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A FERMENTED, ROPY, NON-DAIRY OAT PRODUCT BASED ON THE EXOPOLYSACCHARIDE-PRODUCING STRAIN *Pediococcus damnosus*

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SUMMARY

An attempt has been made to produce a fermented, ropy, non-dairy product (Y2.6) containing the exopolysaccharide(EPS)-producing lactic acid bacterial strain *Pediococcus damnosus* 2.6 in combination with an ordinary yoghurt culture (V2). The fermentation profile, including physical parameters, such as viscosity and ropiness, was measured during a fermentation period of 24 h. The stability of the product was determined during 30 days of cold storage (6 °C). No decrease in viscosity or ropiness was seen during the storage period. The survival of the *P. damnosus* 2.6 strain was high during the whole storage period. Finally, a sensory preference test was done on two differently flavoured Y2.6 products. A traditional, fermented, ropy, dairy product was used as a control product. No significant difference ($P<0.05$) between the fermented, ropy Y2.6 product and the commercial, fermented, ropy dairy product was shown by the sensory preference test. This study shows the possibility of developing a fermented, ropy, non-dairy product derived entirely from oats and water.

KEYWORDS: Non-dairy, Adavena®, *Pediococcus damnosus* 2.6, oats, exopolysaccharides (EPSs), lactic acid bacteria (LAB)

INTRODUCTION

Fermented non-dairy products have been developed in recent years to meet the increasing demand from consumers for non-dairy products [1-4]. In comparison to other cereals and legumes, oats is an interesting raw material for fermented food products because of a well-balanced nutritional composition and containing both soluble and insoluble fiber [5]. It is also generally accepted that the predominant soluble fiber in oats, β-glucans, has a positive effect on blood cholesterol levels [6-7]. High serum cholesterol concentration levels are strongly associated with an increased risk of ischemic heart disease [8]. To increase the interest in oats as a raw material for new functional food products, the use of a fermentation process in combination with new oat-bases (Adavena®) (Ceba Foods AB, Lund, Sweden) rich in other things, in dietary fiber, could provide new foods based on oats. The oat-bases are made entirely of oats and water using a patented enzymatic process (US patent No. 5.686.123) [9]. One application is a non-dairy milk substitute (Ceba Foods AB, Lund, Sweden). This product has been reported to have both high acceptance among consumers and a cholesterol lowering effect [10-11]. It has also been shown that this product can be fermented by lactic acid bacteria (LAB) that have the ability to produce exopolysaccharides (EPSs) [12].

Previous studies indicate that these microbial polysaccharides (EPSs) could have a physiological function similar to polysaccharides from plants [13-14]. The EPS-producing bacterial strain used in this study, *Pediococcus damnosus* 2.6, produces a homopolysaccharide with a β–glucan structure [15]. A fermented product that contains both soluble plant polysaccharides, such as β–glucans from oats, and microbial polysaccharides that are produced *in situ* during the fermentation may, therefore, have improved, beneficial physiological effects.

The main objective of this study was to demonstrate the possibility of producing a new type of fermented, ropy, non-dairy product, which is totally derived from oats and water and fermented with a LAB, that has a documented production of an EPS with a β–glucan structure, in combination with a traditional yoghurt culture.

MATERIALS AND METHODS

Starter cultures

The *Pediococcus damnosus* 2.6 was obtained from the UPV culture collection (Universidad de Pais Vasco, San Sebastian, Spain). The strain was stored at –80 °C in MRS broth [16] plus 25% (v/v) glycerol. Before experimental use the cultures were propagated twice in MRS (Merck, Darmstadt, Germany) at 30 °C. The commercial yoghurt culture, V2, which is a 1:1 mixture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* (Visby Tønder A/S, Tønder, Denmark) was stored according to the manufacturer recommendations (-80 °C) before use. This yoghurt culture was chosen, as it is a starter culture commonly used in Sweden for yoghurt products.

Preparation of the pre-inoculum

The pre-inoculum for the *P. damnosus* 2.6 strain was made by re-constituting oat powder, Adavena® G40, (Ceba Foods AB, Lund, Sweden) to a final dry matter of 20%. This oat powder was analyzed for protein, fat, different carbohydrates, dietary fiber, various vitamins and minerals by an authorized laboratory (AnalyCen Nordic AB, Lidköping, Sweden) (Table 1). After heat-treatment at 90 °C for 5 min with continuous stirring the medium was cooled to fermentation temperature (30 °C). The oat-base medium was inoculated (5%) with an exponentially growing culture of *P. damnosus* 2.6 in MRS broth. The pre-inoculum was incubated at 30 °C for 20 h.

TABLE 1 - Chemical composition (g/100g) of the non-dairy oat base (G 40 medium).

Components	G40 medium (g/100g)
Dry matter (%)	20
Protein (g)	2.2
Fat (g)	1.6
Glucose (g)	8.8
Maltose (g)	0.6
Maltodextrin (g)	5.4
Total fiber (g)	1.6
β-glucan (g)	0.8
α-tocopherol (mg)	0.2
Thiamin (mg)	0.08
Riboflavin (μg)	19.2
Niacin (mg)	0.2
Folic acid (μg)	6.6
Pyridoxine (mg)	0.02
Iron (mg)	0.2
Magnesium (mg)	9.4
Manganese (mg)	0.2
Phosphorus (mg)	54
Sodium (mg)	22
Zinc (mg)	0.2

Experimental procedures

Comparison of fermentation characteristics of *P. damnosus* 2.6 in the presence and absence of a yoghurt culture (V2). Fermentation characteristics were compared in Erlenmeyer flasks (200 ml) at two incubation temperatures (28 and 37 °C) using both the *P. damnosus* 2.6 strain and the V2 culture as pure and mixed cultures. The non-dairy oat base, G40, was prepared to a final dry matter of 16 %, as described earlier [3]. After heat treatment at 90 °C for 5 min with continuous stirring the medium was cooled to fermentation temperature. The *P. damnosus* 2.6 inoculated (5% (v/v)) was taken from a fresh (20 h incubation) pre-inoculum. The commercial yoghurt culture (V2) was inoculated directly (0.02% (w/v)) into it. After incubation the characteristics of the final product, such as pH, viable counts, viscosity and ropiness were measured.

Production of the fermented, ropy Y2.6 product

To investigate the fermentation characteristics during the production of a ropy, oat-based product, fermented both by a commercial yoghurt culture and by an EPS-producing strain (*P. damnosus* 2.6), cultivations were performed in a 12 L fermentor (Chemoferm, Sweden) with an 8 L working volume for 24 h. A concentrated liquid form of the Adavena G40 oat-base with a final dry matter of 16% (v/v) was used as medium. The medium was heated to 90 °C for 5 min with continuous stirring and then transferred to the sterile fermentor. The fermentation temperature was kept at 28 °C without pH control and agitation. The fermentor was inoculated with a fresh 5% (v/v) pre-inoculum, described above, together with a commercial yoghurt culture (V2).

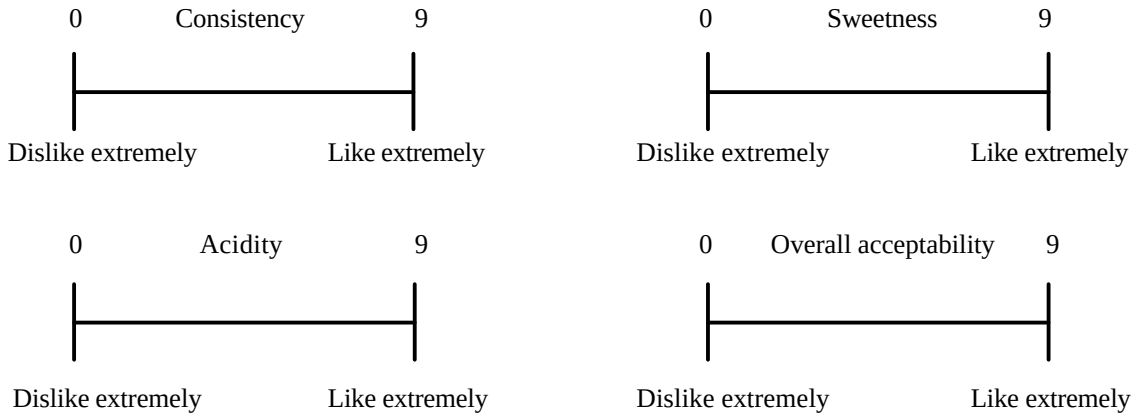
Product stability during refrigerated storage

The storage stability of the Y2.6 products was investigated during cold storage. The products were analysed every fifth day for viscosity, pH, ropiness and bacterial survival of the starter cultures over a storage period of 30 days.

Microbiological analysis

For all microbiological counts, a sample (1 ml) was taken and transferred for serial dilution using 9 ml of peptone water, 0.1% peptone (Difco, Detroit, Michigan, US) and 0.8% NaCl (Merck). Enumeration of *S. salivarius* subsp. *thermophilus* was performed using M17-agar plates (Merck, Darmstadt) [17]. The plates were incubated at 37 °C for 72 h under aerobic conditions. Enumeration of *L. delbrueckii* subsp. *bulgaricus* was performed using MRS-agar plates (Merck). The plates were incubated at 37 °C for 72 h under aerobic conditions. *P. damnosus* 2.6 was enumerated by using MRS-agar plates (Merck) containing 6% v/v ethanol. The plates were incubated at 30 °C for 72 h using anaerobic jars (5% CO_2, 85% N_2, 10% H_2).

FIGURE 1
Hedonic scales used in the ranking for preference evaluation for the different fermented products using four different sensory parameters, consistency, sweetness, acidity and overall acceptability (The unmarked scales ranged from 1 - dislike extremely to 9 - like extremely).



Chemical and physical analysis

Every second hour during a fermentation period of 24 h, samples were withdrawn from the fermentation vessel and analysed for pH, lactic acid concentration, soluble fiber, viscosity and ropiness. Lactic acid concentration (LA) was determined by using a commercial enzymatic kit (Boehringer, Manheim, Germany). pH measurements were carried out using a digital pH meter, MA235 model (Mettler Toledo, Hightstown, USA). To measure the fraction of soluble fiber in the G40 medium during the fermentation, the pH of the samples was adjusted to 6.2. Proteins were hydrolysed by a combination of Alcalase® and Esperase® (Novo Nordisk, Bagsværd, Denmark) at a ratio of 1:1 and a concentration of 1% (v/v). After an incubation period of 16 h at 30 ºC, the samples were centrifuged for 30 min at 4,000 rpm, the supernatant collected and washed through a filter (200 µm) (Spectra Mesh Industries Inc., Houston, USA). The filtrate was collected and precipitated with 4 volumes of cold ethanol (95%), followed by storage overnight at 4 ºC. The precipitate was recovered by centrifugation at 6,000 rpm for 30 min and dissolved and dialysed against distilled water (5 L) for 72 h at 4 ºC. After centrifugation the samples were lyophilised and weighed. The amount of soluble polymer from the G40 medium was expressed as polymer dry mass per litre (mg PDM/L). The viscosity was measured using a Brookfield DV-I Viscometer (Brookfield Viscometers LTD, Harlow, UK) with the S63 spindle, at 28 °C for 2 min at 50 rpm and was expressed as mPas. The ropy characteristic of the products was measured by using an Instron 4442 (Instron Ltd, Buckinghamshire, UK). 25 ml of a sample were transferred to a petri dish. A probe with a diameter of 3.8 cm was brought into contact with the surface of the sample and lifted up to a speed of 100 cm/min. When the product lost its contact with the probe the measurement terminated. The measurements were done in triplicate and at ambient temperature. Maltose content was analysed by high pH anion exchange chromatography (HPHEC) using a Carbopac PA 10 column (Dionex, Jouy-en-Josas, France) with 0.2 M NaOH as the mobile phase at a flow rate of 1.4 ml/min.

Sensory evaluation

Eight panellists of various ages and of both genders determined the organoleptic properties of the Y2.6 product flavoured with two different jams, mixed black berries and exotic fruit jam (Hafi AB, Getinge, Sweden). A fermented, ropy, dairy product, Långfil®, (Milko, Östersund, Sweden) was used as control product on the basis of its ropy texture. The control products were flavoured in the same manner as the Y2.6 products. All products were filled into identical commercial yoghurt containers (100 ml) and labelled with a three-digit code. The products were presented to the panellists as a double blind test. The evaluation was done twice within a period of 14 days.

Sensory properties (appearance, consistency, acidity and sweetness) of the various products were compared and ranked using hedonic scales (Figure 1). The panellists were asked to number the products from 1 to 9 according to their preference, using the same sensory qualifications. The closer the rank sum was to the highest reference score (36.0) the higher was the overall preference for the product.

Statistical analysis

Values were expressed as the means and the standard deviation. The mean values of the treatments were compared by Student's *t* test. Differences were considered significant at $P<0.05$. Results concerning the different products in the sensory preference test were analysed by paired *t*-tests. The significance of the ranking in the preference tests was analysed using Kramer's table [18].

RESULTS

Fermentation characteristics of *P. damnosus* 2.6 (2.6) in the presence and the absence of a yoghurt culture (V2)

There was an increase in both viscosity and ropiness when the *P. damnosus* 2.6 strains were grown in the G40 oat-base (Table 2). This increase did not change when the yoghurt culture was included. The change of incubation temperature had the strongest impact on the final viscosity and ropiness of the product. This was most obvious when the strains were grown at the higher incubation temperature (37 °C), which gave a much lower value for viscosity and ropiness. The *P. damnosus* 2.6 strain showed high viability (> 10^8 cfu/ml) at both incubation temperatures. It was observed that the viability of the two cultures (V2 and *P. damnosus* 2.6) was not decreased when they were grown as mixed cultures.

Fermentation of the ropy Y2.6 product

The fermentation profile of the Y2.6 product is shown in Figure 2. Both viscosity and ropiness increased during the fermentation period and gave the highest value after 24 h. There was a continuous decrease in pH during the whole period. However, it was found that this decrease declined towards the end of the period. The glucose and the β-glucan concentrations were measured before and after the fermentation period (Table 3). It was shown that most of the glucose in the oat-base was not used by the bacterial strains during the fermentation process and consequently was left unfermented.

Stability and bacterial survival during refrigerated storage

The stability of the product in terms of its consistency (viscosity and ropiness), pH and the bacterial survival of the starter cultures were measured (Figure 3). There was an evident increase in viscosity and ropiness during the storage period. The survival of *P. damnosus* 2.6 was high (10^9 cfu/ml) during the whole storage period. The survival of the two yoghurt bacterial strains, *L. bulgaricus* subsp. *delbrueckii* and *S. salivarius* subsp. *thermophilus*, decreased during the storage period to a value of 10^3 cfu/ml after 30 days. The pH of the product was constant during the whole storage period.

Sensory evaluation

The result of the sensorial preference test is shown in Figure 4. The two control products, the ropy dairy products, obtained the highest total in the preference test. The difference, however, is not significant. The two flavoured Y2.6 products received a lower score mainly in terms of the consistency of the product in comparison to both dairy control products. In the two other sensory parameters (sweetness and acidity) there were no significant differences between the two Y2.6 products and the two ropy, dairy control products. It was also observed that the product containing the black berry jam was given the highest total score of the products evaluated in the preference test for both the Y2.6 case and the ropy, dairy control case.

TABLE 2
Fermentation characteristics of the oat-based G40 medium when using a yoghurt culture (V2) consisting of *L. delbrueckii* subsp. *bulgaricus* (Ldb) and *S. salivarius* subsp. *thermophilus* (Sst) and an EPS-producing strain, *P. damnosus* 2.6 (2.6), as pure or mixed cultures.

Culture	Temperature (°C)	Viscosity (mPas)[a]	Ropiness (cm)[b]	pH	Viable count (log (cfu/ml)		
					Ldb	Sst	2.6
V2	28	546 ± 42	1.5 ± 0.1	5.4 ± 0.20	6.3	6.0	ND[c]
V2	37	450 ± 36	1.8 ± 0.1	3.9 ± 0.10	7.5	8.0	ND
2.6	28	2,400 ± 0	28.2 ± 2.4	4.1 ± 0.10	ND	ND	8.2
2.6	37	2,181 ± 169	16.7 ± 3.1	5.7 ± 0.20	ND	ND	8.2
V2 + 2.6	28	2,400 ± 0	22.6 ± 7.6	4.1 ± 0.10	6.3	6.0	9.0
V2 + 2.6	37	958 ± 175	3.1 ± 0.2	3.6 ± 0.10	7.9	7.0	8.5

[a] Viscosity measured after 2 min of shear thinning at 50 rpm and 28 °C. - [b] Ropiness measured at a speed of 100 cm/min. - [c] ND=Not determined.

TABLE 3
Polymer dry mass (PDM) (g l^{-1}) and glucose (%) content in the Y2.6 product before (initial value) and after 24 h of fermentation

	Initial value	Value after 24h of fermentation
PDM (g l^{-1})	4.9 ± 0.1	5.1 ± 0.1
Glucose (%)	7.3 ± 0.1	5.7 ± 0.1

FIGURE 2
Fermentation profile of the Y2.6 product, consisting of *P. damnosus* 2.6 and V2, during 24 h at 28 °C. Viscosity (´), ropiness (?), pH (?), lactic acid (*). The results are the mean values of two fermentations.

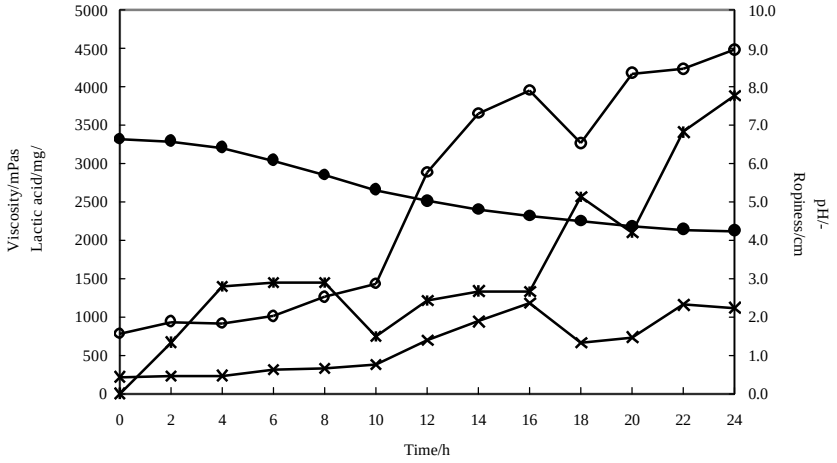


FIGURE 3
Storage stability of the fermented, ropy Y2.6 products containing black berry or exotic fruit jam, during 30 days in cold storage (6 °C). Survival of *Pediococcus damnosus* 2.6 (u), *Lactobacillus delbrueckii* subsp. *bulgaricus* (?), *Streptococcus salivarius* subsp. *thermophilus* (¦), viscosity (´), ropiness (?) and pH (?). The results are the mean values of three determinations.

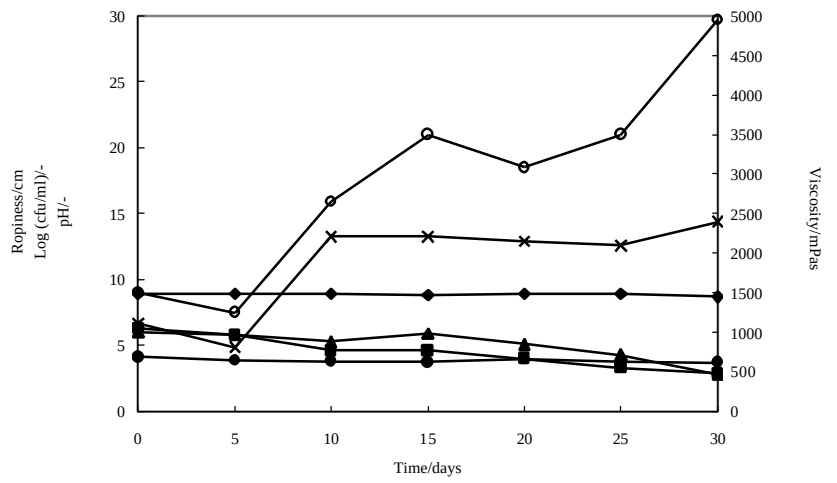
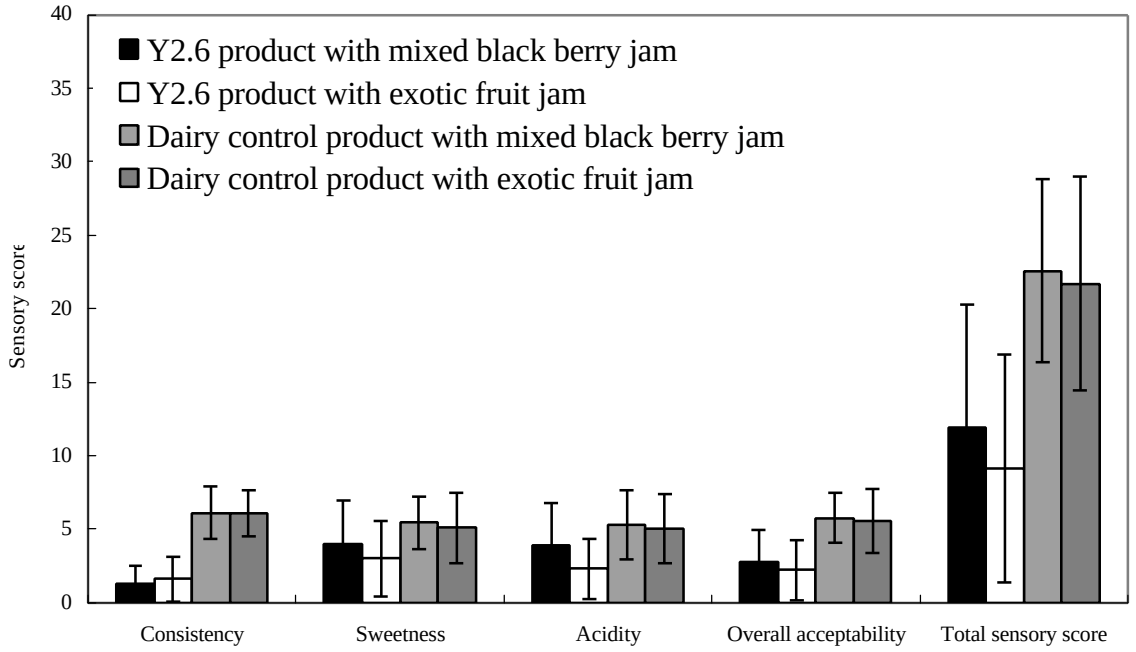


FIGURE 4

Ranking for preference of the Y2.6 product to evaluate four different sensory parameters: consistency, sweetness acidity and overall acceptability, and the comparison with a fermented, ropy dairy product. Four different products were used, two Y2.6 products containing black berry jam (¦) or an exotic fruit jam (?) and two variants of fermented, ropy dairy control products containing black berry jam (?) or exotic fruit jam (?). Figures are mean values (N = 8) of distances marked by the panellists from 1 (dislike extremely) to 9 (like extremely). The total sensory score is the sum of the ranking for preference from 8 (N = 8) panellists. The results are the mean values of two different evaluation occasions



DISCUSSION

The objective of this study was to investigate the possibility of producing a fermented, ropy, non-dairy product made from oats by including the EPS-producing bacterial strain, *P. damnosus* 2.6, as starter culture in combination with an ordinary commercial yoghurt culture (V2). Fermented, ropy, dairy products have been studied earlier because of their unique textures and consistencies [19-21]. A report has also indicated that fermented, ropy dairy product would have cholesterol-lowering activity [22]. In this study we investigated the fermentation characteristics of *P. damnosus* 2.6, with and without the V2 culture, in an oat-base with a high glucose content (G40) at two different temperatures. An increase in viscosity after fermentation was seen only when *P. damnosus* 2.6 was used in the G40 medium. The ropy characteristic of the oat-base after fermentation was lower at a higher incubation temperature (37 °C). It was shown that a lower incubation temperature (28 °C) gave a higher increase in both viscosity and ropiness after a fermentation period of 24 h. This follows earlier findings that the optimal growth for the EPS production by *P. damnosus* 2.6 would be in the range of 25-30 °C [15]. Although this temperature is far from optimal (37 °C) for the V2 culture, A flavour, subjectively described as a palatable aroma recognised as yoghurt flavour, resulted at this incubation temperature (data not shown).

During a fermentation of 24 h at 28 °C using both the *P. damnosus* 2.6 strain and the V2 culture as starter cultures it was seen that both viscosity and ropiness increased during the whole fermentation period. No significant change could be seen in total polymer dry mass (PDM), indicating that the EPS are produced in amounts too small to be detected analytically in this complex, oat-based medium. The significant change in the physical character of the oat-base during the fermentation period is more likely to be due to the interaction between the microbial polysaccharide and macronutrients, such as proteins in the growth medium. This kind of interaction has been described earlier in milk [23].

It was shown that the Y2.6 products retained their stability during 30 days of storage at 6 °C. A high survival of the *P. damnosus* 2.6 strain was also seen during the time of storage, suggesting that the oat-base used is a good support for this strain during a storage period of 30 days. The

survival of the V2 culture was found to be lower. However, these strains have a higher optimal growth temperature (37 °C) than the *P damnosus* 2.6 strain, with its optimal incubation temperature of 30 °C.

In the sensorial preference test four sensory parameters were investigated, consistency, sweetness, acidity and overall acceptability. The panellists evaluated the ropy consistency to have low acceptability. However, the acceptability for this ropy structure was higher when black berry jam was added to the product, as it had a major impact on the colour of the final product. The total rank sum was lower for both of the two Y2.6 products in comparison to the two fermented, ropy, control products. However, the differences between the products were not significant ($P<0.05$).

In this study it is shown that it is possible to produce a fermented, ropy, non-dairy product using both a commercial yoghurt culture and an EPS-producing bacterial strain, *P. damnosus* 2.6. This kind of study can facilitate the development of new, fermented, non-dairy, nutritionally well-balanced food products with unique physical properties.

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OPTIMIZATION OF ß-GALACTOSIDASE PRODUCTIVITY BY *Bacillus macerans* 314 (DSM)

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SUMMARY

Twelve bacterial strains were screened for their ability to produce ß-galactosidase. *Bacillus macerans* 314 (DSM) was the most potent for production of intracellular ß-galactosidase using shaken cultures (200 rpm) after 10 days incubation at 37 °C. Only negligible amounts of extracellular enzyme was produced. Highest yield of intracellular ß-galactosidase (549.5 U/g) was achieved using 0.9% peptone and 1.25% lactose in ultrafiltrated permeate whey as nitrogen and carbon sources, respectively. Addition of 1 mM of a mixture of the metal lons Ca^{2+}, K^{+} and Mg^{2+} optimized the production of the enzyme to 636.66 U/g. Addition of 0.8% K_2HPO_4 to the medium led to an increase of the activity to 912.8 U/ g.

The optimum crude intracellular ß-galactosidase activity (115.11 % of that of control) was obtained at 50 °C and pH 6.5, respectively.

KEYWORDS: ß-Galactosidase, *B. macerans*, Lactase.

INTRODUCTION

In recent years growing interest arised in microorganisms as potential sources for various enzymes that can be applied in food industries and medical fields. Among the microbial enzymes, which was found to be of great interest, is the ß-galactosidase (lactase, ß-D-galactoside galactohydrolase, EC.3.2.1.23) which catalyses the splitting of lactose especially in milk to glucose and galactose, both have a combined sweetness of about 80 % of that of sucrose and are easily adsorbed from the intestine [31].

Because of the growing economic significance of ß-galactosidase, attempts have been reported to produce this enzyme from microbial sources [3, 5, 6, 14, 18, 20, 21, 23, 25]. The present work was undertaken to study the production of ß-galactosidase by 7 imported and 5 local bacterial strains and evaluation of possible use of ultrafiltrated permeate whey as lactose source in the culture medium. The study included the selection of best ß-galactosidase producer and the detection of optimum culture conditions for its production, in addition to some properties of the crude enzyme.

The tudy indicated that optimum ß-galactosidase activity was produced intracellulary by *Bacillus macerans* 314 (DSM).

MATERIAL AND METHODS

Microorganisms

7 imported bacterial stains were used, namely *Bacillus amyloliquefaciens* 312 (**D**eutsche **S**ammlung von **M**ikroorganismen und ellkulturen, DSM), *B. licheniformis* 1969 (DSM), *B. licheniformis 13417* (AMU), *B. macerans* 314 (DSM), *B. macerans* 394 (**N**orthern **R**egional **R**esearch **L**aboratory, NRRL), *B. subtilis* 10400 (**A**merican **T**ype **C**ulture **C**ollection, ATCC) and *Serratia liquefaciens* 1310 (Unesco **MI**CROBIAL **R**ESOURCE **CEN**TRE, MIRCEN).

5 local bacterial strains were obtained from the bacterial collection of the Centre of Culture of the National Research Centre (NRC), Cairo, Egypt, namely; *B. firmus* , *B. megaterium, B. mycoides, B. stearothermophilus,* and *Lactobacillus bulgaricus.*

The cultures were maintained by culturing on nutrient agar medium at 37 °C for 24 hrs and stored at 4 °C.

Ultrafiltrated permeate whey

This was kindly provided by Misr Company for Dairy Products. It contains 4-4.5% lactose, 0.03% NPN (non-protein nitrogen) and 0.3% ash (K^{+}, Na^{+} Ca^{2+}).

Fermentation medium

The modified medium of De Bale and Castillo [12] was used which had the following composition (g/L): Ultrafiltrated perrneate whey containing 1.5% lactose, 5% yeast extract and 5% peptone at pH 7.0.

Cultivation

Experimental cultures were made in 250 ml Erlenmeyer flasks each containing 50 ml culture medium, sterilized for 15 min at 15 psi, cooled, and inoculated with 5% inoculum (v/v) vegetative cell suspension (2-days-old culture). Transfers were made from the stock slants to the same fermentation medium in order to prepare the inocula needed. The flasks were then incubated at 37 °C on a rotary shaker (200 rpm) for different incubation periods. The culture broth from each flask was centrifuged at 6000 rpm in a cooling centrifuge at 0 °C for 10 min to separate the bacterial cells from the culture medium.

Preparation of ß-galactosidase enzymes

a) *Cell permeabilization. This* was carried out with acetone/toluene mixture according to the method of Somkuti and Steinberg [27].

b) *Preparation of cell-free extract.* This was carried out according to the method of Choi *et al*. [11]. The frozen bacterial cells (4 g), which were obtained by growing in optimized fermentaion medium, were thawed, suspended in 100 ml of 50 mM potassium phosphate buffer (pH 7.0) and disintegrated by sonication using Sonifier B-12, Branson Sonic Power for 2 min at 30 s intervals. The disrupted cells were centrifuged at 8000 rpm for 20 min in a refrigerated centrifuge at 0 °C and the supernatant was precipitated at 4 °C (overnight) with streptomycin sulphate (final concentration was 7.0 mg/ml). The precipitate was removed using a cooling centrifuge and the supernatant (cell-free extract) was used to assay ß-galactosidase activity as described below.

Assay of ß-galactosidase

This was carried out according to the method of Shah and Jelen [26] with some modifications. 0.5 ml of the permeabilized cells, cell-free extract or culture filtrate was incubated with 4 ml of 0.3 mM o-nitrophenyl-ß-D-galactopyranoside (ONPG) in 50 mM potassium phosphate buffer (pH 7.0) for 15 min at 37 °C. The reaction was stopped by adding 1 ml of cold 1 M sodium carbonate solution to the reaction mixture. Cells were removed from the assay mixture by centrifugation at 8000 rpm for 10 min at 0 °C. The resulting yellow color of o-nitrophenolate ions was measured at 420 nm. Similarly, the blank was prepared, but sodium carbonate was added before the enzyme solution. One unit of enzyme activity is equivalent to 1 µmole of o-nitrophenol liberated from ONPG per min under the assay conditions specified above.

Protein determination.

This was estimated according to the method of Lowry *et al.* [16].

RESULTS AND DISCUSSION

The results in Table 1 indicate that out of the twelve investigated bacterial strains, only seven produced intracellular ß-galactosidase, namely *Bacillus firmus* (NRC), *B. licheniformis* 13417 (AMU), *B. macerans* 394 (NRRL), *B. mycoides* (NRC), *B. subtilis* 10400 (ATCC), *Lactobacillus bulgaricus* (NRC) and *Serratia liquefaciens* 1310 (MIRCEN). Both extra- and intracellular ß-galactosidases were produced by the three strains *B. licheniformis* 1969 (DSM), *B. macerans* 314 (DSM) and *B. stearothermophilus* (NRC). However, only negligible amounts of extracellular ß-galactosidase were observed compared to the intracellular enzyme by the three latter strains. These results are in agreement with those reported by Blankenship and Wells [4]. No enzyme was detected by the two strains *B. amyloliquefaciens* 312 and *B. megaterium.*

TABLE 1 - Production of ß-galactosidase (intracellular activity) by imported (international collections of microorganisms) and local bacterial strains at different incubation periods (U g^{-1})*.

	Imported strains						Local strains			
Incub. period	*B. mac. 1*	*B. mac. 2*	*B. lich. 1*	*B. lich. 2*	*B. subt.*	*S. lique*	*B. firm.*	*B. stear.*	*L. bulg.*	*B. myco.*
1day	0.73	22.20	0.00	0.00	0.76	0.65	0.28	7.20	3.33	0.10
2 days	0.41	27.20	1.50	0.00	0.45	0.86	0.14	17.30	6.10	1.00
3 days	0.41	30.10	4.50	0.83	0.48	0.89	0.00	18.80	14.20	1.15
4 days	0.29	63.51	9.53	2.36	0.18	0.99	0.00	16.00	7.05	1.30
5 days	Nd	92.10	15.16	3.80	0.12	0.76	Nd	11.00	1.15	1.11

* The incubation period was extended for 12 days, and maximum production of β-galactosidase was achieved after 10 days incubation as recorded in the text. Incub. Period = incubation period; *B. mac.* 1 = *Bacillus macerans* 394 (NRRL); *B. mac.* 2 = *Bacillus macerans* 314 (DSM); *B. lich.* 1 = *Bacillus licheniformis* 1969 (DSM); *B. lich*. 2 = *Bacillus licheniformis* 13417 (AMU); *B. subt.* = *Bacillus subtilis* 10400 (ATCC); *B. firm.* = *Bacillus firmus* (NRC); *B. stear*. = *Bacillus stearothermophilus* (NRC); *L. bulg.* = *Lactobacillus bulgaricus* (NRC); *S. lique.* = *Serratia liquefaciens* 1310 (MERCIN) ; *B. myco.* = *Bacillus mycoides* (NRC).

FIGURE 1 - Effect of different concentrations of lactose in ultrafiltrated permeate whey on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).

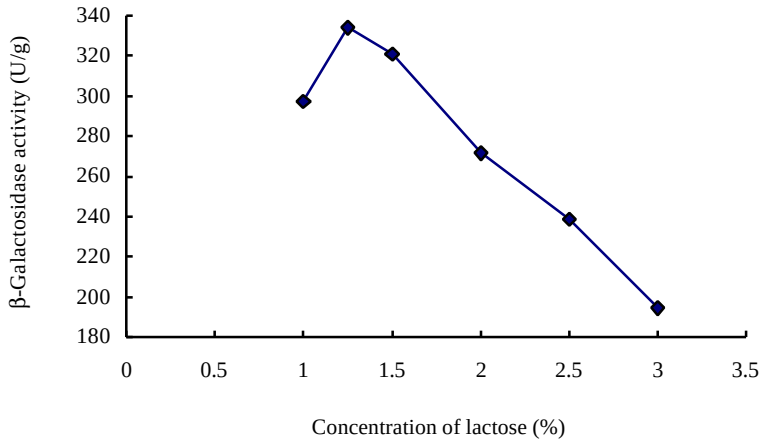


FIGURE 2
Effect of different nitrogen sources on the production of intracelluar b-galactosidase by *B.macerans* 314 (DSM).

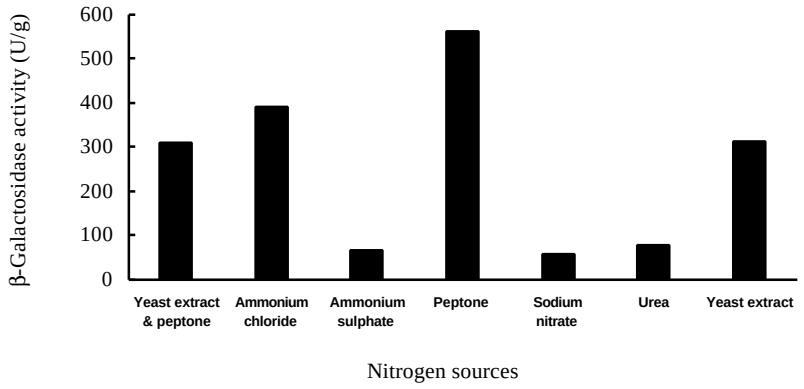


FIGURE 3
Effect of different concentrations of peptone on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).

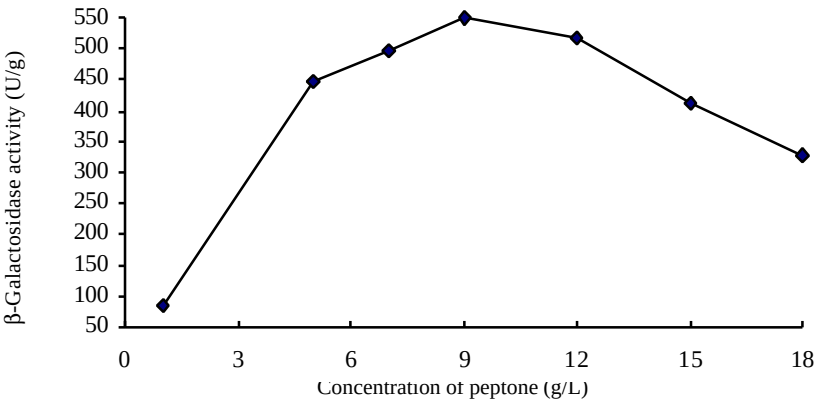


FIGURE 4 - Effect of addition of metal ions on the production of intracellular ß-galactosidase by *B.macerans* 314 (DSM).

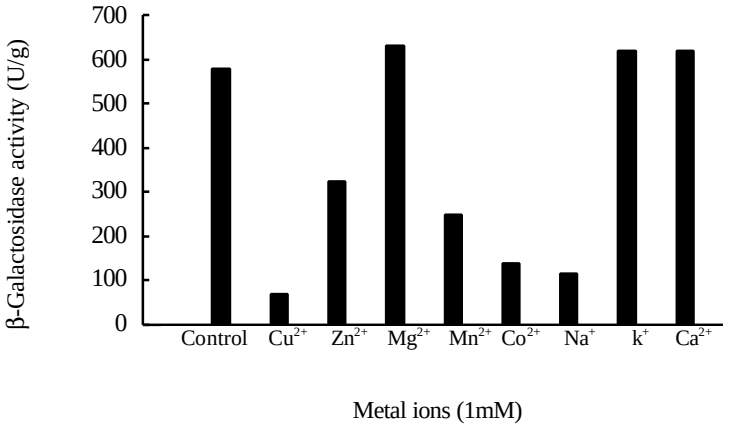


FIGURE 5 - Effect of addition of different concentrations of metal ions on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).

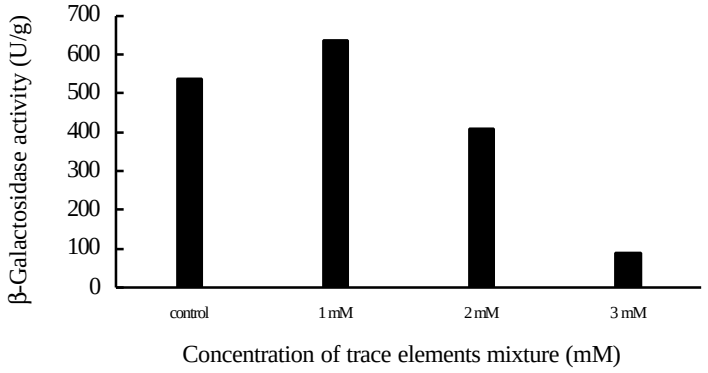
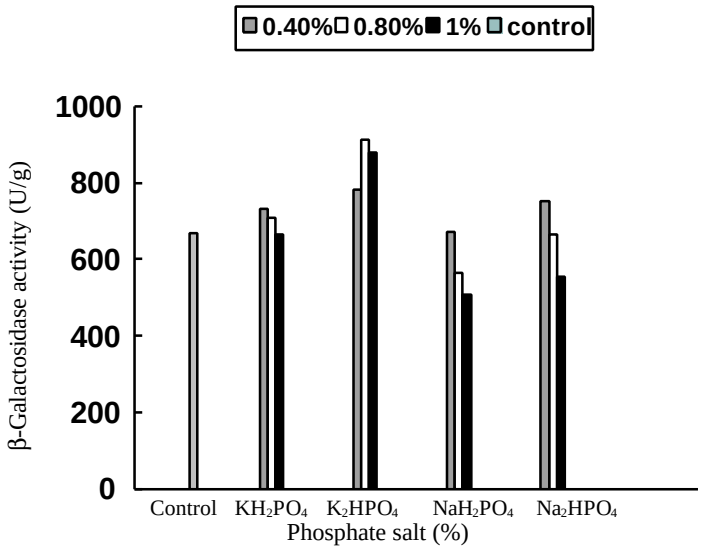


FIGURE 6 - Effect of different concentrations of four phosphate salts on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).



Highest intracellular ß-galactosidase (315.5 U/g) was obtained after 10 days incubation at 37 °C by *B. macerans* 314 (DSM). This strain was, therefore, selected for further investigations. In this respect, some reports on the production of ß-galactosidase from different *Bacillus* species namely *B.* sp. TA-11 [10, 15], *B. circulans* [9] and *B. licheniformis* [30] was mentioned. In addition, Chang and Mahoney [7] and Gündüz and Rejaee [13] reported on the production of ß-galactosidase by *Streptococcus thermophilus*. This enzyme was also produced by *Lactobacillus acidophilus* [24, 29]. In the dairy industry, interest is largely concentrated on *Streptococcus lactobacillus* and *Leuconostoc* strains [13].

The effect of some cultural conditions on the production of intracellular ß-galactosidase by *B. macerans* 314 (DSM) after 10 days incubation using shaken cultures was investigated. Substitution of the ultrafiltrated permeate whey in the culture medium by other carbon sources, on equal carbon basis, led to complete inhibition of enzyme production with either glucose, galactose or glucose + galactose. This indicated that ß-galactosidase of *B. macerans* 314 (DSM) is an induced enzyme, as commonly known for bacterial ß-galactosidase [1]. The effect of lactose concentration (1-3 %) of ultrafiltrated permeate whey in the culture medium showed that lactose concentration higher than 1.25 % caused an adverse effect on the production of intracellular ß-galactosidase (Fig. 1). More or less similar results were obtained by Miyazaki [19] and Chio *et al.* [11] on the production of ß-galactosidase by *B. macerans* 3490 (**I**nstitute for **F**ermentation, **O**saka, Japan; IFO) and the thermophilic *Bacillus* sp. TA-11 at 1% and 1.5% lactose concentration, respectively. However, lower (0.5%) and higher concentrations were optimum for the synthesis of other microbial ß-galactosidase [7, 13]. The effect of nitrogen sources on ß-galactosidase production (Fig. 2), on equal nitrogen bases, showed that among peptone, yeast extract and urea as organic sources the use of peptone in the culture medium led to maximal production of intracellular ß-galactosidase (562.4 U/g). This result is similar to that reported by Ramana Rao and Dutta [22].

In studying the optimum concentration of peptone, it was found that the use of 0.9% (w/v) resulted in the production of maximal intracellular ß-galactosidase activity (Fig: 3).

On studying the effect of metal ions on the intracellular ß-galactosidase activity (Fig. 4), it was found that Mg^{2+}, K^{+} and Ca^{2+} at 1 mM concentration stimulated the enzyme production to 109.37 %, 107.19 % and 107.24 % of that of control, respectively. On the other hand, Cu^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+},and Na^{+} had adverse effects on the production of ß-galactosidase. Thus, a mixture of trace metals of $CaCl_2$, KCl and $MgSO_4.7H_2O$ added to the fermentation medium at 1 mM led to increasing ß-galactosidase activity to about 11.80 % of that of intracellular enzyme (Fig. 5). This may be due to the fact that Ca^{2+}, K^{+} and Mg^{2+} may act as cofactors of many enzymes of bacterial cells.

The effect of the addition of different concentrations of phosphate to the culture medium was investigated using different phosphate salts (KH_2PO_4, K_2HPO_4, NaH_2PO_4 and Na_2HPO_4) (Fig. 6). It was shown that addition of phosphate at 0.8 % as K_2HPO_4 was most favorable for increasing the production of intracellular ß-galactosidase to 169.8 % of that of control. Using either higher or lower concentrations (1 % or 0.4 %) of this salt led to decrease of enzyme activity (131.99 % and 117.1 % of that of control). This result is similar to that reported by Chio *et al.* [11] who used K_2HPO_4 as a source of phosphate at 0.15 % (w /v) concentration.

Also, the inoculum size and incubation period showed significant effect on ß-galactosidase production (Fig. 7). Highest intracellular enzyme activities (886.5 U/g) were obtained using 5 % inoculum after 2 days incubation. This is in agreement with results of Miyazaki [19]. In this respects, Chang and Mahoney [7] used 2 % inoculum size, while Sridhar and Dutta [28] used 1 % inoculum size for 36 hrs incubation period. The rate of shaking the culture broth affected the enzyme production. 200 rpm was the most favorable and increasing or decreasing rpm resulted in lowering the enzyme yield (Fig. 8). The optimum temperature for ß-galactosidase production was found to be 37 °C, while Chio *et al*. [11] reported on the production of ß-galactosidase at 50 °C by the thermophilic strain *Bacillus* sp. TA-11. In addition, the thermophilic bacterium *Thermus aquaticus* was grown at 70 °C [17]. Other psychrotrophic bacteria may grow at lower temperature. Thus, *Buttiauxella agrestis* NC4 was propagated at 30 °C for ß-galactosidase production [2]. Production of the ß-galactosidase by *B. macerans* 314 (DSM) was considerably affected by the initial pH values. Thus, it can be noticed (Fig. 9) that pH 7.0 was the most favorable for the ß-galactosidase activity. Similar results were reported for ß-galactosidase production by *Streptococcus venzuelae* (135) at pH 7.0 [8]. In addition, Chang and Mahoney [7] and Chio *et al.* [11] reported that *Streptococcus thermophilus* produced ß-galactosidase at pH 6.0 and alkalophilic and thermophilic *Bacillus* sp. TA-11 at pH 9.5, respectively.

Furthermore, the optimum temperature of the crude intracellular ß-galactosidase of the enzyme reaction was investigated at different incubation temperatures (25 °C – 60 °C) at pH 7.0. Maximum relative activity (178.43 % of that of control) was observed at 50 °C (Fig. 10).

FIGURE 7
Effect of inoculum age and size on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).

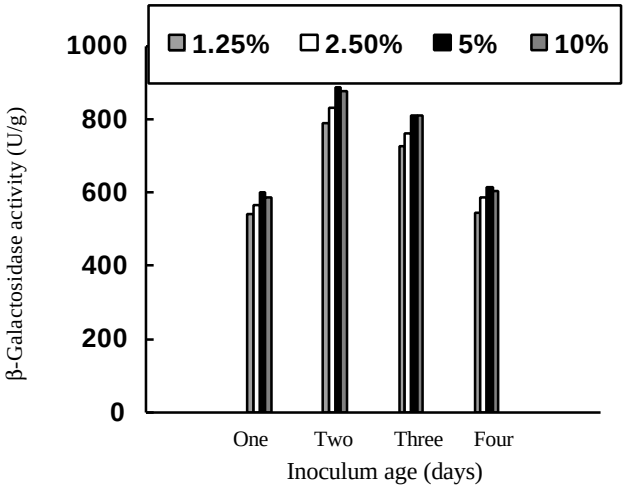


FIGURE 8
Effect of rate of shaking on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).

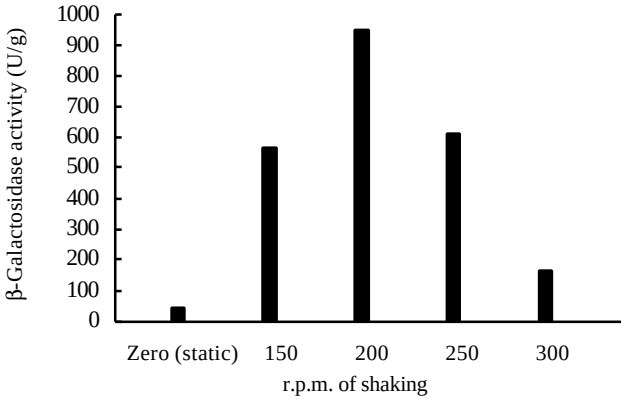


FIGURE 9 - Influence of initial pH levels of the medium on the production of intracellular b-galactosidase by *B.macerans* 314 (DSM).

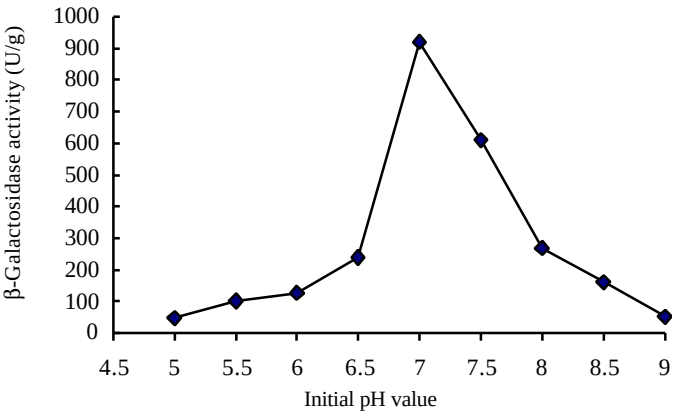


FIGURE 10 - Effect of temperature of the reaction mixture on the activity of crude intracellular b-galactosidase produced by *B.macerans* 314 (DSM).

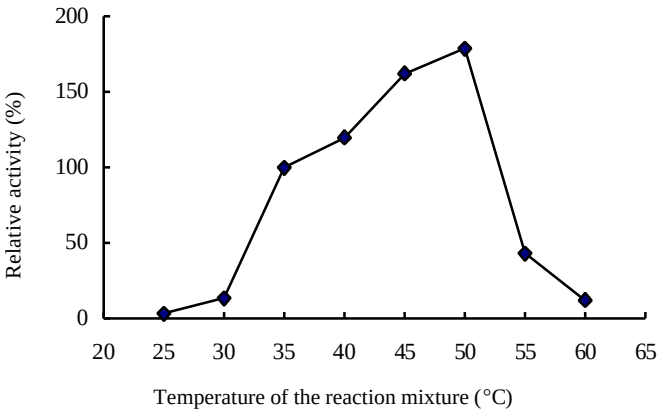


FIGURE 11
Thermal stability of the crude intracellular b-galactosidase produced by *B.macerans* 314 (DSM).

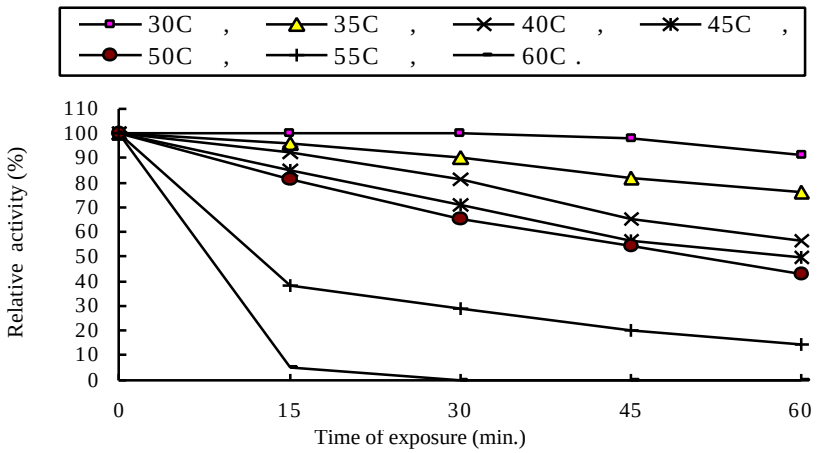
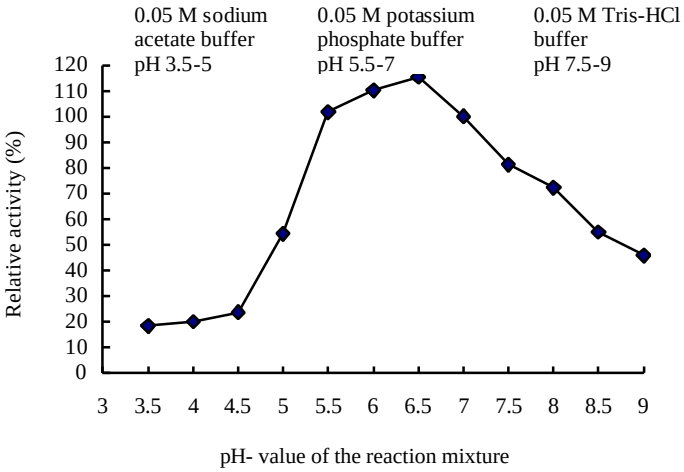


FIGURE 12 -Effect of pH-value of the reaction mixture on the activity of crude intracellular b-galactosidase produced by *B.macerans* 314 (DSM).



The thermal stability of the enzyme was dependent on the temperature and storing time. Therefore, the enzyme was stable below 40 °C and lost its activity at 60 °C after 30 min (Fig. 11). The use of different buffers (pH values 3.5-9.0) at 50 °C showed that optimum enzyme activity (115.11 % of that of control) was obtained at pH 6.5 (Fig. 12). This result is in agreement with that reported by Maciunska *et al.* [17] for *Thermus aquaticus* ß-galactosidase.

Collectively, these results may justify the suitability of the bacterial strain *B. macerans* 314 (DSM) for commercial production of ß-galactosidase using inexpensive material.

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VALUABLE FOOD PROTEIN PREPARATION FROM SOYBEAN

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SUMMARY

Georgian varieties of soya: Kartuli-7, Kolkhuri and the variety Armaviri widely distributed in the former Soviet Union, distinguished by high protein content, were studied. In defatted flour of each variety the amount of albumins, globulins and glutelins and their specific content in total protein have been defined. The method of obtaining valuable food protein, which will satisfy the requirements of international standards by its protein and amino acid composition has been elaborated. The method is based on simultaneous initial isolation of albumins and globulins from flour. For this reason, combined fraction of albumins and globulins have been isolated from defatted flour of each variety and their amino acid composition studied. Based on total protein and valuable albumin and globulin fraction content, as well as amino acid composition (in particular indispensable amino acids) the variety Kolkhuri has been chosen for further investigations. The combined preparation of albumins and globulins isolated from the selected variety of soybean, was precipitated as a result of adjusting the pH of the solution to 4.5. Further separation of albumins and globulins was carried out by dialysis. By mixing 40% albumin-alginate and 20% globulin suspensions on water bath at 95 ^{O}C the coagulate was formed. After treatment of the coagulate first with alkali, then by weak acid a thick porous product was obtained. The product obtained exhibits stability to thermal and mechanical treatments, is well cut and at cooking in oil manifests meat odour and colour. As high nutritional value product, according to its protein and amino acid composition it is not inferior to meat and can be used as a supplement to meat products. The protein preparation could be an excellent initial material for vegetarian food production, used as a meat analogue at dietary nutrition.

KEYWORDS:
Soybean, albumins, globulins, glutelins, valuable food protein.

INTRODUCTION

As an essential building block of life, protein is one of the few food ingredients that cannot become obsolete. Throughout the industrialized world which has traditionally relied on meat for nearly all its protein, a growing concern for health has aroused an interest in protein sources that are low in fat and cholesterol. In developing nations, a greater concern is the prudent managing of resources and the availability of protein sources. For whatever the reason, the world's attention is being focused on protein alternatives [1].

Plant protein sources provide 65% of the world supply of edible protein [2]. The protein content of soybean plant seed is about 40%. After the hulls and the oil are removed, the remaining defatted flake, which is the starting material for most commercial protein ingredients, has a protein content of approximately 50% [3].

Both human and animal studies have proven soy products to be excellent sources of protein. Soy bean proteins are easily assimilated by the body [4]. Soy protein provides all the essential amino acids needed to fulfill human nutritional requirements for growth, maintenance, or physical stress. This amino acid pattern is among the most complete of all vegetable protein sources and resembles, with the exception of the sulphur-containing amino acids, the pattern derived from high-quality animal protein sources [5].

Both human clinical studies and animal research have demonstrated that soy protein products are comparable in digestibility to other high-quality protein sources, such as milk, fish and egg [6-7].

Despite the large number of products on the market, there are only three types of soy protein: flour, isolate and concentrates. Types of Soy Protein Concentrates Preparation Produced by three basic processes are acid leaching (at about pH 4.5), extraction with aqueous alcohol (70% to 90%), and denaturing of the protein with moist heat prior to extraction with water. Soy isolates are prepared from dehulled and defatted soybeans by removing most of the non-protein components.

TABLE 1 - Protein content in soybean flour of Kartuli-7, Kolkhuri and Armaviri varieties.

Variety	Total protein, %	Fraction	% in flour	% in Total Protein
Kartuli-7	**50,4**	**Albumins**	**7,96**	**15,8**
		Globulins	**35,28**	**70**
		Glutelins	**7,16**	**14,2**
Kolkhuri	**53,2**	**Albumins**	**7,55**	**14,2**
		Globulins	**36,34**	**68,3**
		Glutelins	**97,1**	**17,5**
Armaviri	**49**	**Albumins**	**5,31**	**14,6**
		Globulins	**31,95**	**65,2**
		Glutelins	**9,9**	**20,2**

FIGURE 1
Isoelectrofocusing of soybean flour albumins. Phast gel IEF 3 – 9
Armaviri. Protein concentration: 1 - 0,45 mg/ml; 4 - 0,9 mg/ml
Kolkhuri. Protein concentration: 2 - 0,5 mg/ml; 5 - 1,0 mg/ml
Kartuli -7. Protein concentration: 3 - 0,45 mg/ml; 6 - 0,9 mg/ml

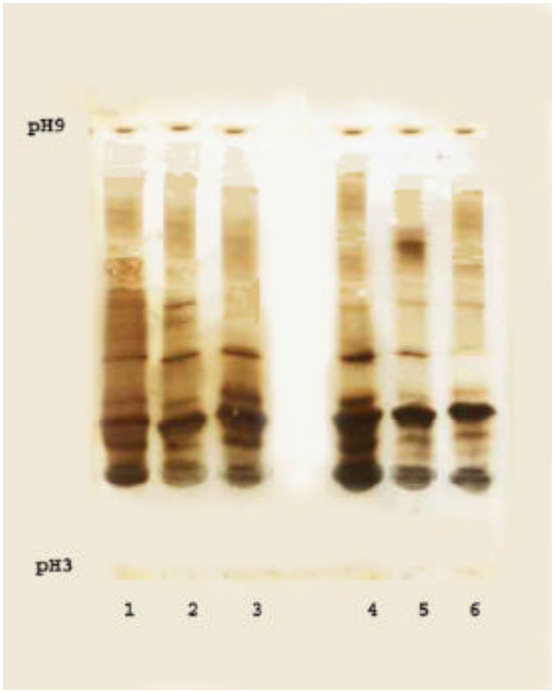

TABLE 2
Amino acid compositiont in total albumin-globulin preparation of Kartuli-7, Kolkhuri and Armaviri varieties.

Vareity	Amino Acids µmol/l										
	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
Kartuli-7	72	18	35	27	98	7	17	18	15	36	73
Kolkhuri	96	20	22	30	73	7	23	30	18	48	58
Armaviri	70	20	20	13	69	9	24	24	13	35	41

The goal of the presented work was to study the seed proteins and amino acid composition of different soybean varieties and possibility of their application for the preparation of valuable food proteins, inexpensive than the traditional texturized soybean protein. Production of different food on the basis of valuable soybean proteins will contribute to provision of population of Georgia and other countries with transitional economics with cheap, high caloric food.

MATERIALS AND METHODS

Investigation was carried out on Georgian soybean *(Glycine max)* varieties: Kartuli-7 and Kolkhuri. For comparison a variety of Armaviri widely distributed in the former Soviet Union and distinguished by high protein content was used.

Soybean flour was prepared by grinding and screening of seeds. Flour defatting was carried out in one of several types of counter current extraction systems with dimethylether as a solvent. After the defatted flour leaves the extractor, any residual solvent remaining was removed by heat and vacuum.

Total protein was determined by modified micromethod of Kjeldahl using Nesler reagent.

Albumins, globulins and glutelins were isolated from previously defatted bean flour according to Osborn [8]. Extraction of albumins was carried out with 0.1M phosphate buffer, pH 7.5, globulins with 1 M NaCl containing 0.1 M phosphate buffer pH 7.5 and glutelins with 0.05 M sodium hydroxide.

Protein content in the fractions obtained was determined according to Bradford [9] and/ or Lowry et al [10].

The isoelectrofocusing of albumins of each variety was conducted in Phast system, used Pharmacia LKB Biotechnology S-75182 uppsala apparatus and the method and phast gel IEF 3-9 recommended by Pharmacia.

The silver staining technique used in this work was derived from the method of Heukeshoven and Dernik [11]. The method has been optimized for silver staining Phast Gel IEF and gradient media. The staining strategy consists in fixing and removal of buffer ions, "sensitization" of the proteins in a glutardialdehyde solution, removal of excess glutardialdehyde, reaction with silver ions in a silver nitrate solution, developing in a basic formaldehyde solution and stopping the development in acetic acid. After development the gel was dried and mounted in frames. The run took 500 Vh or approximately 30 minutes. Prefocusing step lasted 10 minutes.

The elaborated method of valuable food protein preparation consists in simultaneous isolation of albumins and globulins initially. For this reason total albumin and globulin fractions were isolated by extraction with 1 M NaCl containing 0.2 M phosphate buffer, pH 7.5 [12] from soybean defatted flour. Insoluble fibrous residue was removed by centrifugation at 6000 g 20 min, adjusting the resulting extract to pH 4.5, where most of protein precipitates as a curd. Separation of the curd from the soluble oligosaccharides was conducted by centrifugation at similar conditions.

The precipitate, containing selected albumins and globulins of variety Kolkhuri was dissolved in minimum amount of 0.2 M NaCl containing phosphate buffer, pH 7.5. Further separation of albumins and globulins were carried out by dialysis. A 20% suspension of globulins was incubated at pH 9.4 at room temperature for 5 hours [13]. Simultaneously a suspension of albumin-alginate complex (40% by protein) was prepared at the same pH 9.4. The suspensions were mixed on water bath at 95 ^{O}C under continuous stirring during 10 minutes.

Amino acid composition of combined preparation of albumins and globulins used for valuable food protein preparation was studied on HPLC Pico Tag-type amino acid analyzer. For this reason, a precipitate of albumins and globulins obtained after acidic treatment was purified: washed up first by alcohol, then by ether, dried and weighed. A 5g of each sample was placed into an ampoule, 4ml of 6 M HCl was added and defrosted under vacuum. The ampoules remained at 110 ^{O}C, for 24 hours.

RESULTS AND DISCUSSION

Soybean varieties Kartuli-7 and Kolkhuri are characterized by high albumin and globulin content (Table 1). Besides, Kolkhuri is distinguished by the highest content of protein in flour (53%). As for variety Armaviri, in spite of high protein content (49%) of its flour, because of high glutelin percentage in total protein (20%), the amount of valuable fractions is low.

As shown from the isoelectrofocusing picture albumins are manifested mainly as mild acid or neutral proteins, as most of them are located in respective region (Fig. 1).

Isoelectrofocusing in polyacrylamide gel did not reveal noticeable differences among protein spectra of investigated varieties, except Kolkhuri variety, where one albumin fraction, developed in neutral region is expressed more clearly.

The amino acid composition of combined preparation of albumins and globulins of each studied soybeen variety is presented in Table 2.

Recommended FAO/WHO the Protein Digestibility-Corrected Amino Acid Score (PDCAAS), where great attention is paid to a high content of sulphur-containing amino acids, methionine and cystine, in protein food products. For example, in soy protein products this content in the most cases is low. Thus, it was very important to know amino acid composition of the initial protein spectrum, necessary for the production of valuable food products. Amino acid composition of preparation ob-

tained, containig albumins and globulins meets the FAO/WHO requirements, as in the varieties studied the total content of sulphur-containing amino acids is rather high. At the same time it should be noted that high content of methionine conditions high level of sulphur-containing amino acids. In spite of comparatively low content of cystine (Table 2), methionine content exceeds the content of almost all indispensable amino acids, and is nearly 10 times higher than cystine content. Methionine content is the highest in Kartuli-7 and Kolkhuri and negligable in Armaviri. Variety Kolkhuri evidently differs from Kartuli-7 and in particular from Armaviri according to the main indispensable amino acid content. In its total fraction the content of histidine, isoleucine, lysine, phenylalanine, threonine and tryptophane is highest. Variety Kartuli-7 is characterised by high content of indispensable amino acids. But in variety Armaviri, comparatevely low content of methionine, as compared to other varieties, is compensated by higher content of cystine.

Kolkhuri variety, which is distinguished by high content of albumins and globulins, and having high nutritional value has been chosen for further investigations.

The method of isolation of food protein from defatted flour was currently elaborated and was different from traditional methods of preparation of soy protein isolate, concentrate and their textured products.

Different from traditional method of soy protein isolate preparation [14] the pH of extraction buffer according to elaborated method was lower. This allowed to obtain the fraction, containing albumins and globulins and avoid the presence of glutelin in the preparation. Presence of the latter fraction decreases nutritional value of the isolate giving advantage to protein concentrates to some extent [1]. At the same time the content free from glutelin albumins and globulins fraction determines high content of indispensable amino acids and especially methionine, which is extremely important from the point of methionine value.

Further separation of albumins and globulins was carried out by dialysis. By mixing of 40% albumin-alginate and 20% globulin suspensions on water bath at 95^{O}C the coagulate was formed. After treatment first with alkali, then by weak acid, a thick phorous product was obtained. After cooling and filtration the coagulate was exposed to consecutive treatment with alkali at pH 9-9.4 and by boiling up in 0.1 N acetic acid. The thick phorous protein product obtained exhibits stability towards thermal and mechanical treatment, is well cut and during cooking in oil manifests characteristic of meat odour and colour. This allows to use the product obtained as meat alternative.

Thus, the described method enables to obtain high nutritional value soybean protein preparation, with phorous structure and approximately the same wastes as for soy protein isolate. Besides this method is cost effective and permits to avoid extra expenses needed for production of texturized soy protein isolate.

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ANTIOXIDANT ACTIVITY OF GRAPE BIOFLAVONOIDS AND SOME FLAVONOID STANDARDS

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SUMMARY

The antioxidant activity of grape bioflavonoid and some flavonoid standard (unsubstituted flavone, apigenin, fisetin, morin, quercetin and dihydroquercetin) samples have been studied. All samples were incubated in hydrophilic and lipophilic systems and oxidation was measured according to the formation of hydroperoxides and malondialdehyde. The activity of antioxidants (concentration 10 μM) was calculated based on the decrease in the formation of hydroperoxides and malondialdehyde. The order of antioxidant activity in hydrophilic and lipophilic system was nearly the same: quercetin > grape bioflavonoids > morin > fisetin > dihydroquercetin > α-tocopherol > apigenin > unsubstituted flavone. In both systems approximately the same activity sequence was found in the case of malondialdehyde. In hydrophilic as well as in lipophilic systems the antioxidant activity of the flavonoids increased with the increase of the number of hydroxyl groups and was dependent on the position of the hydroxyl groups.

KEYWORDS: Antioxidants, flavonoids, hydroperoxides, malondialdehyde, linoleic acid.

INTRODUCTION

Food additives increase the overall nutritional quality of food products including improvement of storage stability and acceleration of technological processing. Among the food additives great attention is paid to antioxidants. They cause harmless destructive effects on the molecular oxygen active forms in cells and inhibit the formation of free radical oxidation products. Among natural antioxidants, the flavonoid compounds, widely spread in the plant world, are well determined. These aromatic and heterocyclic compounds possess a high physiological activity (bioflavonoids) exerting multipe biological effects including antioxidative activity and regulation of the activity of enzymes of different classes. Furthermore, flavonoids possess wide spectra of pharmacological activity including cardioprotective, spasmolytic, anti-inflammatory, radioprotective, antiallergic, hepatoprotective, antisclerotic, diuretic and other kinds of actions [1-5]. According to the latest data [6] maximum radical scavenging activity of a flavonoid molecule needs to meet the following criteria: ortho-dihydroxy structure in the B-ring; $C_{2,3}$-double bond in conjugation with a 4-oxo group in the C-ring; and hydroxyl groups in C_3 and C_5 positions.

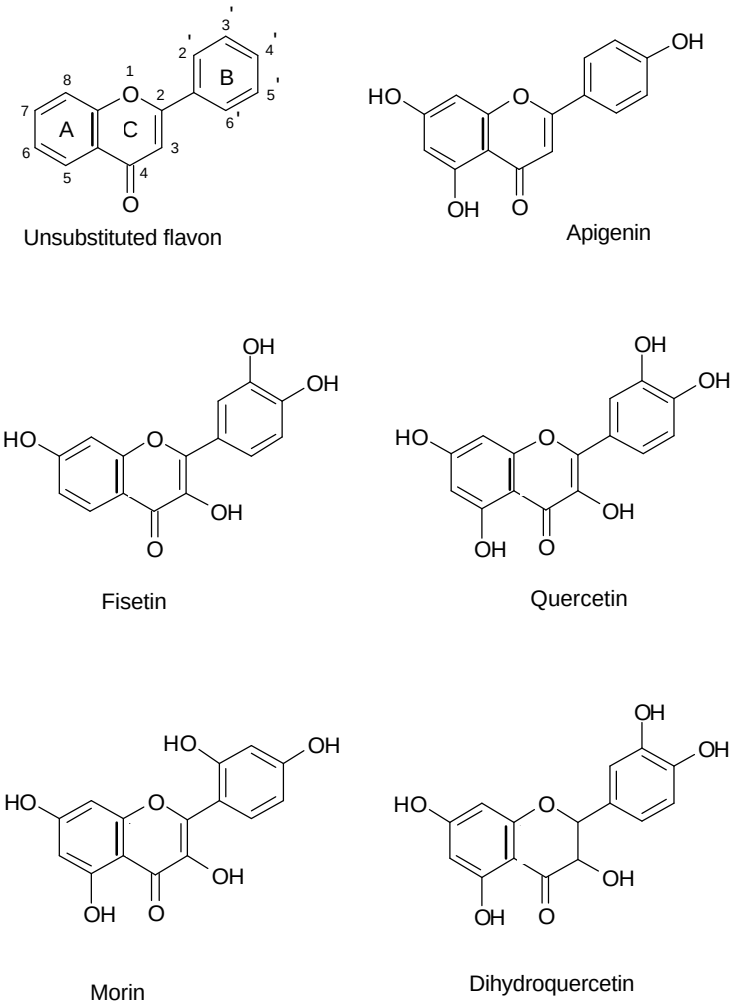
In this study the antioxidant action of grape bioflavonoids and some flavonoid standards were evaluated according to their ability to inhibit the autoxidation of linolic acid in hydrophilic and lipophilic model systems. Chemical structures of the tested flavonoids are presented in Fig. 1. α-Tocopherol was used as a reference antioxidant.

MATERIALS AND METHODS

Materials

Grape bioflavonoids were prepared from grape seeds of "Rkatsiteli" variety cultivated in Georgia. Grape bioflavonoids are phenolic compounds which may be determined according to Folin-Denis method [7]. Linoleic acid (Reachim, Russia) was used as oxidizing substrate without further purification. Initial hydroperoxide content was 32 mmol/kg. Unsubstituted flavone was supplied by Fluka, apigenin from Serva, fisetin and dihydroquercetin from Austrowaren (Vienna), morin from Ferk (Berlin), quercetin from Chemapol (Prague), α-tocopherol, dimethyl sulphoxide and thiobarbituric acid from Sigma.

FIGURE 1 - Chemical structures of different flavonoids.



Methods

Autoxidation of linoleic acid was studied in hydrophilic (water/ethanol/dimethyl sulphoxide solution) and lypophilic (butanol) homogeneous model systems using the thiocyanate and thiobarbituric acid methods [8, 9]. Peroxide quantity was measured spectrophotometrically at 480 nm with ammonium rhodanate by colour reaction. The formation of thiobarbituric acid reacting substances (malondialdehyde) was measured at 532 nm. Antioxidant activity of flavonoids was calculated as percent inhibition of hydroperoxide production {[(C-S)/C] × 100}, where C is the amount of hydroperoxides formed in control sample (α-tocopherol) and S in the samples containing the flavonoids tested [10].

Malondialdehyde concentration in reaction mixtures was calculated using the following formula [11],

$$C_{MDA} = \frac{A_{532} \cdot 100(V_1 + V_2 + V_3)}{152V_1}$$

where A_{532} is the optical density of solution, V_1 the incubation volume, V_2 the volume of chloroform-ethanol mixture and V_3 the volume of thiobarbituric acid solution.

The reaction mixtures of both model systems contained 0.5 g of linoleic acid, 10 μM of the flavonoids studied or α-tocopherol as reference dissolved in 0.5 ml dimethyl sulphoxide, 0.3 ml ethanol, 0.2 ml phosphate buffer (pH 7.0) for the hydrophilic and in 1 ml n-butanol for the lipophilic system. Control samples contain only α-tocopherol as reference antioxidant and no flavonoids studied. Tests were carried out in 100 ml stoppered flasks, at 40 °C in the darkness for 10 days. Samples were taken out for analysis after 1, 3, 7, and 10 days. Two parallel tests were conducted and the results were treated statistically.

RESULTS AND DISCUSSION

The antioxidant activity of flavonoids in hydrophilic and lipophilic systems depends on their solubilization and penetration ability into the substrate [12]. Probably, antioxidative activity of any flavonoid compounds can be better estimated if the process is carried out in a homogeneous system. Therefore, we have chosen the hydrophilic water-ethanol-dimethyl sulfoxide buffered system, which was already tested for its applicability [12], and n-butanol as solvent for the homogeneous lipophilic system. In both cases concentration of the flavonoids studied was 10 μM.

Under identical conditions of incubation, autoxidation of linoleic acid was more intensive in the hydrophilic system. The quantity of the hydroperoxides formed in the hydrophilic system control samples significantly exceeds that of the lipophilic system (Figs. 2 and 3). In both systems the hydroperoxide content was high in the samples containing unsubstituted flavone, apigenin and α-tocopherol. This indicates their low antioxidant activity at a concentration of 10 μM. The antioxidant activity of flavonoid compounds determined in the inhibition experiments of hydroperoxide

FIGURE 2
Effect of flavonoids on the formation of hydroperoxides by autoxidation of linolic acid in hydrophilic system.
1 – Control; 2- unsubstituted flavon; 3 – apigenin; 4 – fisetin; 5 – morin; 6 – quercetin; 7 – dihydroquercetin; 8 – grape bioflavonoids; 9 - a - tocopherol. Each column consists of four bands, which one corresponds (from left to right) to exposure time - 1, 3, 7 and 10 days.

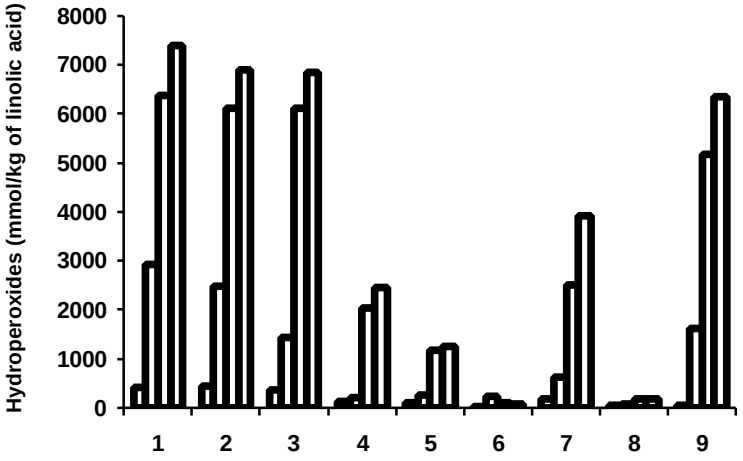


FIGURE 3
Effect of flavonoids on the formation of hydro-peroxides by autoxidation of linolic acid in lipophilic system.
1 – Control; 2- unsubstituted flavon; 3 – apigenin; 4 – fisetin; 5 – morin; 6 – quercetin; 7 – dihydroquercetin; 8 – grape bioflavonoids; 9 - a -tocopherol. Each column consists of four bands, which one corresponds (from left to right) to exposure time - 1, 3, 7 and 10 days.

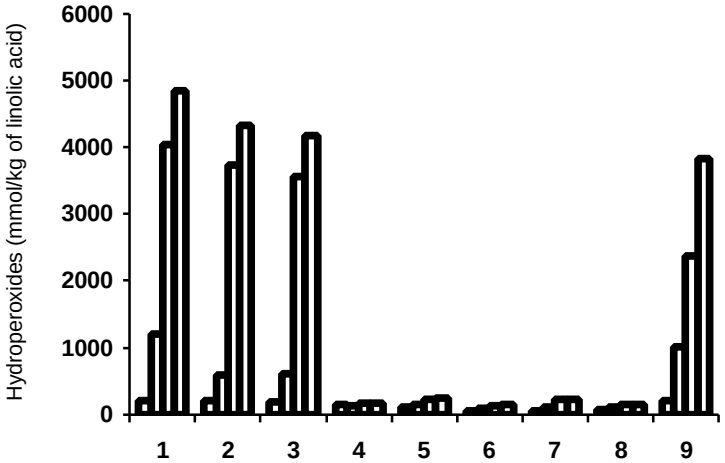


TABLE 1
Inhibition of hydroperoxide formation at linolic acid autoxidation (%)

Antioxidans	Autooxidation duration (days)							
	1	3	7	10	1	3	7	10
	Hydrophilic system				Lipophylic system			
Grape bioflavonoids	78,5±2,4	95,4±0,5	96,5±0,1	97,0±0,1	58,1±2,9	88,2±0,5	95,4±0,3	96,1±0,2
Unsubstituted flavon	7,6±3,9	15,3±0,9	4,2±2,6	6,6±2,9	4,8±1,9	48,9±4,5	8,0±0,9	10,4±4,0
Apigenin	9,7±3,8	50,2±1,9	4,0±2,9	7,5±2,8	5,8, ±4,2	48,4±3,9	11,6±1,0	13,1±3,8
Fisetin	57,4±4,4	91,5±0,1	67,2±1,6	66,2±1,8	25,5±10,0	86,8±0,5	95,0±0,3	95,9±0,3
Morin	67,3±2,9	89,1±0,6	81,0±0,2	82,4±2,1	37,6±5,2	85,3±1,0	93,6±0,5	94,4±0,2
Quercetin	83,7±1,8	89,3±1,3	97,5±0,4	98,3±0,1	66,4±1,5	90,0±0,4	96,1±0,1	96,5±0,1
Dihydroquercetin	47,7±7,3	76,7±0,7	60,4±3,7	46,8±5,4	64,6±1,3	88,2±1,2	93,7±0,8	94,6±0,2
α-Tokoferol	3,6±3,2	43,6±1,3	19,0±5,8	13,7±2,8	5,3±3,2	14,3±7,8	40,9±3,4	21,0±3,4

FIGURE 4
Effect of flavonoids on the formation of malondialdehyde by autoxidation of linolic acid in hydrophilic system.
1 – Control; 2- unsubstituted flavon; 3 – apigenin; 4 – fisetin; 5 – morin; 6 – quercetin; 7 – dihydroquercetin; 8 – grape bioflavonoids; 9 - a -tocopherol. Each column consists of three bands, which one corresponds (from left to right) to exposure time - 3, 7 and 10 days.

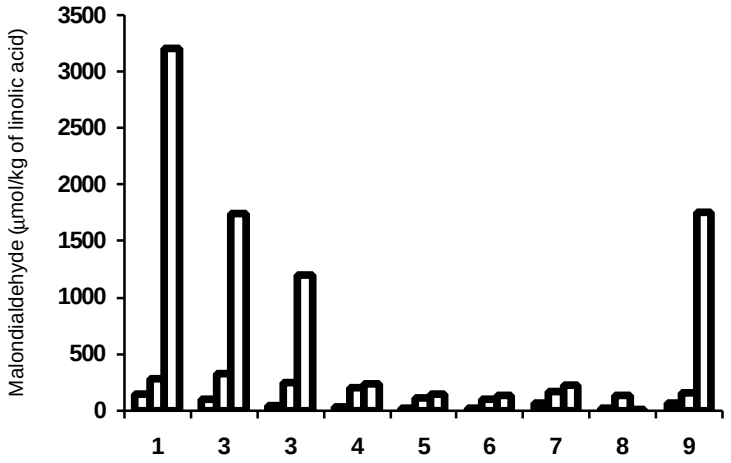
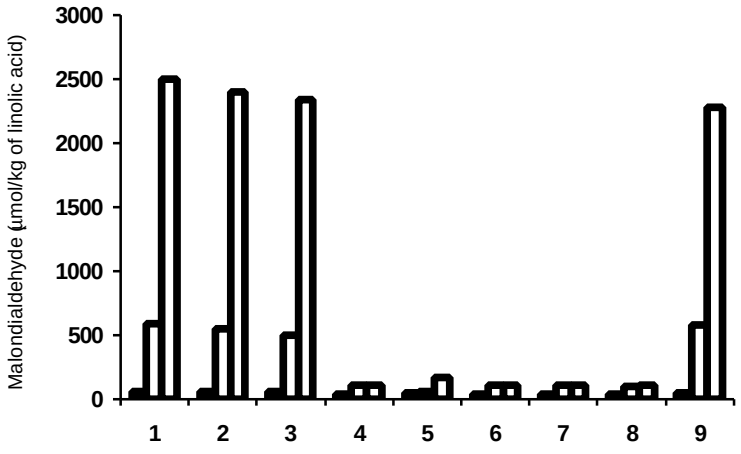


FIGURE 5
Effect of flavonoids on the formation of malon-dialdehyde by autoxidation of linolic acid in lipophilic system.
1 – Control; 2- unsubstituted flavon; 3 – apigenin; 4 – fisetin; 5 – morin; 6 – quercetin; 7 – dihydroquercetin; 8 – grape bioflavonoids; 9 - a -tocopherol. Each column consists of three bands, which one corresponds (from left to right) to exposure time - 3, 7 and 10 days.



formation in hydrophilic system showed the following order: quercetin > grape bioflavonoids > fisetin > morin > dihydroquercetin > apigenin > α-tocopherol > unsubstituted flavone. In the lipophilic system this sequence was as follows: quercetin > grape bioflavonoids > dihydroquercetin > fisetin > morin > apigenin >unsubstituted flavone > α-tocopherol (Figs. 2 and 3). Flavonoids antioxidant activity was increased with the increase of the number of hydroxyl groups in their molecules. In the lipophilic system dihydroquercetin, fisetin and morin exhibited higher antioxidant activity than in hydrophilic system.

During the experiments the effects of quercetin and grape bioflavonoids on inhibition of hydroperoxides formation during autoxidation of linoleic acid increased and reached its maximum in hydrophilic as well as lipophilic system on the 10th day (Table 1). Antioxidant activity of dihydroquercetin, morin and fisetin also increased in the lipophilic system, whereas in the hydrophilic system antioxidant activity of the above three compounds reached its maximum already on the 3rd day of incubation and then decreased (Table 1). Among the flavonoids used, unsubstituted flavone showed the lowest activity as it has no hydroxyl groups and antioxidant activity was only caused by the $C_{2,3}$-double bond in the C-ring (Fig. 1). Apigenin, with substituted hydroxyl in C_5 and C_7 positions in A-ring and $C_{4'}$ position in B-ring, evidenced higher antioxidant activity compared to unsubstituted flavone. The inhibitory effect of the wellknown antioxidant α-tocopherol at 10 μM concentration was significantly low and would be more efficient at higher concentrations as recommended in some references [13, 14].

The concentration of malondialdehyde, one of the products of hydroperoxide destruction, was higher in control samples of the hydrophilic system (Figs. 4 and 5), but corresponds to the level of hydroperoxide formation in both systems (Figs. 2 and 3). The antioxidant activity of flavonoid compounds used in experiments can be divided into two groups. The more active group is represented by quercetin, grape bioflavonoids, morin, ficetin and dihydroquercetin and the less active one by unsubstituted flavone, apigenin and α-tocopherol (Figs. 4 and 5). This classification is affirmed by the following data: at the 10th day of incubation in hydrophilic system containing quercetin as the active flavonoid, the concentration of malondialdehyde did not exceed 130 μM/kg linoleic acid, while in the samples with unsubstituted flavone as representative of the less active group, concentration of malondialdehyde was 1700 μM/kg linoleic acid. The observations in lipophilic system were similar (Figs. 4 and 5).

The effect of the C-ring structural peculiarities has been studied by comparison of the antioxidant activities of unsubstituted flavone, quercetin and dihydroquercetin. Activities of these three flavonoids in hydrophilic and lipophilic systems decreased in the following order: quercetin > dihydroquercetin > unsubstituted flavone (Figs. 2 and 3). Quercetin more efficiently inhibited hydroperoxide formation compared to dihydroquercetin and unsubstituted flavone. In the case of linoleic acid autoxidation, the antioxidant effects of flavonoids depend on the C-ring structural peculiarities. Quercetin has a $C_{2,3}$ double bond and a hydroxyl group in C_3 position, while in dihydroquercetin the $C_{2,3}$ double bond is reduced and unsubstituted flavone has no hydroxyl group at all (Fig. 1). This points to the fact that the $C_{2,3}$ double bond and the hydroxyl group in C_3 position are mainly responsible for the high antioxidative effect of quercetin. The results also prove the already existing data that –C(=O)-C=C- group and the substituted hydroxyl group in C_3 position of the pyrone ring are the main factors of antioxidant activity of flavonoid molecules [15].

The effect of B-ring structural peculiarities was studied with samples of unsubstituted flavone, apigenin, quercetin and morin. Morin has hydroxyl groups in meta-position (B-ring, $C_{2',4'}$ positions), quercetin in $C_{3',4'}$ ortho-position, apigenin in $C_{4'}$ position and unsubstituted flavone has no hydroxyl group at all (Fig. 1). Activity of these flavonoids in both systems decreased in the following order: quercetin > morin > apigenin > unsubstituted flavone (Figs. 2 and 3). Thus, the increase of hydroxyl group number in the B-ring of flavonoid molecules caused a sharp rise of antioxidant effect. At the same time it should be noted that the position of hydroxyl groups in the B-ring in meta-position (morin), in contrast to quercetin, decreases its antioxidant activity. The high antioxidant activity of quercetin can be explained by the ortho-position of hydroxyl groups in the B-ring [15]. Thus, it can be concluded from the experimental data, also the number of hydroxyl groups in the B-ring and their position in flavonoid molecules significantly determines their antioxidant activity. In particular, the activity is increased with the increase of the number of hydroxyl groups in B-ring and it depends on their position [13, 14].

The effect of A-ring structural peculiarities was studied by comparison of antioxidant activities of unsubstituted flavone, quercetin and fisetin . The A-ring of quercetin has substituted hydroxyl group at C_5 and C_7 positions, fisetin at C_5 position and unsubstituted flavone no hydroxyl group (Fig. 1). The antioxidant activity of these flavonoids to prevent linoleic acid autoxidation decreased in hydrophilic as well as in lipophilic system in the following order: quercetin > fisetin > unsubstituted flavone (Figs. 2 and 3). Ensuing from the above-mentioned order, flavonoids antioxidant activity preventing autoxidation of linoleic acid is, to a definite extent, affected by the hydroxyl groups $C_{5,7}$ positions of the A-ring.

Quercetin was found to be the most active flavonoid. Its high antioxidant activity was confirmed in a number of publications: quercetin inhibits hydroperoxide formation in methyl linoleate [13] and peroxidation of linoleic acid by lipoxygenase [16]. Photosensitized hemolysis of hu-

man erythrocytes by hematoporphyrin was suppressed by flavonols such as quercetin and rutin at submillimolar concentrations [9]. Grape bioflavonoids exposed a similar antioxidant activity as quercetin (Figs. 2 and 3). The main components of grape bioflavonoid fraction (catechins [17, 18] and proanthocyanidins [19]) also possess vitamin P [20] and antioxidant activities [3].

Thus, according to experimental data, flavonoids antioxidant action is mainly influenced by $C_{2,3}$-double bond and the substituted hydroxyl group in C_3 position in the C-ring, by $C_{3',4'}$ ortho-position of hydroxyl groups in B-ring, and $C_{5,7}$ meta-position of hydroxyl groups in A-ring. The loss of activity, more pronounced in hydrophilic than in lipophilic systems at 10 μM concentration, was approximately identical with the exceptions of dihydroquercetin, morin and fisetin acting in lipophilic system. Therefore, grape bioflavonoids are of great interest and their inhibitory effect on hydroperoxide formation approximated that of quercetin, which was the most active antioxidant among the flavonoids used in the experiments.

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PURIFICATION AND ENZYMIC PROPERTIES OF KDGAL ALDOLASE FROM *Aspergillus niger*

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SUMMARY

D-Galactonate dehydratase (EC 4.2.1.6) and 2-keto-3-deoxy-D-galactonate (KDGal) aldolase (lyase division; similar to EC 4.1.2.21, but without phosphorolytic reaction) were purified from cell-free extracts of *Aspergillus niger* by ion exchange column chromatography using diethylaminoethyl (DEAE)-cellulose. The highest specific activity was found in fraction numbers 35 and 28 corresponding to a 30 and 73-fold purification for these two enzymes, respectively. Some properties of the purified KDGal aldolase were studied. The enzyme exhibited the optimum pH and temperature at 7.5 and 50 °C, respectively. Potassium phosphate buffer showed the highest KDGal aldolase activity when compared to the analogous activity obtained with the other buffers used. The results also indicated that the catalytic activity of the purified enzyme was proportional to the amount of protein in question and a linear relationship was obtained. The effect of the concentrations of the three substrates namely KDGal, pyruvate and glyceraldehyde on the activity of KDGal aldolase was studied. From a Lineweaver-Burk plot of the reciprocal of initial velocities and substrate concentrations the K_m (Michaelis constant) values were calculated and found to be 7.69, 12.20 and 6.25 mM, respectively, indicating the highest affinity of the enzyme for glyceraldehyde.

KEYWORDS:
Aspergillus niger, degradation, KDGal aldolase, purification.

INTRODUCTION

Investigators divided the pathways for carbohydrate degradation into phosphorolytic, partially non-phosphorolytic and non-phosphorolytic pathways according to the presence or absence of adenosine triphosphate (ATP) participation in the catalytic reactions. Many of them have demonstrated the alternative pathways of D-galactose and D-galactonate breakdown in microorganisms [1-7].

Most of these studies indicated the presence of phosphorolytic step(s) in these pathways, e.g., Leloir pathway [8-10] and De Ley-Doudoroff pathway [1, 11, 12]. We previously reported a degradative pathway for D-galactonate in extracts of *Aspergillus terreus* NRRL 265 [13], and the properties of the enzymes involved in D-galactonate degradation were also studied [14]. Recently, we demonstrated an evidence for a non-phosphorylated route of galactose breakdown in cell-free extracts of *Aspergillus niger* [15]. Some factors affecting D-galactonate degradation by the same extract were also studied [16]. The objective of the present work is concerned with the purification and enzymic properties of KDGal aldolase in extracts of *Aspergillus niger*.

MATERIALS AND METHODS

Organism

A local strain of *Aspergillus niger*, available in the Department of Microbial Chemistry, Division of Genetic Engineering, National Research Centre, Egypt, was used in this study.

Media

Aspergillus niger was cultivated on slants of modified solid Czapek-Dox medium [17]. The composition of this medium is as follows (g L^{-1}): D-galactose, 30; $NaNO_3$, 2.0; KH_2PO_4, 1.0; $MgSO_4$. $7H_2O$, 0.5; KCl, 0.5 and agar 20. The medium was sterilized by autoclaving under 1.5 atmosphere for 20 min. In case of solid media, the sterilization time was prolonged to 30 min under the same pressure conditions.

Cultivation of organism

Conidia were scrapped from mycelia, which were grown on slants for 12 days at 30 °C and suspended, by hand shaking, in sterile distilled water. Two-ml aliquots of this suspension were used to inoculate, under aseptic conditions, 250 ml Erlenmeyer flasks each containing 50 ml of

sterile medium. The inoculated flasks were incubated statically at 30 °C for specific time intervals.

Harvest of mycelia

After different incubation periods the mycelia were collected and harvested by filtration, then washed thoroughly with cold distilled water and, finally, blotted dry with absorbent paper.

Preparation of cell-free extracts

The blotted-dry mycelia were ground with approximately twice its weight of washed cold sand in a cold mortar and extracted with 0.1 M potassium phosphate or Tris-(hydroxymethyl) aminomethane (Tris-HCl) buffer at pH 8. The slurry so obtained was centrifuged at 5,500 rpm for 5 min. The supernatant was used as the crude enzyme preparation.

Preparation of aldonic acid and KDGal

Potassium salt of aldonic acids was prepared according to the method described by Moore and Link [18]. This method is based on the oxidation of aldoses by hypoiodite in methanol. KDGal was prepared by the method of Portsmouth [19].

Enzyme assay

D-galactonate dehydratase and 2-keto-3-deoxy-D-galactonate (KDGal) aldolase were estimated by determination of µmoles KDGal formed from D-galactonate or pyruvate and glyceraldehyde, respectively, according to the method of Weissbach & Hurwitz [20] as previously described [21].

Chemical methods

2-Keto-3-deoxy-aldonate was determined by the method of Weissbach and Hurwitz [20]. Protein was determined by the method of Lowry *et al* [22]. The protein content in the purified enzyme fractions was determined by the UV absorbance technique of Warburg and Christian [23].

Treatment of diethylaminoethyl-cellulose (DEAE)

DEAE-cellulose was thoroughly washed with distilled water followed by several washings with 0.5 N NaOH until no more colour was removed. After the last alkaline wash the resin was rinsed with distilled water until it was free of alkali. Then it was suspended in about 3 volumes of 0.01 M potassium phosphate buffer at pH 7.5 and portions of 0.2 M KH_2PO_4 were added until the pH of the suspension was within 0.1 pH unit of the starting buffer. The adjusted resin was then washed five times, each with about 3 volumes of the starting buffer, and transferred to a bottle where it was resuspended in about 3 volumes of the starting buffer. At this stage the supernatant fluid was almost clear indicating the removal of most of the particles. This preparation was used for column packing.

Purification of D-galactonate dehydratase and 2-keto-3-deoxy-D-galactonate (KDGal) aldolase

The cell-free extracts of *A. niger* grown on D-galactonate, which is considered as the crude enzyme preparation (8 ml), was placed on the DEAE-cellulose chromatographic column (1.5 x 50 cm) which was pre-equilibriated with 0.01 M potassium phosphate buffer (pH 7.5). Elution was carried out by batch-wise addition of 25 ml portions of increasing molarities (0.05 - 0.6 M) of solutions of NaCl in 0.01 M potassium phosphate buffer pH 7.5. 5 ml fractions were collected and then assayed for D-galactonate dehydratase, KDGal aldolase and protein determination.

RESULTS AND DISCUSSION

Purification of D-galactonate dehydratase and KDGal aldolase

Fig. 1 represents a typical elution diagram of D-galactonate dehydratase and 2-keto-3-deoxy-D-galactonate (KDGal) aldolase from the DEAE-cellulose chromatographic column (1.5 x 50 cm). The highest specific activities of D-galactonate dehydratase were found in fraction numbers 34, 35, 36, 37 and 38 corresponding to a 15.25-, 30.19-, 20.32-, 17.36- and 11.59-fold purification, respectively. The highest specific activities of KDGal aldolase were found in fraction numbers 26, 27, 28, 29 and 30 corresponding to a 22.06-, 34.56-, 73.53-, 30.78- and 22.82-fold purification, respectively. A summary of the purification procedure is given in Tables 1 and 2.

FIGURE 1 - DEAE-cellulose chromatography of D-galactonate dehydratase and KDGal aldolase.

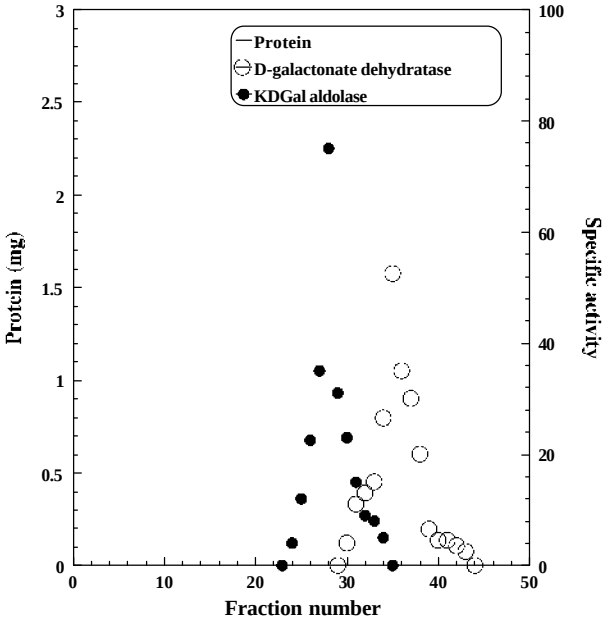


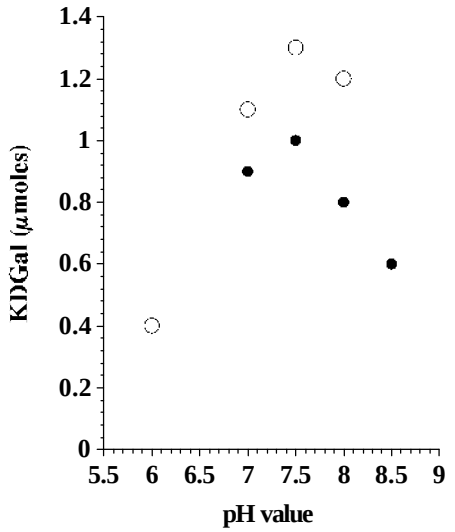
TABLE 1 - Purification of D-galactonate dehydratase using DEAE-cellulose column ion exchange chromatography.

Step	Total protein (mg)	Total KDGal (µmoles)	Specific activity	Recovery (%)	Purification fold
Crude extract	42.24	73.60	1.74	100.00	1.00
Fraction 32	0.40	5.20	13.00	7.07	7.47
Fraction 33	0.35	5.25	15.00	7.13	8.62
Fraction 34	0.25	6.63	26.52	9.01	15.24
Fraction 35	0.15	7.88	52.53	10.71	30.19
Fraction 36	0.20	7.07	35.35	9.61	20.32
Fraction 37	0.20	6.04	30.20	8.21	17.36
Fraction 38	0.25	5.04	20.16	6.85	11.59
Fraction 39	0.30	1.95	6.50	2.65	3.74
Fraction 40	0.30	1.35	4.50	1.83	2.59
Fraction 41	0.25	1.12	4.48	1.52	2.57
Fraction 42	0.20	0.70	3.50	0.95	2.01
Fraction 43	0.20	0.50	2.50	0.68	1.44

TABLE 2 - Purification of 2-keto-3-deoxy-D-galactonate aldolase using DEAE-cellulose column ion exchange chromatography.

Step	Total protein (mg)	Total KDGal (µmoles)	Specific activity	Recovery (%)	Purification fold
Crude extract	42.24	43.04	1.02	100.00	1.00
Fraction 24	0.35	1.40	4.00	3.25	3.92
Fraction 25	0.35	4.20	12.00	9.76	11.76
Fraction 26	0.30	6.75	22.50	15.68	22.06
Fraction 27	0.20	7.05	35.25	16.38	34.56
Fraction 28	0.10	7.50	75.00	17.43	73.53
Fraction 29	0.20	6.28	31.40	14.59	30.78
Fraction 30	0.25	5.82	23.28	13.52	22.82
Fraction 31	0.30	4.50	15.00	10.46	14.71

FIGURE 2 - Effect of pH value on KDGal aldolase activity.



Reaction mixture conditions: Sodium pyruvate, 10 µmoles; glyceraldehyde, 5 µmoles; (o) potassium phosphate or (•) triethanolamine hydrochloride buffer (pH as indicated), 60 µmoles; protein enzyme, 84 µg; total volume, 1.5 ml; temperature, 50 °C and reaction time, 12 min.

Some properties of the purified KDGal aldolase of *A. niger*

Since the stability of D-galactonate dehydratase is more sensitive to temperature and storage the study was directed toward examining some of the properties of the purified 2-keto-3-deoxy-D-galactonate (KDGal) aldolase of *A. niger* in the reverse direction (i.e. the direction of the condensation of pyruvate and glyceraldehyde).

Effect of pH

Potassium phosphate and triethanolamine hydrochloride buffers were used to cover the pH range (6.0 - 8.5) studied. All the reaction mixtures were incubated in a waterbath adjusted to 50 °C. The results in Fig. 2 show that maximum activity of KDGal aldolase was obtained with potassium phosphate buffer at pH 7.5. This range of pH coincides with that obtained in the preceding study concerning crude enzyme preparation [16].

Effect of the nature of the buffer

Different types of buffers at the same pH value (pH 7.5) were added separately to each reaction mixture to

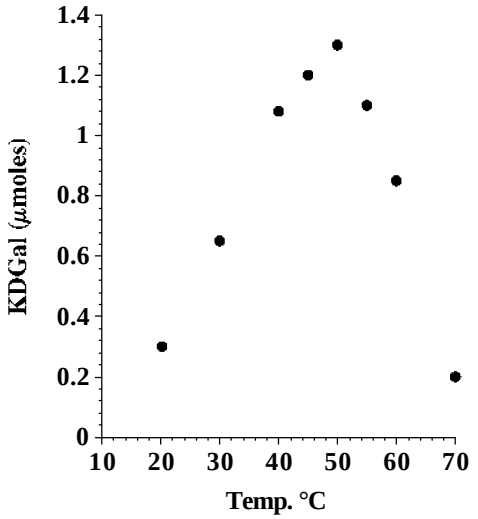
study the effect of the nature of the buffer system on KDGal aldolase activity. These buffers are potassium phosphate, triethanolamine hydrochloride (TEA), Tris-HCl, N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES), imidazole and pyrophosphate. The results in Table 3 indicate that potassium phosphate shows the highest KDGal aldolase activity when compared to the activity obtained with the other buffers. Also TEA buffer shows considerable amount of enzyme activity. Pyrophosphate buffer caused 83.08% inhibition of enzyme activity which can be considered as the lowest activity for KDGal aldolase.

TABLE 3
Effect of the nature of the buffer system on KDGal aldolase activity.

Type of buffer (0.1 M)	KDGal (µmoles)
Potassium phosphate	1.30
TEA	1.00
Tris-HCl	0.73
HEPES	0.52
Imidazole	0.41
Pyrophosphate	0.22

Reaction mixture conditions: Sodium pyruvate, 10 µmoles; glyceraldehyde, 5 µmoles; different buffers pH 7.5; protein enzyme, 84 µg; total volume, 1.5 ml; temperature, 50 °C and reaction time, 12 min.

FIGURE 3 - Formation of KDGal from pyruvate and glyceraldehyde as a function of temperature.



Reaction mixture conditions: Sodium pyruvate, 10 µmoles; glyceraldehyde, 5 µmoles; potassium phosphate buffer pH 7.5, 60 µmoles; protein enzyme, 84 µg; total volume, 1.5 ml; temperature, as indicated and reaction time, 12 min.

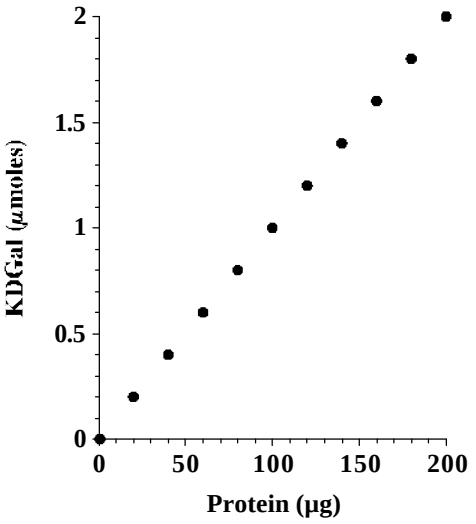
Effect of temperature

Eight reaction mixtures were prepared each containing the same amount of substrate (pyruvate, glyceraldehyde), potassium phosphate buffer pH 7.5 and protein enzyme. After fixed time intervals aliquots were withdrawn for each reaction mixture and the amounts of KDGal formed were analyzed. The optimal temperature for KDGal formation from the two substrates (pyruvate and glyceraldehyde) by the enzyme KDGal aldolase was 50 °C (Fig. 3). The activity of enzyme was decreased gradually by increasing or decreasing the temperature. At 45 °C and 55 °C the activity was about 8% and 16% lower than at 50 °C, respectively. However, at 60 °C there was a pronounced decrease (35%) in activity.

Effect of enzyme concentration

Several reaction mixtures containing the same amount of substrate (pyruvate and glyceraldehyde) and potassium phosphate buffer but varying amounts of protein enzyme were examined. The results in Fig. 4 indicate that the catalytic activity of KDGal aldolase is proportional to the amount of protein enzyme in question and a linear relationship was obtained.

FIGURE 4 - Effect of enzyme concentration on enzyme activity.



Reaction mixture conditions: Sodium pyruvate, 10 µmoles; glyceraldehyde, 5 µmoles; potassium phosphate buffer pH 7.5, 60 µmoles; protein enzyme (µg), as indicated, total volume, 1.0 ml; temperature, 50 °C and reaction time, 12 min.

KDGal aldolase activity as a function of substrate concentration

As previously demonstrated, the enzyme KDGal aldolase of *A. niger* catalyzes the reversible reaction

KDGal $\rightleftharpoons$ **pyruvate + glyceraldehyde**.

It is apparent that the study of this enzyme activity as a function of substrate concentration should include the three substrates namely KDGal, pyruvate and glyceraldehyde. The presence of D-galactonate dehydratase in the extracts will not cause any interference with aldolase activity since the reaction

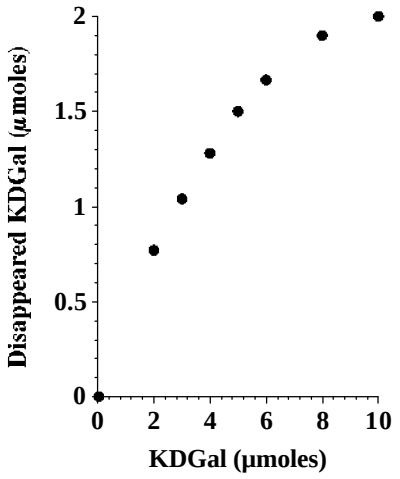
D-galactonate $\longrightarrow$ **KDGal**

is known to be irreversible.

With regard to the reverse reaction of aldolase, when the concentration of glyceraldehyde or pyruvate was changed, the other substrate was added to saturation. In this way the activity determined only reflects the changes of one substrate concentration.

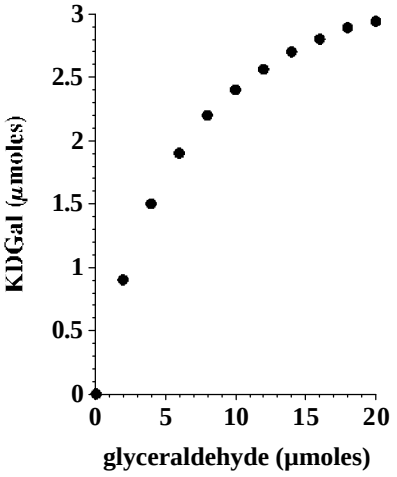
Figs. 5, 6 and 7 demonstrate the effect of KDGal, glyceraldehyde and pyruvate concentrations on enzyme activity, respectively, also showing a Lineweaver-Burk plot [24] of the reciprocal of initial velocities and concentrations of KDGal, glyceraldehyde or pyruvate as substrates. From these plots K_m (Michaelis constants) were calculated and found to be 7.69, 6.25 and 12.20 mM, respectively.

FIGURE 5 - Effect of KDGal concentration on enzyme activity.



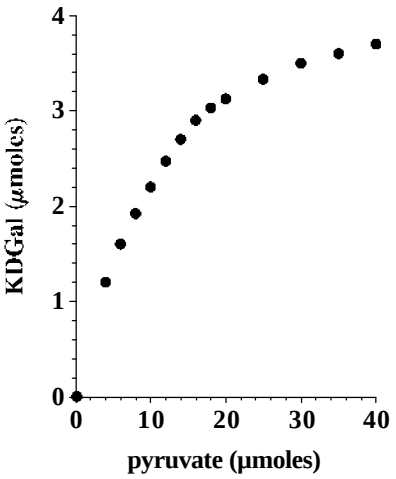
Reaction mixture conditions: KDGal, µmoles as indicated; potassium phosphate buffer pH 7.5, 80 µmoles; protein enzyme, 100 µg; total volume, 1.0; temperature, 50 °C and reaction time, 10 min.

FIGURE 6
Effect of glyceraldehyde concentration on enzyme activity.



Reaction mixture conditions: Sodium pyruvate, 20 µmoles; glyceraldehyde, as indicated; potassium phosphate buffer pH 7.5, 60 µmoles; protein enzyme, 84 µg; total volume, 1.0 ml; temperature, 50 °C and reaction time, 10 min.

FIGURE 7 - Effect of pyruvate concentration on enzyme activity.



Reaction mixture conditions: Glyceraldehyde, 10 µmoles; sodium pyruvate, as indicated; potassium phosphate buffer pH 7.5, 60 µmoles; protein enzyme, 84 µg; total volume, 1.0 ml; temperature, 50 °C and reaction time, 10 min.

The Entner-Doudoroff (ED) pathway, which was first elucidated in *Pseudomonas saccharophila* metabolism [25, 26], is also the major route of glucose utilization in other microorganisms [27-34]. We previously indicated an evidence for a non-phosphorylated route of galactose and galactonate breakdown [15] in cell-free extracts of *Aspergillus niger* and the factors affecting D-galactonate degradation by extracts of the same fungus [16]. This route was nearly similar to the Entner-Doudoroff pathway but without the phosphorolytic steps. In the ED pathway comprising the two enzymes involved D-gluconate degradation, namely 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate (KDPG) have been characterized extensively in *Pseudomonas putida* by W.A. Wood and his coworkers [35, 36]. Kersters and De Ley have also examined the distribution of these enzymes in a variety of Gram-negative bacteria [37, 38]. Many authors reported the occurrence of D-galactonate catabolic pathway in saprophytic strains of the genus Mycobacterium namely *Mycobacterium sp.* strains 607; *M. jucho; M. friburgensis; M. butyricum* and *M. sp.* strain 279 [5, 39, 40]. The pathway involved galactonate dehydratase (EC 1.1.4.8) and 2-keto-3-deoxy-galactonate kinase (EC 4.1.2.21), which are the enzymes previously found in *Pseudomonas saccharophila* [1]. D-Galactonate dehydratase has been purified and enzymologically characterized [41]. The enzyme has been purified 325-fold from the crude extracts of galactose-grown *Mycobacterium butyricum* and its molecular weight of about 270,000 determined by Sephadex G-200 filtration. The results obtained in this work indicate that D-galactonate could be degraded non-phosphorolytically by cell-free extracts of *A. niger* grown statically on Dox's medium containing D-galactose as the

only carbon source and both pyruvate and glyceraldehyde were produced with the formation of 2-keto-3-deoxy-D-galactonate (KDGal) as an intermediate. This pathway shows great similarity with the corresponding pathway for D-galactonate catabolism in *A. terreus* (Elshafei *et al*. [14]), especially in that that the maximum aldolase activity obtained at 50 °C and pH 7.5 was in accordance with the results obtained using cell-free extracts of *A. terreus.* Also similarity in the results was observed calculating the apparent K_m values for D-galactonate, pyruvate and glyceraldehyde. A similar pathway was also discovered by Szumilo [42], in which D-galactonate-grown cells of *Mycobacterium sp*. 607 could utilize D-galactonate by a pathway involving D-galactonate dehydratase, 2-keto-3-deoxy-galactonate kinase and 6-phospho-2-keto-3-deoxy-galactonate (KDGal) aldolase. The enzymes have been separated by ion exchange chromatography on DEAE cellulose or ultrafiltration on Sephadex G-100. These results indicated that the catabolism of D-galactonate was effected by phosphorylative reactions. However, our results suggest the presence of a non-phosphorylative route for D-galactonate degradation in *A. niger*.

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PRESENCE OF *Staphylococcus aureus* IN FRESH PASTA AND FRESH EGG PASTA

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SUMMARY

In the present study, 30 samples each of eggs, fresh egg pasta and fresh pasta were analyzed to identify *Staphylococcus* spp., and, among these, especially *Staphylococcus aureus* strains. Isolates were tested for coagulase and thermonuclease production. Twenty strains identified as *S. aureus* were additionally tested for the production of enterotoxins A - D. The results revealed the presence of an enterotoxin-producing *S. aureus* in one fresh egg pasta sample confirming the importance of both egg quality and hygienic standards to prevent uncontrolled microbial proliferation during the production process.

KEYWORDS:
Fresh egg pasta, fresh pasta, *Staphylococcus aureus.*

INTRODUCTION

The Italian food industry is traditionally dominated by pasta production. Indeed, a large amount of both fresh and dried pasta is consumed not only in Italy, but also in many other countries [1]. However, fresh pasta is characterized by some facts such as A_w 0.95, pH value approximately 6, many handling phases and high risk of environmental contamination during production, that make it perishable compared to the dried pasta product. Since there is no cooking stage during dough preparation, both heat-sensitive and heat-resistent microorganism may be present in the dough and can even grow rapidly [2, 3]. The presence of some pathogens such *as Salmonella* spp.and *S. aureus,* may be due to contaminated raw materials (i.e. eggs) and to unhygienic handling [4]. In particular, a real microbiological problem in pasta manufacturing is represented by enterotoxigenic *Staphylococcus aureus* strains, which may be found in both fresh and dried products, even in very good production conditions [4]. Furthermore, it is important to underline that the cooking of the pasta does not assure the complete destruction of heat-resistent enterotoxins. An outbreak of food-borne disease caused by enterotoxigenic *S. aureus* strains occurred in Europe in winter 1984. The source identified was an Italian pasta factory, in which high levels of organisms were detected in packages of dried lasagne distributed in Luxemburg, England, France and Italy [5]. The aim of the present study was to identify and count *S. aureus* strains either on eggs used in the pasta production process or on fresh pasta produced with and without eggs.

MATERIALS AND METHODS

Samples

30 samples each of fresh egg pasta, fresh pasta and liquid shelled eggs (used for fresh egg pasta production) were examined. The samples were directly collected in refrigerated (4 °C) plastic or cardbord containers from different home-making producers at different times, in order to represent several production lots.

Microbiological analysis

10 g aliquots of each sample was added to 90 ml of sterile peptone water 0.1 % (w/v) and blended (stomacher 400 Colworth) two times for 30 sec with an interval of 5 min. Several decimal dilutions were prepared with physiological saline and 0.1 ml of each dilution was spread on Baird-Parker Agar (BPA, Biolife, Milan, Italy) and incubated at 37 °C for 24 h. *S. aureus* colonies were typically formed, 1.0-1.5 mm in diameter, black, shiny, convex with a narrow white entire margin and surrounded by clear zones extending 2-5 mm into the opaque medium. Typical colonies were counted and cultured in Brain Heart Infusion Broth (Biolife) and *S. aureus* presence was confirmed by both coagulase and thermostable nuclease production tests. Strains with more than 10^4 cfu/g were considered positive and 10 colonies of each positive strain were randomly picked and tested for the production of enterotoxins A - D by passive inverse agglutination to latex in microtitre plates (Set-RPLA, Oxoid).

TABLE 1
Frequency distribution (%) of *Staphylococcus* spp. [a] (cfu/ml for eggs, cfu/g for pasta and fresh egg-pasta samples).

Product	No. of samples	Range [b]			
		$<10^1$	10^2 - 10^3	10^3 - 10^4	$> 10^4$
Egg (cfu/ml)	30	33.0	13.0	20.0	34.0
Fresh egg-pasta (cfu/g)	30	33.0	6.7	13.3	46.7
Fresh Pasta (cfu/g)	30	53.3	6.7	6.7	33.3

[a] Mean values of counts (eggs: 1.5×10^2, fresh egg pasta: 3.7×10^5, fresh pasta: 4.2×10^4)
[b] Each range includes the lowest value and excludes the highest one.

TABLE 2
Frequency distribution (%) of *Staphylococcus aureus* [a] . (cfu/ml for eggs, cfu/g for pasta and fresh egg pasta samples).

Product	No of samples	Range [b]			
		$<10^2$	10^2 - 10^3	10^3 - 10^4	$>10^4$
Egg (cfu/ml)	30	2	23	*50*	25
Fresh egg-pasta (cfu/g)	30	10	10	40	40
Fresh Pasta (cfu/g)	30	9	10	81	-

[a] Mean values of counts (eggs: 3.8×10^5, fresh egg pasta: 7.8×10^5, fresh pasta: 4×10^3)
[b] Each range includes the lowest value and excludes the highest one.

RESULTS AND DISCUSSION

The percentage frequency of distribution of staphylococci isolated from the egg and pasta samples used in this study is shown in Table 1. In particular, fresh egg pasta samples were the most contaminated, with 46.7% of the samples having $>10^4$ cfu/g. The lowest contamination was detected in fresh pasta, 53.3% of the samples showing a count $<10^2$ cfu/g, while liquid egg samples were intermediately affected (34% showing $>10^4$ cfu/ml).

Table 2 shows the frequency of distribution of *S. aureus* in both eggs and fresh pasta produced with and without eggs. About 75% of shelled eggs had S. *aureus* counts between 10^2 and 10^4 cfu/ml, while 60 and 100 % of fresh egg pasta and fresh pasta resulted in counts below 10^4 cfu/g. The levels of *S. aureus* isolated in this investigation were higher than those reported by Cesaroni *et al.* [3] and Spicher [6]. The latter found that about 66% of fresh egg pasta samples contained less than 10^2 *S. aureus* per g, and only 6.7% of samples examined had more then 10^3 staphylococci per g.

In the present study we found that one fresh egg pasta sample reached a *S. aureus* count of 3×10^6 cfu/g. Nevertheless, none of the 10 strains tested was able to produce enterotoxins A - D. Only in one sample a production of enterotoxin A was observed (data not shown). However, the count was only 8×10^4 cfu/g and it has been demonstrated that a concentration of 10^6 cfu/g is needed in order to cause the typical intoxication [7].

Considering that the M parameter, fixed by the Italian Ministry of Health, is 10^4 cfu/g for *S. aureus* in fresh egg pasta [8], in the present study 25% of egg samples and 40% of egg pasta samples exceeded these low limits (Table 2). In the light of the present findings and from other data available in literature [3, 9], eggs and hygienic standards in fresh pasta production may be considered the main factors influencing the final product [10, 11].

In conclusion, our results confirm that the chief cause of staphylococci contamination in fresh egg-pasta is represented by the entire quality of the eggs which can be considered as a very critical raw material. The second factor influencing the quality of fresh pasta (produced with or without eggs) is the manufacturing process itself and every successive handling. Based on these conclusions, egg raw materials should be thoroughly controlled and used only if suitable for fresh pasta production. Furthermore, it is important to assure hygienic manufacture.

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Innovative Products from Plant Proteins at the Food Ingredients 2002 symposium 18 and 19 June 2002, Freising, GERMANY

The Fraunhofer Institute for Process Engineering and Packaging IVV, Giggenhauser Str. 35, 85354 Freising, Germany is organising this symposium on 18 and 19 June 2002.

Experts from the Fraunhofer IVV as well as experts from other organisations in Germany and abroad will report on the most recent product developments.

The symposium is directed at all involved in food processing, including food producers and users of food ingredients and also food technologists, product developers and nutritional-physiologists.

The symposium will be split into four sessions:

- Session 1 covers the demands of consumers for functional foods and the market potential of such foods. Special attention here will be put on the legal situation in Europe and on the development and testing of functional foods.
- Session 2 will inform delegates about the nutritional-physiological properties and functionality of new protein products made from rape, potatoes and lupins. This will primarily concern how these proteins affect the taste and texture of foods.
- Session 3 will cover the chemical and technological structure and function of food ingredients. Here it will be shown, for example, how enzymatic crosslinking of plant proteins enables new properties to be introduced into products and what interactions there are between lipids and phenolic substances in proteins.
- Session 4 deals with how products for promoting health can be produced from foods which contain special Food Ingredients. Here we are for example concerned with bioactive peptides from plant protein hydrolysates and the use of functional elements from red wine grapes in various foods. A report will also be given about a series of tests on the effect of antioxidants in bread spreads on reducing the risk of thrombosis.

There will also be a few special presentations covering related technical areas such as the latest packaging solutions for high-quality foods. Fraunhofer scientists will present their work on high-barrier films and packaging materials with an oxygen-absorbing functionality and integrated light-protection.

The languages of the symposium will be German and English. The German presentations will be simultaneously translated into English. The venue for the symposium is Erding, close to Munich Airport.

The Fraunhofer IVV reports its latest findings on Food Ingredients on a regular basis. In both 1999 and 2001 symposia were held which were well-received internationally. The aim of this year's symposium is not merely to report on further developments but also to promote interaction between those in the producing, modifying and processing sectors of the food industry and related sectors.

The symposium programme and further information can be obtained from the Fraunhofer IVV:

Your contact person there is Karin Agulla
Tel. +49 (0) 81 61/4 91-120, Fax -222,
E-mail: agulla@ivv.fhqde.

The symposium fee is € 490. Press representatives are invited to attend free of charge. They are asked to apply in advance.

Fresenius Seminar "Qualitätsmanagement in der Süß- und Backwarenindustrie" - Was bringt der neue Internationale Auditstandard? Praxisnahe Informationen über die neuen Qualitätsanforderungen sowie Details zum neuen Auditstandard)

Aus aktuellem Anlass bietet die Akademie Fresenius (Dortmund) in Kooperation mit dem Institut Fresenius (Taunusstein) dieses eintägige Praxisseminar an.

Der deutsche Lebensmittelhandel hat auf Basis des bereits erfolgreich eingeführten Standards des "British Retail Consortiums" (BRC) einen neuen Auditstandard erstellt, der die Mindestanforderungen der "Global Food Safety Initiative" (GFSI) und des deutschen Lebensmittelhandels vereinigt. Der neue Auditstandard wird in eine DIN-Norm eingebunden werden, nach der sich die Produktionsbetriebe zertifizieren lassen können.

Das Seminar vermittelt die notwendigen Hintergrundinformationen über die neuen Qualitätsanforderungen und gibt Hilfestellungen zur Ausrichtung auf den neuen Auditstandard: Zertifizierungsmöglichkeiten, Vorbereitungen, Kosten. Außerdem diskutieren die Teilnehmer Erfahrungen mit dem Benchmarking von Produktionsstandorten und Lieferanten. Alle Teilnehmer erhalten ein Zertifikat.

Angeboten wird der Workshop an zwei **Terminen**:
06. Juni 2002 (Stuttgart, Novotel Stuttgart-Nord) und
19. Juni 2002 (Hannover, Mercure Hotel Hannover City).

Preis: 445 € zzgl. MwSt.

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Mineralstoffe – Mengen-, Spuren- und Ultraspurenelemente in der Prävention

Manfred Anke, Ralf Müller, Ulrich Schäfer (Hrsg.)

(aus der Schriftenreihe der Gesellschaft für Mineralstoffe und Spurenelemente e.V.)

383 Seiten, **93** Abbildungen, **124** Tabellen; Wissenschaftliche Verlagsgesellschaft mbH Stuttgart, **2001**; **ISBN 3-8047-1838-8**; Kartoniert **€ 50.11**.

Dieser weitere Tagungsband der Gesellschaft für Mineralstoffe und Spurenelemente enthält insgesamt 39 Beiträge von Wissenschaftlern aus 12 Ländern Europas und Asiens, vorgestellt auf der 16. Jahrestagung in Jena, 1.-2.12. 2000). Die Beiträge befassen sich sowohl mit Unter- als auch Überversorgung an Mengen-, Spuren- und Ultraspurenelementen bei Mensch und Tier. Fragestellungen aus den Bereichen Analytik, Diagnostik, Ernährung, Stoffwechsel und Intoxikation werden unter dem Aspekt der Prävention unerwünschter Wirkungen dieser anorganischen Nahrungs-, Körper- und Umweltbestandteile erörtert.

Im Zentrum dieser interdisziplinären und internationalen Betrachtung der oben erläuterten Thematik stehen Arbeiten, die sich mit der chemischen Bestimmung, biologischen, ernährungswissenschaftlichen, medizinischen und toxikologischen Bedeutung sowie der rechtlichen Bewertung der Mengenelemente Na, K, Mg, Ca, der Spurenelemente Fe, Zn, Mn, Se, J, Mo, Cu und Ultraspurenelemente Hg, Cd, Pb, Cr und Cs beschäftigen.

Das Buch ermöglicht mit Hilfe eines Autoren- und Sachregisters eine rasche und umfassende Orientierung und ist besonders empfehlenswert für Ernährungswissenschaftler, Lebensmittelchemiker, Klinische Chemiker, Ärzte, Veterinäre, Landwirte, Apotheker und Molekularbiologen.

Biotechnologie der Arzneistoffe – Grundlagen und Anwendungen

Wolfgang Kreis, Diethard Baron, Günther Stoll

(aus der Reihe „Wissen und Praxis")

368 Seiten, **92** Abbildungen, **57** Tabellen; Deutscher Apotheker Verlag Stuttgart, **2001**; **ISBN 3-7692-2310-1**; Kartoniert **€ 50.11**.

Dieses Buch beschäftigt sich speziell mit der „Pharmazeutischen Biotechnologie", d.h. dem Einsatz von Organismen oder Teilen davon bei der Herstellung bzw. Verbesserung von Arzneistoffen in technischen Verfahren. Gerade in den letzten Jahren kamen zu den eher *traditionell* biotechnisch hergestellten Antibiotika, Steroidhormonen und Aminosäuren die *modernen*, rekombinant hergestellten Proteine dazu.

In allen Bereichen der Biotechnologie, speziell bei der Arzneimittelentwicklung, kommt der Gentechnologie mittlerweile eine immer wichtiger und deutlicher werdende Schlüsselstellung zu. Zur Zeit befinden sich in der EU mehr als 40 gentechnisch hergestellte Proteine in ca. 60 für die Anwendung beim Menschen zugelassenen Medikamenten auf dem Markt. Die Umsatzzahlen gentechnisch hergestellter Arzneimittel wachsen derzeit zweistellig. Dies ist vor allem den Entdeckungen und methodischen Fortschritten in der Molekularbiologie zu verdanken. Als interdisziplinäre, angewandte Wissenschaft führt die Biotechnologie Fortschritte und Erkenntnisse aus den Teilbereichen Mikrobiologie, Biochemie, Molekulare Genetik, Pflanzen- und Tierzucht, Chemieingenieurwesen, Analytische Chemie, Mess- und Regeltechnik sowie Apparatebau zusammen.

In dem vorliegenden empfehlenswerten Buch wird die interdisziplinäre Darstellung der Biotechnologie für Pharmazeuten vorgestellt mit folgenden Schwerpunkten: Bio- und gentechnische Methoden; Produzenten und Verfahren; Produkte mikrobieller, pflanzlicher, tierischer und menschlicher Herkunft. Ergänzt wird das Buch durch die Gesetzlichen Grundlagen, das Kapitel „Biotechnologie und Gesellschaft" sowie ein Sachregister.

African Traditional Medicine A Dictionary of Plant Use and Applications

Hans-Dieter Neuwinger

(with Supplement: Search System for Diseases)

589 pages, **46** pp. supplement; medpharm Scientific Publishers Stuttgart, **2000**; **ISBN 3-88763-086-6**; Hardcover **€ 102.00**.

This book is only a reference book, not one to be read for entertainment. In the form of a dictionary the knowledge of African plants and their use in traditional medicine is documented. More than 5,400 plants and over 16,300 medicinal applications are listed, more or or less detailed. The taxonomy of all the plants is, as far as has been possible, up-to-date. Unfortunately, the basis for scientific research, i. e. the handed-down and collected mass of knowledge about the medicinal use of plants is not complete, is partly being rapidly lost, remains unwritten or is passed on orally. The expertise of many local healers is still unknown and has yet to be recorded.

All plants are listed in the particular flora of an African country and also in the Kew Index. Information on active principles has been consistently omitted because this is not the purpose of this book.

This interesting handbook is mainly made up of literary research based on scientific journal entries and monographs. It is dedicated to scientists searching for medicinally active plants for further research. The alphabetical

arrangement of plants can also be of interest for botanists, biologists and ethnologists. Those readers more interested in the medicinal side can find a search system for diseases in a supplement included. This book is intended as a scientific overview and it is not a handbook for homeopaths or self-medication because many of the plants are toxic and may cause severe side effects or poisoning due to insufficient knowledge.

Stereochemie & Arzneistoffe – Grundlagen, Betrachtungen, Auswirkungen.

Hermann J. Roth, Christa E. Müller, Gerd Folkers

324 Seiten, **364** Abbildungen (**40** farbig), **27** Tabellen; Wissenschaftliche Verlagsgesellschaft mbH Stuttgart, **1998**; **ISBN 3- 8047-1485-4**; Kartoniert **€ 29.65**.

Biochemische Reaktionen und pharmakologische Effekte sind bisher häufig nur zweidimensional betrachtet worden. Daher sind auch heute noch totalsynthetische chirale Arzneistoffe zum überwiegenden Teil als Racemate im Handel. Es ist aber schon lange bekannt, dass sich die R- und S-Enantiomeren in ihren biologischen Effekten qualitativ und quantitativ deutlich unterscheiden. Da aber alle Moleküle dreidimensionale Gebilde darstellen, sind befriedigende und gesicherte Erkenntnisse des biologischen Geschehens oder der pharmakologischen Wirkung nur durch stereochemische Betrachtung möglich. In diesem Buch werden die statische Stereochemie (räumlicher Aufbau der Moleküle) und die dynamische Stereochemie (räumlicher Ablauf chemischer Reaktionen) unter dem Aspekt der Struktur und Wirkung von Arzneistoffen lebensnah vermittelt und der Einstieg in die dreidimensionale Welt biologisch aktiver Moleküle wird erleichtert. Wann immer möglich, wird auf die Grundlagen der Stereochemie Bezug genommen. Deshalb ist dieses Buch für einen breiteren Leserkreis interessant und besonders empfehlenswert für Pharmazeuten, Chemiker, Biologen und Mediziner.

AUS DEM INHALT

1. Stereoisomerie - 2. Historische Betrachtungen - 3. Die Entstehung der homochiralen Welt- 4. Stereochemie der Arzneistoffe I: Chirale Verbindungen - 5. Stereochemie der Arzneistoffe II: Verbindungen mit E/Z-(cis/trans) Isomerie und Ringsysteme - 6. Konformation - 7. Gewinnung enantiomerenreiner Arzneistoffe - 8. Stereochemische Charakterisierung von Verbindungen - 9. Stereochemie pharmazeutisch interessanter Zucker - 10. Stereochemie der Nucleinsäuren und ihrer Bauelemente - 11. Stereochemie der Proteine und ihrer Bauelemente - 12. Stereochemische Aspekte des Arzneistoff-Schicksals - 13. Glossar - 14. Stereochemische Bezeichnungen und Symbole.

Encyclopedia of Food Mycotoxins

Martin Weidenbörner

295 pages, **96** figures, **9** tables; Springer-Verlag Berlin – Heidelberg – New York – Barcelona – Hong Kong – London – Milan – Paris – Singapore – Tokyo, **2001**; **ISBN 3- 540-67556-6**; Hardcover **US $ 99.00**.

Various species of fungi (only the names of food-relevant molds are given) like to grow on food, releasing toxins which might bear a health risk to the consumer. The main emphasis of the present book is an alphabetical list and overview of all foods which have been reported to be contaminated with mycotoxins. Data on the degree of contamination, the concentration of the toxins, the country of origin and/or detection of the contaminated food are included. The multiple listing of some countries should not implicate a high rate of mycotoxin contamination of foods there, but primarily document the efforts being made to detect toxic fungal metabolites in food. These data concerning mycotoxin contamination of food listed in this encyclopedia based on results predominantly published in recommended journals and scientific monographs in this special field.

This book is a valuable supplement in the library of specialists in this scientific field, especially microbiologists, food technologists and chemists.

Handbook of Environmental Data on Organic Chemicals

Karel Verschueren (Ed.)

(Series in conjunction with Environmental Protection Magazine Dallas, Texas)

Fourth edition; two-volume print set with **2391** pages; JOHN WILEY & SONS, INC. New York – Chichester – Weinheim – Brisbane – Singapore – Toronto, **2001**; **ISBN 0-471-37490-3** (Two volume print set), **0-471-37488-1** (CD-ROM), **0-471-41188-4** (Two volume print set + CD-ROM); Hardcover **495.00 US $**, CD-ROM **545.00 US $.**

This handbook is a trusted reference for over two decades and a must for wastewater engineers, groundwater scientists, industrial hygienists, toxicologists, ecologists, geologists and also regulatory personnel. With this reference book you get fast, hands-on access to the latest data on organic compounds and their environmental impact.

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concentration in air at different temperatures, solubility, and liquid or solid density at room temperature), air pollution factors (conversion factors, odor treshold values and characteristics, atmospheric reactions, natural and man-made sources, emission control methods, and methods of sampling and analysis), water and soil pollution factors (biodegradation processes, odor and color of contaminated water or microorganisms, quality of surface water and undergroung water and sediment, natural and man-made sources of pollution, wastewater treatment methods and results, and methods of sampling and analysis), biological effects (residual concentrations, bioaccumulation values, and toxicological effects of exposure in ecosystems, plants, animals, and humans).

These environmental data are gathered from worldwide sources and edited by an internationally renowned expert. They offer ready access to the commonly accepted names,. synonyms, molecular and structural formulas, CAS numbers, etc. Worthful are also the extensive appendices containing glossary, abbreviations, and more than 5,000 bibliographic references. Explanatory notes clarify the categories for each entry and describe how the data can be used for pollution prevention and control. To have all these data on a CD-ROM is very comfortable.

Frying: Improving quality

J. B. Rossell (Ed.)

Woodhead Publishing in Food Science and Technology

369 pages, numerous figures and tables; CRC Press Boca Raton - Boston - New York - Washington D.C., published in Europe by Woodhead Publishing Ltd Cambridge England, **2001**; **ISBN 1-85573-556-3**; Hardcover **£ 125.00/ € 206.00** (plus p&p).

This interesting new book provides an authoritative review of key issues in improving quality in the manufacture of fried products. In the first part of the book an introduction is given with the differing types of fried products, their markets and also the regulatory context. A discussion of the role of dietary lipids, the impact of frying on lipid intake and its influence on consumer health is included. Part 2 deals in detail with frying oils, the most important common influence on fried product quality. These oils have to withstand the stresses of high temperatures during frying, but also have to maintain their quality during subsequent storage of fried products. The factors affecting frying oil quality and the methods for measuring this quality are included. The final part looks at quality factors relating to fried products (pre-fried potato products such as French fries or potato crisps). The last chapters of this part give details on an effective process control of the frying operations, flavour development in fried foods and possibilities of analysing and improving the texture and colour of fried products.

This up-to-date presentation of frying under the aspect of improving quality of fried products is part of the series "Woodhead Publishing in Food Science and Technology" and especially recommendable for food technologists.

Wasseranalysen – richtig beurteilt; Grundlagen, Parameter, Wassertypen, Inhaltsstoffe, Grenzwerte nach Trinkwasserverordnung und EU-Trinkwasserrichtlinie.

Walter Kölle

357 Seiten, **43** Abbildungen, **44** Tabellen; WILEY-VCH Weinheim - New York - Chichester - Brisbane - Singapore - Toronto, **2001**; **ISBN 3-527-30169-0**; Hardcover **EUR 85.90**.

Trinkwasser ist unser wichtigstes Lebensmittel. Wasseranalysen müssen regelmäßig durchgeführt werden und geben Aufschluß über den ökologischen Zustand der Wasserressourcen, die Möglichkeiten der technischen Aufbereitung und auch über die Leistungsfähigkeit der Laboratorien. Der blanke Vergleich von Messergebnissen reicht zur Beurteilung der Wasserqualität längst nicht mehr aus, zumal derzeit die Umsetzung der EU-Trinkwasserrichtlinie stattfindet.

Dieses empfehlenswerte Buch ist ein geeignetes Hilfsmittel zur richtigen Beurteilung von Wasseranalysen. Der Autor hat seine jahrzehntelange Erfahrung eingebracht und den gesamten Themenkomplex systematisch und praxisorientiert vorgestellt. Zusätzlich wertvoll ist die Untermauerung der Theorie durch zahlreiche Tabellen und konkrete Fallbeispiele mit vielen nützlichen Daten.

AUS DEM INHALT

Vorwort; Grundlagen; Wasser-Typen; Physikalische, physikalisch-chemische und allgemeine Parameter; Anorganische Wasserinhaltsstoffe, Hauptkomponenten und Spurenstoffe; Organische Wasserinhaltsstoffe; Calcitsättigung; Mikrobiologische Parameter und Desinfektionsmittel; Radioaktivität; Kürzel und Begriffe; Tabellenanhang; Analysenanhang; Literatur und Register.

Thermal technologies in food processing

Philip Richardson (Ed.)

Woodhead Publishing in Food Science and Technology

294 pages, numerous figures and tables; CRC Press Boca Raton - Boston - New York - Washington D.C., published in Europe by Woodhead Publishing Ltd Cambridge England, **2001**; **ISBN 1-85573-558-X**; Hardcover **£ 115.00/ € 190.00** (plus p&p).

Thermal technologies are irreplaceable in food processing. Heat application is both an important method of food preservation as well as a means of developing texture, flavour and colour initiating the Maillard reaction. Aim of this application is not to damage other desirable sensory and nutritional qualities in a food product. This book is a state-of-the-art presentation of this major issue edited by an expert in this field in collaboration with a distinguished international team of contributors.

The book begins by reviewing conventional retort and continuous heat technologies. Then the key issues of measurement and control in ensuring not only the effectiveness of the thermal process but also the minimization of undesirable changes in the food products. A lot of chapters deal with temperature and pressure management, validation of heat processes, modelling and simulation of thermal processes and the measurement/control of changes during thermal processing. In the last part there are introduced different thermal technologies, which are becoming more and more used in the food industry (radio frequency heating, microwave processing, infrared heating, instant and high-heat infusion, ohmic heating). Finally thermal combined with high pressure processing is considered, a new technology guaranteeing safe and minimally-processed food products.

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