or without the after-fermentation fluid. Considering the nutritive and economic aspects, spray-drying of biomasses not containing the after-fermentation fluid appeared to be most desirable and resulted in protein-vitamin preparations showing the highest biological value.

It was demonstrated in previous investigations that cheesewhey can be used as growth medium for bacterial and yeast bacterial biomasses. The characteristics of these biomasses showed that they can be considered as vitamin (bacteria) and protein-vitamin (yeast and bacteria) concentrates for addition to food and feed [15, 16]. The purpose of this study was to evaluate the effect of the mode of drying (replacing model freeze-drying by spray-drying) on the nutritive components and biological value of the final biomasses obtained. An other purpose was to check the possibility of drying the whole culture in order to utilize all its nutritive components.

MATERIAL AND METHODS

FERMENTATION CONDITIONS

One step fermentation by propionic acid bacteria (mixed cultures of *P. shermanii* 1, *P. freudenreichii J.* and *P. petersonii J.*) and two step fermentation by yeast and propionic acid bacteria (*Kluyveromyces fragillis* CBS-397 and the same mixture of propionic acid bacteria strains)

were carried out according to the previous investigations [15, 16].

Selected strains of propionic acid bacteria and yeast were derived from the local culture collection. The growth medium was prepared from fresh liquid cheese-whey (acid rennet whey) obtained from the main local dairy. The cheese-whey used in this study had the following characteristics: dry matter 5.57%, protein content (N×6.25) 0.75%, lactose content 4.05%, pH 4.4.

METHODS OF DRYING THE BACTERIA AND YEAST-BACTERIA BIOMASSES

Freeze-drying and spray-drying techniques were used alternatively in the investigations on the effect of the drying method on the quality of the final product. For this purpose Loire equipment for freeze drying and the Niro spray-drier with rotating spray-disc was used; evaporating capacity was 25 μg H_2O/h. For drying purposes the air temperature was maintained at 160-170°C (inlet) and 80-85°C (outlet). The dry product was kept in hermetic containers at 4°C prior to analysis.

The following analytical methods were used:

Dry weight of whey and biomasses (fresh and dried) was estimated by drying to constant weight at 105°C. The fluid and half-fluid products were pre-dried at 60°C and then at 80°C. The following amounts were used:

fresh whey	— 5 g
fresh biomass	— 0.5 g
dried material	— 0.5 g

Total nitrogen and protein contents (N × 6.25) was determined by the Kjeldahl method.

Protein nitrogen (N × 6.25) was determined by the micro-Kjeldahl procedure using 20% TCA precipitate (10 ml TCA per 25 ml of extract).

Total corrinoid contents was assayed microbiologically by the agar plate procedure with Escherichia coli 113-3 as test organism. The conditions described by Harrison et al. [2] and Bogucka [1] were followed, as slightly modified in this laboratory. Growth zones (after 18 h incubation) corresponding to several dilutions of cristalline vitamin B_{12} (0.03125 — 1 μg/ml) served for the preparation of a standard curve. The corrinoid contents was also measured spectrophotometrically at 580 nm. Before readings were taken the corrinoids were converted to the di-CN-form (2 h incubation with KCN). Thus, for quantitation the extinction coefficient $E\,{}^{1\%}_{1\,cm} = 105$ was applied.

Vitamins of the B group were assayed microbiologically with the following test organisms according to [6, 8, 19]:

Vitamin B_1	— *Lactobacillus fermenti* P-36
Vitamin B_2	— *Lactobacillus casei* E-ATCC 7469

Vitamin B_6	— *Saccharamyces carlsbergensis* ATCC 9080
Folic acid	— *Streptococcus faecalis* R-ATCC 8043
Nicotin amide, Panthotenic acid and Biotin	— *Lactobacillus arabinosus* ATCC 8014

Quantitative amino acid analysis was conducted by means of single column resin chromatography (M-82 type resin) in an automatic amino acid analyser (Beckman-Multichrom 4255 type) following the standard procedure. Acid hydrolysis (prior to chromatography) of the material was conducted in 6 N HCl (analytical grade) in a vacuum sealed tube under nitrogen. Hydrolysis was carried out at 110°C for 22 hrs.

Quantitative gas chromatography separation of volatile fatty acids (mainly acetic and propionic acids) was carried out with Willy Griede model GCHF 18.34 gas chromatograph equipped with a flame ionization detector [13, 11]. Column dimensions: 2 meter length, 3 mm diameter, filled with SP-1200 (Supelco Inc.) on Chromosorb W 80 1/100 mesh. Flow rate of nitrogen was 50 ml/min. Column temperature — 150°C.

The nucleic acid content in the investigated biomasses was determined by adopting the conditions reported in [4, 9]. The samples were centrifuged after the homogenization of the biomasses with ethyl alcohol. The sediment was washed with 70% ethanol containing 0.1% $HClO_4$ and centrifuged again. Than nucleic acids were extracted three times from the sediment by means of 0.5 $HClO_4$. All extracts were combined and subject to RNA and DNA content determination. RNA was determined with the orcinol reagent at 660 nm for concentration ranging from 5-50 μg per 1 ml. DNA was determined with diphenylamine reagent at 595 nm for amounts from 10-300 nm/1 ml.

The "in vitro" enzymatic digestibility of proteins was contacted according to the methods described by Mauron [5] and Prończuk [12] and some modifications [17] with pepsin and pancratin. The enzymatic digestibility of biomasses with pronase from *Streptomyces griseus* was performed under optimal conditions for this enzyme preparation [18]. For comparative reasons, acid hydrolysis of the investigated biomasses was conducted in 6 N HCl, at 110°C for 22 hrs. The rate of enzymatic and acid hydrolysis was determined by the amount of α-NH_2 nitrogen liberated. The latter was estimated by the ninhydrin procedure at 570 nm. Fat and vitamin free casein (BDH) was used as standard protein.

Determination of the enzymatic digestibility "in vitro" of the biomasses examined was combined with the determination of free amino acids after digestion with the same enzyme complexes: pepsin+pancreatin and pronase.

For this purpose, the dialysates obtained after digestion procedure were evaporated to a small volume (approx. 10 ml) and the same volume of 6% sulphosalicylic acid was added to precipitate the peptides. The samples were centrifuged, evaporated and then the content of free amino acid was evaluated.

The following chemical indexes of the nutritional value were calculated.

Chemical Score (CS) according to Block and Mitchell [7] was calculated on the basis of the ratio of exogenous amino acid content in the preparations to their content in the hen egg proteins, being the standard.

Essential Amino Acid Index to Oser (EAA), [10] was calculated using the following formula:

$$EAA = \sqrt[n]{\frac{ileu\ p}{ileu\ s} \times \frac{leu\ p}{leu\ s} \times \cdots \frac{wal\ p}{wal\ s}}$$

p — content of the indicated amino acid in the protein being examined,
n — amount of exogenous amino acids,
s — content of certain amino acids in the hen egg proteins.

If the content of a certain amino acid in the examined preparations exceeded its content in the egg proteins, the value of the $\frac{p}{s}$ ratio was taken as 1.

Biological Value (BV) according to Oser [14] was calculated on the basis of the following formula:

$$BV = 1.09 \times EAA - 11.73$$

Pronase Digest Dialysats (Pron. DD index) Index was calculated according to the method of Sheffner et all [14] after protein hydrolysis with pronase and taking casein as a standard.

RESULTS AND DISCUSSION

The products investigated in this were abbreviated as follows: Biomass of Propionibacteria mixed culture

— without the after fermentation fluid
  freeze dried — 1
  spray dried — 2

— with the after fermentation fluid — whole culture
spray dried — 3
Yeast-Propionibacteria biomass
— without the culture fluid
freeze dried — 4
spray dried — 5
— with the culture fluid
spray dried — 6

The basic characteristics of the products obtained are presented in Table 1.

Table 1. General characteristics of biomasses

Biomass	Dry weight of preparation (freeze or spray dried) in %	Dry weight of fresh biomass in %	Protein contents in % dry weight	Nucleic acid contents in % dry weight		Acetic acid / propionic acid	Ash in % dry weight
				DNA	RNA		
1	92.03	22.0	58.9	3.43	4.28	9.14 13.77	10.23
2	93.67	21.5	58.3	2.77	4.08	2.60 6.47	9.89
3	80.86	—	54.1	2.30	2.59	50.94 179.80	17.81
4	94.99	25.0	56.2	2.46	3.06	4.03 11.38	10.71
5	96.13	24.59	50.58	1.17	2.45	0.52 2.89	12.30
6	93.94	—	41.50	0.99	2.04	0.66 3.08	25.70

Tables 1 and 2 present data which illustrate the influence of the drying technique of the biomasses, with or without the culture fluid on the quality of the products of bacterial or yeast-bacterial origin. The analysis of experimental results concerning the biomasses No. 1, 2, 3, 4, 5 and 6 indicated that in the yeast-bacteria cultures a higher yield of biomass of lower nucleic, propionic acid and acetic acids content was obtained, as compared with the biomasses produced by the bacteria cultures.

The drying technique influenced considerably the content of propionic and acetic acids in the product. It was demonstrated both in the bacterial biomasses No. 1 and 2 and in the yeast-bacterial preparations No. 4 and 5 that spray-drying as compared with freeze-drying caused removal of a major part of these acids, thus resulting in their lower content in the

final product. Also the content of nucleic acids in both kinds of products inderwent positive changes when using the spray-drying technique.

Drying procedure of biomasses with the culture fluid resulted in an increased yield of the products obtained, solid components of the fluids, an increase of the content of the propionic and acetic acids in the final products (comparison of the bacterial products No. 2 and 3, and yeast-bacterial products No. 5 and 6). This was noticed particularly in the bacteria cultures in which propionic acid was the major metabolite.

Table 2. The corrinoid and B group vitamin contents in mg per 100 g biomass dry weight

Biomass	Corrinoids		B_1	B_2	PP	B_6	Folic acid	Ca pantothenate	Biotin
	Total contents	B_{12}							
1	61.08	54.98	0.55	2.2	31.3	5.5	1.8	2.7	0.15
2	57.70	52.00	0.82	1.6	21.2	1.0	0.98	1.8	0.10
3	29.01	26.11	0.52	2.1	29.6	1.2	1.1	2.2	0.12
4	30.73	24.73	0.82	0.62	27.3	0.98	0.62	2.6	0.21
5	11.86	9.60	0.59	2.08	22.87	3.22	2.60	5.00	0.10
6	10.77	8.71	0.85	2.76	11.18	6.91	1.87	6.17	0.11

An increased content of these two acids in the yeast-bacterial products was also observed. However, the total content resulting from the management conditions of these cultures was so small that it seemed to create no problems. In this case, however, a considerable, i.e. twofold increase of ash content in the final products was observed. The analysis of data concerning the corrinoids content and B-group vitamins in the investigated products (see Table 2 and allows to make the following comments.

As a result of the culturing conditions, the bacteria-yeast products No. 4, 5 and 6 contained, in general, lower amounts of corrinoids than the bacterial biomasses. However, the content of other B-group vitamins was generally similar, independently of the drying technique and the composition of the dried product (with or without the culture fluid).

Moreover the drying technique had, in principle, no influence on total content of corrinoids in Saeterial products (No. 1 when compared with 2), as well as in the yeast-bacteria products (No. 4 when compared with 5). Drying procedure of the culture with the post fermentation fluid containing, as shown previously, some traces of vitamin B_{12}, decreased the proportional amount of the total corrinoids, calculated per dry matter of the product. The content of other vitamins of B-group in the bacteria products dried with the culture fluid (No. 3 when compared with biomasses No. 1 and 2) and the results pertaining to the yeast-bacteria products demonstrated no clear relationship in this respect,

as far as biomass No. 6 when compared with 5 is concerned. The drying techniques were demonstrated to have an influence on the content of the other vitamins of B-group, contrary to the vitamin B_{12} content. The spray-drying technique when applied to the bacteria biomasses (No. 1 compared with 2) without the post fermentation fluids decreased the content of the majority of vitamin. This relationship however was not observed in the yeast-bacteria biomasses, and in the case of vitamins B_{12} and B_6, folic acid and panthotenic acid, the spray-dried products demonstrated a higher content of the vitamins specified above as compared with the freeze-dried products (see No. 4 and 5). This might be attributed to some disruption and availability of higher amounts of these vitamins to the test microorganisms used for their determinations.

Table No. 3 presents enzymatic digestibility data of proteins in the investigated biomasses.

Table 3. Enzymatic digestibility of proteins in the investigated biomasses in relation to acid hydrolysis (assumed as 100%)

Biomass	Enzymatic system			
	pepsin	pancreatin	pronase	
	amount of N alpha NH_2 liberated in g per 100 g dry weight	effectiveness of enzymatic hydrolysis in % *)	amount of N alpha NH_2 liberated in g per 100 g dry weight	effectiveness of enzymatic hydrolysis in % *)
1	1.77	20.23	1.89	21.60
2	1.21	15.31	2.32	29.36
3	1.41	18.80	2.31	30.80
4	1.06	13.51	1.85	23.65
5	1.15	14.6	2.06	26.1
6	1.21	15.4	2.20	28.0

*) A complete/100% acid hydrolysis was assumed

The experimental results demonstrated, that when using the two model enzymatic systems (pepsine+pancreatine and pronase), the digestibility of bacteria biomasses was slightly higher than that of the yeast-bacteria ones. The application of pronase ensured higher effectiveness of digestibility of both types of products as compared with the digestion procedure with pepsine-pancreatine system only. The spray-drying technique when compared with the freeze-drying one resulted in a product of higher susceptibility to the action of proteolytic enzymes. This was demonstrated in terms of the higher values of the digestibility effectiveness, irrespective of the enzymatic system used. The comparison of results concerning the products obtained from biomasses fried with

or without the culture fluid indicated that the enzymatic digestibility was slightly higher in the former, of bacterial (compare No. 2 and 3) as well as yeast-bacterial) compare No. 5 and 6) origin.

Some additional data concerning the degree of amino acid liberation (mainly of the exogenous ones) show that irrespectively of the kind of biomass the digestion with proteolytic enzymes of the digestive tract (pepsin+pancreatin) resulted in a liberation of 8 to 11 amino acids, whereas acids, whereas the digestion with pronase resulted in the liberation of all amino acids. These findings were reflected in the results of the total amounts of amino acids liberated by pepsin+pancreatin (9.90-18.04 grams of amino acids) 16 g N and pronase (30.18-46.78 grams of amino acids 16 g N).

These results correspond with the values of enzymatic digestibility, discussed earlier (see Table 3). The experimental results indicated that higher amounts of exogenous amino acids such as lysine, leucine, isoleucine and tyrosine were liberated during the digestion of yeast-bacterial origin. As demonstrated in Table 4 the amino acid composition of the products (biomasses) obtained was interesting and valuable from the nutritional point of view.

Table 4. The amino acid composition of biomasses

Biomass / Amino acid	1		2		3		4		5		6	
	A	B	A	B	A	B	A	B	A	B	A	B
Lysine	6.73	7.71	7.31	7.90	7.44	8.88	7.87	9.25	8.55	8.75	8.53	8.79
Histidine	1.24	1.42	1.55	1.68	0.93	1.11	1.40	1.64	1.06	1.08	1.17	1.21
Arginine	3.14	3.60	3.48	3.77	2.09	2.50	2.72	2.19	1.97	2.02	3.74	3.86
Aspartic acid	9.85	11.30	10.65	11.51	9.18	10.96	9.90	11.64	12.70	13.00	12.05	12.43
Threonine	4.29	4.92	4.52	4.89	4.64	5.54	4.36	5.09	5.98	6.12	5.05	5.20
Serine	3.99	4.58	3.99	4.32	3.47	4.14	4.04	4.74	5.35	5.47	2.54	2.62
Glutamic acid	16.18	18.56	16.77	18.13	16.33	19.49	15.24	17.91	20.84	21.34	18.67	19.26
Proline	2.62	3.01	2.76	2.99	3.11	3.71	2.99	3.52	0.25	2.25	2.39	2.46
Glycine	2.85	3.27	3.15	3.41	2.70	3.23	2.58	3.03	2.88	2.94	3.07	3.17
Alanine	8.02	9.20	8.02	8.67	8.82	100.53	6.45	7.58	—	8.59	7.13	7.35
Cystine 1/2	0.63	0.72	0.96	1.04	0.96	1.15	1.30	1.52	—	—	0.12	0.12
Valine	4.68	5.37	4.92	5.32	5.51	4.19	4.29	5.04	5.58	5.72	5.75	5.97
Methionine	3.28	3.76	3.58	3.87	3.13	3.74	2.45	2.87	1.69	1.73	2.44	2.52
Isoleucine	3.94	4.51	4.46	4.82	3.94	4.70	4.04	4.75	6.14	6.28	5.23	5.39
Leucine	9.45	10.83	9.71	10.50	8.27	9.87	9.76	11.48	11.66	11.92	12.44	12.84
Tyrosine	3.99	4.57	3.99	4.31	3.26	3.89	3.26	3.83	2.21	2.25	3.08	3.18
Phenyloalanine	2.31	2.65	2.64	2.86	1.98	2.37	2.48	2.91	2.41	2.64	3.43	3.54
Total	87.19	99.98	92.47	99.99	83.78	100.00	85.13					
The amount of NH_3 in % of total N	16.45%		6.62%		18.03%		12.95%		7.75%1		9.07%	

Certain chemical coefficients of the nutritional value were calculated out of the amino acid composition to obtain comparable values demonstrating the nutritional value of these biomasses produced as shown in Table 5. The nutritional value was limited by the sulphur amino acids i.e. methionine and cysteine in the yeast-bacteria biomasses and by the aromatic amino acids tyrosine and phenyloalanine in biomasses produced by bacteria.

These findings were in a compliance with the paper of Sheffner [14] who reported, however, lower nutritional values for the yeast-bacteria proteins, when compared with the results presented in this report. It

Table 5. Chemical indexes of the nutritional value of several biomasses examined

Biomass Coefficient	1	2	3	4	5	6
C.S. (amino acid lim.)	63 (tyr + phen)	65 (tyr + phen)	69 (tyr + phen)	62 (cys + met)	55 (met)	41 (cys + met)
EAA	88	88	87	87	86	91
BV	84	84	83	83	82	87
Pron. DD index	61	43	47	42	58	56

should be pointed out that the indexes of nutritional value shown in Table 5 can be regarded as high when compared with various protein sources reported in the literature [13, 14]. For these reasons, the examined protein-vitamin products my be considered as highly valuable protein sources.

The values of coefficients shown in Table 5 indicated, in principle no differences resulting from various techniques applid to the production and drying of the biomasses.

CONCLUSIONS

1. The spray drying technique when applied instead of liophylization to bacterial (mixed propionic acid) and yeast-bacterial biomasses (*Kluyveromyces fragillis* CBS-397 and mixed propionic acid bacteria: *P. shermanii*-1, *P. freudenreichii J.* and *P. petersonii J.*) does not effect the biological value of the final vitamin-protein or protein-vitamin preparations.

2. Spray-drying of both bacterial and yeast-bacterial cultures also seems to be advantages in terms of the overall utilization of the post fermentation fluids (usually creating the polution problem).

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WPŁYW WARUNKÓW PRZYGOTOWANIA I SUSZENIA BIOMAS DROŻDŻOWO-BAKTERYJNYCH NA ICH WARTOŚĆ ODŻYWCZĄ

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Streszczenie

Biomasy drożdżowo-bakteryjne uzyskano w wyniku hodowli drożdży *Culveromyces fragilis* i bakterii z rodzaju *Propionibacterium* na podłożu serwatkowym. Uzyskane biomasy suszono rozpyłowo i liofilizacyjnie wraz z podłożem lub po jego oddzieleniu metodą wirowania. Jako kryteria wartości uzyskanych preparatów przyjęto podstawową ich charakterystykę oraz zawartość witamin z grupy B, zwła-

szcza witaminy B_{12}, kwasów nukleinowych, kwasu propionowego, strawność enzymatyczną „in vitro" oraz wartość biologiczną oznaczoną na zwierzętach doświadczalnych, mikrobiologicznie i na podstawie wskaźników chemicznych.

Badania wykazały, że zastosowana metoda prowadzenia skojarzonych hodowli drożdżowo-bakteryjnych na serwatce pozwala na osiągnięcie wysokowartościowych preparatów białkowo-witaminowych. Uzyskane wyniki wskazują ponadto, że suszenie rozpyłowe tych biomas jest poza względami ekonomicznymi bardziej uzasadnione od liofilizacyjnego, głównie z uwagi na obniżenie ilości kwasów nukleinowych i podwyższenie podatności preparatów na działanie enzymów proteolitycznych przewodu pokarmowego (strawność enzymatyczna „in vitro"). Nie wykazano jednocześnie negatywnego wpływu tego suszenia na zawartość witamin z grupy B ani na profil korynoidów.

Suszenie omawianych biomas z płynem pofermentacyjnym prowadzi do uzyskania produktu o znacznej zawartości kwasu propionowego i związków popiołowych przy jednoczesnym obniżeniu zawartości witamin. Optymalne właściwości badanych preparatów zapewnia więc suszenie metodą rozpyłową biomas oddzielonych od płynów pofermentacyjnych.